19th MEETING

3-5 November 2021

Lleida

ABSTRACT BOOK

SPANISH SOCIETY OF NEUROSCIENCE

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Sections

Plenary

Lectures

ALL-OPTICAL INTERROGATION OF BRAIN CIRCUITS USING OPTOGENETICS AND HOLOGRAPHY

Genetic targeting of neuronal cells with activity reporters (calcium or voltage indicators) has initiated the paradigmatic transition whereby photons have replaced electrons for reading large-scale brain activities at cellular resolution. In parallel, optogenetics has demonstrated that targeting neuronal cells with photosensitive microbial opsins, enables the transduction of photons into electrical currents of opposite polarities thus writing, through activation or inhibition, neuronal signals in a non-invasive way.
These progresses have in turn stimulated the development of sophisticated optical methods to enable “all optical” in depth brain circuits interrogation with high spatial and temporal resolution on large volumes.
Here, we will review the most significant breakthroughs of the past years, which enable reading and writing neuronal activity at the relevant spatiotemporal scale for brain circuits manipulation, with particular emphasis on the most recent advances in what we named *circuit optogenetics*: a combination of approaches including holographic light illumination, temporal focusing, opsins engineering and laser development for the control of single or multiple targets independently in space and time with single-neuron and single-spike precision, at large depths.

ON THE COMPLEXITIES OF IMMOBILITY

Our work concerns the general problem of adaptive behaviour in response to predatory threats, and of the neural mechanisms underlying choice and implementation of defensive strategies. When faced with a threat, an animal must decide whether to freeze, reducing its chances of being noticed, or to flee to the safety of a refuge. Animals, from fish to primates, freeze, when faced with distant or inescapable threats, staying completely immobile for prolonged periods. We recently found that fruit flies also freeze when faced with a visual inescapable threat. Using this model organism, we demonstrated that threat-induced freezing corresponds to a distinct internal state from that of spontaneous immobility, as measured by the animal's cardiac activity. Furthermore, by measuring sugar levels and resistance to starvation we found that freezing behaviour is energetically costly, contradicting a widespread belief that freezing is an energy sparing preparatory state. In mammals the large number of brain regions involved in the expression of freezing suggests that this seemingly simple behaviour requires the integration of multiple sources of information. We believe that describing how contextual cues modulate freezing will be instrumental for our understanding of the organization of survival circuits in the brain. Towards this end, we are studying how flies process contextual cues, focusing on the social and spatial environment, and how these come to gate freezing. Given the knowledge regarding sensory detection of visual looming threats and descending neuron involved in the expression of freezing, we are now in a unique position to understand how information about a threat is integrated with cues from the environment to guide the choice of whether to freeze.

NEURAL SUBSTRATES OF ASSOCIATIVE, ELECTIVE, AND COOPERATIVE LEARNING TASKS

J.M. Delgado-García
Division of Neurosciences, Pablo de Olavide University, 41013-Seville, Spain

While contemporary Neuroscience is paying increasing attention to subcellular and molecular events underlying the acquisition, storage, and retrieval of newly acquired motor and cognitive abilities, a similar attention should be paid to the study of electrophysiological phenomena taking place at cortical and subcortical sites during the very moment in which complex forms of learning processes are being acquired. These *in vivo* approaches to the study of individual and social learning will allow the proper integration of the important information already collected from *in vitro* and delayed molecular studies. During my presentation, I will summarize studies carried out in our laboratory on activity-dependent changes in unitary activity, synaptic strength, and local field potentials taking place in hippocampal, motor, prefrontal and related cortical and subcortical circuits during the acquisition of associative, elective, and cooperative learning paradigms. Available data allow to suggest that different hippocampal synapses are selectively modified in strength during the acquisition of classical, but not instrumental, conditioning paradigms. In contrast, selected prefrontal and striatum synapses are more directly modified by instrumental conditioning tasks. These studies also show that besides NMDA receptors, many other neurotransmitter, intracellular mediating, and transcription factors participate in these two types of associative learning. Interestingly, structures as the medial prefrontal cortex, the amygdala, the claustrum and the accumbens nucleus are selectively involved in the behavioral and/or cognitive components of other complex forms of associative learning as cooperative behaviors, and decision making and go/no-go situations. The differential roles of these cortical and subcortical structures during these different types of associative learning will be described and the distributed and timed nature of associative learning abilities will be stressed.

INNATE IMMUNITY IN NEURODEGENERATIVE DISEASE

Michael T. Heneka[1,2]
[1] *Dept of Neurodegenerative Disease and Geriatric Psychiatry, University of Bonn, Bonn, Germany, michael.heneka@ukbonn.de*
[2]*German Center for Neurodegenerative Disease (DZNE), Bonn, Germany.*

The accumulation of neurotoxic amyloid beta peptides along with neurofibrillary tangle formation are key pathological hallmarks of Alzheimer's disease. The brain has been considered as an immune-privileged organ, however, increasing evidence from translational, genetic, and pathological studies suggests that activation of distinct innate immune pathways are a third important disease hallmark which actively contributes to disease progression and chronicity.
Microglia play a pivotal role in this immune response and are activated by binding of aggregated proteins or aberrant nucleic acids to pattern recognition receptors. This immune activation leads to the release of inflammatory mediators, but also distracts microglia cells from their physiological functions and tasks, including debris clearance and trophic factor support. NLRP3 inflammasome activation microglial clearance capacities and such contributes to the increase amyloid beta burden of the brain. Additionally, NLRP3 is a negative regulator of hippocampal long-term potentiation and spatial navigation memory. Chronic activation of the NLRP3 inflammasome causes microglial pyroptosis and thereby the release of ASC specks. The latter contribute to spreading of pathology by enhancing the propensity of beta-amyloid peptides to aggregate. Importantly, sustained NLRP3 activation can increase tau pathology and the formation of neurofibrillary tangles in vitro and in vivo, through a bidirectional regulation of tau kinases CAMKII-apha and GSK3-beta on one hand and phosphatase PP2A on the other.
In keeping with this immune hypothesis of neurodegeneration, inhibition of NLRP3-related immune pathways protect from neurodegeneration in cellular and murine models of Alzheimer's disease and primary tauopathies, collectively suggesting that this pathway could be exploited for future therapeutic interventions.

Symposia

Symposium S01
BINDING CELL ASSEMBLIES INTO MEMORY ENGRAMS

TITLE OF THE SYMPOSIUM: BINDING CELL ASSEMBLIES INTO MEMORY ENGRAMS

Topic: Systems, cognitive and computational neuroscience.

Mechanistic research on memory formation is currently a hot topic in neuroscience. Based on the new tools available to investigate the formation of memory engrams, the dominant view is that memory is encoded in a group of cells (not all) that was activated during the experience to be remembered, and involves several (not all) brain structures. However, a clear definition, with spatial and temporal limits, of what we understand by memory engram at present does not exist. In this symposium we will discuss from complementary computational and experimental investigations, and sometimes with competing points of view, how neurons dispersed in distributed cell assemblies might be recruited and bound into an engram holding a specific memory. The problem of stable vs. transient membership of neurons to engrams will be also discussed.

SPEAKER #1

- Full Name: Marlene Bartos, Cellular and Systemic Neurophysiology Dept. University of Freiburg
- Enter three recent publications of the Speaker related to the topic of the symposium:
    1) Hainmueller T, Bartos M. Dentate gyrus circuits for encoding, retrieval and discrimination of episodic memories. Nat Rev Neurosci., 21(3):153-168. 2020.
    2) Hainmueller T, Bartos M. Parallel emergence of stable and dynamic memory engrams in the hippocampus. Nature, 558(7709):292-296. 2018.
    3) Strüber et al. Distance-dependent inhibition facilitates focality of gamma oscillations in the dentate gyrus. Nat Commun., 8(1):758. 2017.
- Relevance for the symposium: Dr. Bartos is an Advanced ERC grant holder focused on understanding memory engram formation. She will discuss the role of entorhinal cortex inputs forcontext-specific engrams in the hippocampus. She is a world-leader in the study of the physiology of the dentate gyrus. Together with Peter Jones they discovered the multiple roles that dentate inhibition plays in regulating information transmission in the hippocampus. More recently, Dr. Bartos has unveiled the distinct temporal properties of cell assemblies formed in CA3 and CA1 (dynamic) vs. the more stable dentate assemblies, providing an explanation to the capacity of dentate gyrus to reactivate memories. She will share with us her ideas about how the dentate gyrus may serve as a memory repository.

- Abstract:

Prof. Dr. Marlene Bartos
University of Freiburg
Institute for Physiology, Dept. I
Cellular and Systemic Neurophysiology
Hermann-Herder Str. 7
79104 Freiburg
Germany

THE HIPPOCAMPUS CONVERGES VOLATILE ENTORHINAL INPUTS IN STABLE SPATIAL MAPS

The dentate gyrus (DG) is the entrance gate of the hippocampus and translates the rich input stream from the entorhinal cortex into sparse non-overlapping memories. The network mechanisms underlying sparse coding are however largely unknown. In this talk, I will highlight new insights on the role of the various cellular components of the DG network, glutamatergic granule cells (GCs) and GABAergic inhibitory interneuron types in the sparse coding of information and the spatio-temporal emergence of DG population activity during learning. I will provide new insights on the relationship between the rich input stream provided by the medial entorhinal cortex to the DG and the hippocampal areas CA1 and CA3 and the spatial code generated by the principal cell output, which is forwarded to downstream brain areas for mnemonic associations. I will present our recently published and unpublished data obtained with /state-of-the-art techniques/ including single unit recordings and 2-photon population imaging of neuron types in behaving rodents, to test their role in cell assembly formation for the representation of space and context during learning.

SPEAKER #2

- Full Name: Panayiota Poirazi, from the Foundation for Research and Technology-Helas, Crete.
- Enter three recent publications of the Speaker related to the topic of the symposium:
    1) Tzilivaki A, Kastellakis G and Poirazi P. Challenging the point neuron dogma: FS basket cells as 2-stage nonlinear integrators. Nat Commun. 10(1):3664. 2019.
    2) Gidon et al. Dendritic action potentials and the computation in human layer 2/3 cortical neurons. Science, 367(6473):83-87. 2020
    3) Shuman et al. Breakdown of spatial coding and interneuron synchronization in epileptic mice. Nat Neurosci. 23(2):229-238. 2020.
- Relevance for the symposium: Dr. Poirazi is a Consolidator ERC grant holder focused of the role of neuronal dendrites on brain network computation and memory engram formation. She has contributed to the discovery of important dendritic functions that boost the computation capacities of excitatory neurons. Her team has recently extended these functions to inhibitory interneurons and showed their contribution to computations such as pattern separations. She has further proposed a role for dendrites in the sparseness of cell populations recruited into engrams. She will provide a computational modeling perspective to the questions of how cell assemblies bind into memory engrams.

- Abstract:

ACTIVE DENDRITES AND THEIR ROLE IN MEMORY ASSEMBLIES

Panayiota Poirazi [1*]
[1] *Institute of Molecular Biology and Biotechnology (IMBB), Foundation for Research and Technology-Hellas (FORTH), Heraklion, Crete, GREECE*
* *poirazi@imbb.forth.gr*

Abstract:
The goal of this presentation is to provide a set of predictions generated by biophysical and/or abstract mathematical models regarding the role of dendrites in information processing, learning and memory across different brain regions. I will present modelling studies from our lab –along with supporting experimental evidence- that investigate how dendrites may be used to facilitate the learning and coding of both spatial and temporal information at the single cell, the microcircuit and the neuronal network level. I will present the main findings of a number of projects in lab dealing with dendritic nonlinearities in PV interneurons and their consequences on memory engram formation [1], the role

of dendrites in solving nonlinear problems in human neurons [2] and microcircuit contributions to spatial learning in the CA1 [3,4].

[1] Tzilivaki A, Kastellakis G, Poirazi P. Challenging the point neuron dogma: FS basket cells as 2-stage nonlinear integrators. Nat Commun. 2019 Aug 14;10(1):3664. doi: 10.1038/s41467-019-11537-7.

[2] Gidon A, Zolnik TA, Fidzinski P, Bolduan F, Papoutsi A, Poirazi P, Holtkamp M, Vida I, Larkum ME. Dendritic action potentials and computation in human layer 2/3 cortical neurons. Science. 2020 Jan 3;367(6473):83-87. doi: 10.1126/science.aax6239.

[3] Turi GF, Li WK, Chavlis S, Pandi I, O'Hare J, Priestley JB, Grosmark AD, Liao Z, Ladow M, Zhang JF, Zemelman BV, Poirazi P, Losonczy A. Vasoactive Intestinal Polypeptide-Expressing Interneurons in the Hippocampus Support Goal-Oriented Spatial Learning. Neuron. 2019 Mar 20;101(6):1150-1165.e8. doi: 10.1016/j.neuron.2019.01.009. Epub 2019 Jan 31.

[4] Shuman T, Aharoni D, Cai DJ, Lee CR, Chavlis S, Page-Harley L, Vetere LM, Feng Y, Yang CY, Mollinedo-Gajate I, Chen L, Pennington ZT, Taxidis J, Flores SE, Cheng K, Javaherian M, Kaba CC, Rao N, La-Vu M, Pandi I, Shtrahman M, Bakhurin KI, Masmanidis SC, Khakh BS, Poirazi P, Silva AJ, Golshani P. Breakdown of spatial coding and interneuron synchronization in epileptic mice. Nat Neurosci. 2020 Feb;23(2):229-238. doi: 10.1038/s41593-019-0559-0.

SPEAKER #3

- Full Name: Claudio Mirasso, Instituto de Física de Sistemas Complejos (IFISC), CSIC-Universidad de las Islas Baleares.
- Enter three recent publications of the Speaker related to the topic of the symposium:

1) Ziaeemehr et al. Frequency-dependent organization of the brain's functional network through delayed-interactions. Neural Netw.,132:155-165. 2020.
2) López-Madrona VJ, et al. Different theta frameworks coexist in the rat hippocampus and are coordinated during memory-guided and novelty tasks. Elife, 9: e57313. 2020.
3) Pariz A, et al. High frequency neurons determine effective connectivity in neuronal networks. Neuroimage, 166:349-359. 2018.

- Relevance for the symposium: Dr. Mirasso is co-organizer of the symposium together with Dr. Canals. Dr. Mirasso is interested in understanding communication in complex systems, including the brain and photonic networks, to mention some examples. He investigates how specific activity patters and synchronization between different nodes of a large network contribute to information transmission. In this symposium, he will bring a computational perspective of network communications which is highly relevant to excitation/inhibition balance in brain networks and the routing of information.

- Abstract:

REGULATION OF INHIBITORY CIRCUITS IN THE DENTATE GYRUS: ROLE ON TEMPORAL CODING AND PATTERN SEPARATION

C. Estarellas[1,2], E. Álvarez[2], S. Canals[2] and C. Mirasso[1]
[1]Instituto de Física Interdisciplinar y Sistemas Complejos (IFISC, UIB-CSIC), Campus UIB, E-07122, Palma de Mallorca, Spain.
[2] Instituto de Neurociencias, Consejo Superior de Investigaciones Científicas, Universidad Miguel Hernández, E-03550 Sant Joan d'Alacant, Spain.

Electrophysiological recordings in the hippocampus revealed a tight control of inhibitory interneurons over the Granule Cells (GCs) of the Dentate Gyrus. In in-vivo experiments of Long-Term Potentiation (LTP) of the Perforant Pathway, it is found that the expected potentiation of glutamatergic synapses is also accompanied by a reduction of the feedforward inhibitory activity, facilitating activity propagation in the circuit. To further investigate this phenomenon, we built a

population model where neurons were described by Izhikevich's equations and synapsis mediated by AMPA, NMDA and GABAA receptors. The results obtained from the numerical integration of the model equations, before and after the application of the LTP, support both the counterintuitive experimental observation of synaptic depression in the feed-forward inhibitory connection after LTP induction as well as the change in the correlation between excitatory and inhibitory inputs over GCs. We find that LTP increases the efficiency of the glutamatergic input to recruit the inhibitory network of the hilar region, resulting in an average reduction of the basket cell population activity. The predictions of the model were experimentally corroborated by intracellular patch-clamp recordings in an in vitro preparation, after in vivo LTP induction in mice. To gain insight into the functional role of the feedforward inhibition reduction over GCs, we built a neuronal model of GCs including their dendritic arbor. The results obtained from this model predict that the reorganization induced by the LTP increases the occurrence of bursts in GCs and improves the temporal coding and information transmission from the entorhinal cortex to the CA3 via GCs. This finding is supported by previous experiments where it was observed that the activity of CA3 pyramidal cells is tremendously facilitated upon highfrequency presynaptic GCs activity while producing minor changes on the inhibitory neurons' activity.

SPEAKER #4

- Full Name: Santiago Canals, Instituto de Neurociencias de Alicante, Consejo Superior de Investigaciones Científicas (CSIC)-Universidad Miguel Hernández de Elche (UMH)
- Enter three recent publications of the Speaker related to the topic of the symposium:

1) Caramés JM et al.Hippocampal dentate gyrus coordinates brain-wide communication and memory updating through an inhibitory gating. BioRxiv. 2020.doi: https://doi.org/10.1101/2020.07.14.202218

2) López-Madrona VJ, et al. Different theta frameworks coexist in the rat hippocampus and arecoordinated during memory-guided and novelty tasks. Elife, 9: e57313. 2020.

3) Del Ferraro et al. Finding influential nodes for integration in brain networks using optimal percolation theory. Nat Commun., 9(1):2274. 2018.

- Relevance for the symposium: Dr. Canals is co-organizer of the symposium. The talk of Dr. canals will bring the key regulatory control of dentate gyrus inhibition in the selection of the cell assemblies that conform an engram and hold the memory. This experimental research tries to find the mechanism that gate information in brain networks to associate different sensory inputs and assimilate them, when novel, into the memory engram.

- Abstract:

THE DENTATE GYRUS COORDINATES BRAIN-WIDE FUNCTIONAL NETWORKS DURING MEMORY FORMATION.

Elena Pérez-Montoyo, José María Caramés, Raquel Garcia-Hernandez and Santiago Canals
Instituto de Neurociencias de Alicante (CSIC-UMH), Sant Joan d'Alacant, Alicante, Spain.

Memory is thought to be encoded through modifications in the weights of synaptic connections. The circuits enabled by these synaptic modifications, and the corresponding activated cell assemblies, form brain-wide memory engrams that hold specific memory. However, the mechanisms linking task-activated neuronal populations in the brain are not well understood. Using cell-specific manipulations of inhibitory neuronal activity, we discovered a key role of the dentate gyrus (DG) in coordinating dispersed neuronal populations during memory formation. In whole-brain fMRI and

electrophysiological experiments, we found that parvalbumin (PV) interneurons in the DG control the functional coupling of the hippocampus within a wider network of neocortical and subcortical structures including the prefrontal cortex (PFC) and the nucleus accumbens (NAc). In a novel object-location task, regulation of PV interneuron activity enhanced or prevented memory encoding and, without effect upon the total number of task activated c-Fos+ cells, revealed a correlation between activated neuronal populations in the hippocampus-PFC-NAc network. These results are consistent with a previously described contribution of long-term synaptic plasticity in the DG to brain-wide activity propagation, and the importance of the NAc in this network reorganization. These data suggest a critical regulatory role of PV interneurons in the dentate gyrus in brain-wide polysynaptic communication channels and the association of cell assemblies across multiple brain regions.

Symposium S02
DECIPHERING BRAIN CIRCUITS: INSIGHTS FROM REWARD AND NEURONAL EXCITABILITY

TITLE OF THE SYMPOSIUM: Deciphering Brain Circuits: Insights from Reward and Neuronal Excitability

Present symposium proposal aims to present and discuss novel findings regarding themechanisms by which cells communicate and form circuitries in brain regions responsible for specific behavior output in health and disease. All the speakers will also be presenting several cutting edge techniques useful for every area of neuroscience.

The speakers of this symposium are prestigious scientists that have highly contributed to most of the above evidence in recent publications all in high-ranking journals. Therefore, we believe they constitute an outstanding panel to present up-to-date and innovative data. Finally, this symposium will provide an opportunity for Neuroscientist to gain deeper insights into novel techniques and methodologies to study cell circuitries.

SPEAKER #1

-Full Name: **Ana João Rodrigues, PhD**
University of Minho
ICVS | School of Medicine
Campus Gualtar
4710-057 Braga
Portugal
Tel:+351 253 604 929/835
email:ajrodrigues@med.uminho.pt

-Enter three recent publications of the Speaker related to the topic of the symposium:

1. Role of laterodorsal tegmentum projections to nucleus accumbens in reward-related behaviors. Coimbra, B., Soares-Cunha, C., Vasconcelos, N.A.P., Domingues, A.V., Borges, S., Sousa, N., Rodrigues, A.J. (2019) Nature Communications, 10 (1), art. no. 4138, .
2. Nucleus accumbens medium spiny neurons subtypes signal both reward and aversion. Soares-Cunha, C., de Vasconcelos, N.A.P., Coimbra, B., Domingues, A.V., Silva, J.M., Loureiro-Campos, E., Gaspar, R., Sotiropoulos,I., Sousa, N., Rodrigues, A.J. (2019) Molecular Psychiatry, https://doi.org/10.1038/s41380-019-0484-3.
3. Impairments in laterodorsal tegmentum to VTA projections underlie glucocorticoid-triggered reward deficits. Coimbra, B., Soares-Cunha, C., Borges, S., Vasconcelos, N.A.P., Sousa, N., Rodrigues, A.J. (2017) eLife, 6, art. no. e25843.

.-Relevance for the symposium:
Dr. Rodrigues is a group leader at the University of Minho. Herpresentation will be focused on the neuronal mechanismsthat drivemotivated behaviors and how the brain processespositive and negative stimuli, and the impact of stress in the underlying neuronal networks.Sheusesa combination of behavioral, neuroanatomical, electrophysiology, molecular and optogenetic tools to dissect the neurobiological basis of valence (reward and aversion) and motivation.

- Abstract:

NEUROBIOLOGICAL BASIS OF REWARD AND AVERSION: A FOCUS ON THE NUCLEUS ACCUMBENS CIRCUITRY

Ana João Rodrigues
University of Minho, ICVS/School of Medicine, Braga, Portugal

The nucleus accumbens (NAc) is a key player in reward/aversion and motivated behaviors. The NAc is mainly composed of medium spiny neurons (MSNs), segregated into those that express dopamine receptor D1 or D2. D1-MSNs have been associated with positive reinforcement and reward, whereas D2-MSNs neurons are associated with negative reinforcement and aversion. However, recent evidence from our team and others challenged this view of functional opposition.
In this seminar, we will show that by differentially controlling the activation pattern of either type of nucleus accumbens MSNs, one can trigger both reward and aversion. We will further show that even for the same type of MSN, distinct subpopulations exist that respond differently to different stages of reward-related behaviors.
The complexity of the findings suggest that we need to revise the proposed model of striatal functional opposition of MSNs, and that additional studies are needed to unravel the role of each type in behavior.

SPEAKER #2

-Full Name: **Jan Tonnesen, PhD**

Achucarro Basque Center for Neuroscience
Science Park of the UPV/EHU
Sede Building, 3rdfloor, Barrio Sarriena s/n
48940 Leioa Spain
phone: (+34) 946018300
email: jan.tonnesen@ehu.es

-Enter three recent publications of the Speaker related to the topic of the symposium:

1. Super-Resolution Imaging of the Extracellular Space in Living Brain Tissue. Tønnesen J, Inavalli VVGK, Nägerl UV. Cell. (2018)
2. Neurobiological Mechanisms of Autism Spectrum Disorder and Epilepsy, Insights from Animal Models. Sierra-Arregui T, Llorente J, Giménez Minguez P, Tønnesen J, Peñagarikano O. Neuroscience. (2020)
3. Spine neck plasticity regulates compartmentalization of synapses. Tønnesen J,Katona G, Rózsa B, Nägerl UV. Nat Neurosci. (2014)

-Relevance for the symposium:
Dr. Tonnesen is a Ramon y Cajal Researcher at the Achucarro Basque Center for Neuroscience. His talk will be focused on the mechanisms that influence neuronal excitabilityin neurological diseases as epilepsy. His studies are centered onthe changes that occur in individual dendrites and synapses, as well as extracellular factors mostly regulated by astrocytes that directly affect the activity of specialized neurons. Dr. Tonnesen also outstands for this symposium for the use of state of the art techniques to visualize in vivo synapses.

- Abstract:

REVEALING THE FUNCTIONAL ANATOMY OF THE NEUROPIL USING SUPERRESOLUTION STED MICROSCOPY

Jan Tønnesen
Achucarro Basque Center for Neuroscience and University of the Basque Country, Bilbao, Spain

Stimulated emission depletion (STED) microscopy is unique among the commonly applied super-resolution techniques in being a laser beam scanning fluorescence microscopy modality. This makes it particularly suited for imaging inside live brain tissue, where it can resolve the morphology and dynamics of the smallest cellular structures, including dendritic spines, boutons and glia processes. More recently, we have conceived a new STED based approach to visualize also the extracellular space in live brain slices, termed super-resolution shadow imaging (SUSHI). Because the SUSHI approach is based on fluorescently labeling of the interstitial fluid, it inherently reveals all cellular structures in the field of view as shadows, and these can be analyzed in the context of the extracellular space. When SUSHI is combined with conventional STED imaging of fluorescently labelled cells, it allows analysis of these in the context of the neuropil.
In this talk, I will first introduce STED/SUSHI and the proof-of-principle findings it has brought about in revealing structural extracellular space dynamics on multiple spatiotemporal scales. I will move on to our current work that aims to understand diffusion in the extracellular space by developing a computational model based on live tissue SUSHI imaging, and finally I will outline our future plans for investigating the interplay between microglia cells and the extracellular space using our newly custom-built STED microscope.

SPEAKER #3

-Full Name: **Marta Navarrete Llinás, PhD**
Instituto Cajal, CSIC
28002Madrid
Spain
Tel:+3491 585 4648
email:mllinas@cajal.csic.es

-Enter three recent publications of the Speaker related to the topic of the symposium:

1. A specific prelimbic-nucleus accumbens pathway controls resilience versus vulnerability to food addiction. Domingo-Rodriguez, L., Ruizde Azua, I., Dominguez, E., Senabre, E., Serra, I., Kummer, S., Navandar, M., Baddenhausen, S., Hofmann, C., Andero, R., Gerber, S., Navarrete, M., Dierssen, M., Lutz, B., Martín-García, E., Maldonado, R. (2020) Nature Communications, 11 (1), art. no. 782.
2. Astrocytic p38α MAPK drives NMDA receptor-dependent long-term depression and modulates long-term memory. Navarrete, M., Cuartero, M.I., Palenzuela, R., Draffin, J.E., Konomi, A., Serra, I., Colié, S., Castaño-Castaño, S., Hasan, M.T., Nebreda, Á.R., Esteban, J.A (2019) Nature Communications, 10 (1), art. no. 2968, .

3. Melanopsin for precise optogenetic activation of astrocyte-neuron networks. Mederos, S., Hernández-Vivanco, A., Ramírez-Franco, J., Martín-Fernández, M., Navarrete, M., Yang, A., Boyden, E.S., Perea, G.(2019) GLIA, 67 (5), pp. 915-934.

-Relevance for the symposium:
Dr. Navarrete is a Ramon y Cajal Researcher at the Cajal Institute (CSIC). Her research is focused on the study of the properties that define the bidirectional communication between astrocytes and

neurons, and its physiological and pathological implications in brain function. In this symposium she will show her recent discoveries related with the role of gliotransmitters released by astrocytes on basal ganglia circuits andtheir impact on animal behavior.
- Abstract:

ASTROCYTIC NETWORK HETEROGENEITY IN THE NUCLEUS ACCUMBENS
M. Navarrete
Instituto Cajal, Consejo Superior de Investigaciones Científicas, Madrid, Spain.

Unraveling the principles of information processing in complex cell circuits requires techniques capable of target and modulates specifically the activity of those elements involved.
Neuro-astrocyte networks display a surprising degree of complexity and state-of-the-art complementary tools are required to understand astrocyte involvement in circuit modulation and behavior. Although the evolution of genetic tools to study and control these circuits has focused mainly on neuronal activity, in this talk, I will show newly developed techniques in our laboratory to specifically dissect the active astrocyte circuits with spatio-temporal precision, i.e. CaMPARIGFAP (calcium-modulated photoactivatable ratiometric integrator under GFAP promoter) and Astro-Light (calcium- and light-gated switch to induce gene expression in activated astrocytes). Furthermore, I will discuss our recent data about mapping the functional astrocytic-circuitries in the Nucleus Accumbens (NAc) that reveal the existence of specific-astrocyte circuits in the NAC.
In short, I will present data, acquired using cutting-edge tools, which supports the idea that NAc astrocytic networks are critical players in understanding the way that the NAc integrates information.

Supported by: RYC-2016-20414, MINECO (RTI2018-094887-B-I00)

SPEAKER #4

-Full Name: **Juliana Martins Da Rosa, PhD**
Hospital Nacional de Parapléjico
sFinca La Peraleda, s/n
45071 Toledo, Spain
phone: (+34) 925247700 ext 47275
email: jmartinsd@externas.sescam.jccm.es

-Enter three recent publications of the Speaker related to the topic of the symposium:

1. Rosa JM, Morrie RD, Baertchs HC, Feller M. Contributions of Rod and Cone Pathways to Retinal Direction Selectivity Through Development, The Journal of Neuroscience (2016) 36(37): 9683-9695.
2. Rosa JM, Ruhle S, Ding H, Lagnado L. Crossover Inhibition Generates Sustained Visual Responses in the Inner Retina, Neuron(2016) 90(2):308-19.
3. Rosa JM, Bos R, Sack GS, Fortuny C, Agarwal A, Bergles DE, Flannery JG, Feller M. Neuron-glia interaction in developing retina mediated by neurotransmitter spillover, eLife (2015) 10.7554.

-Relevance for the symposium:
Dr. Rosa is a Ramon y Cajal Researcher at the HNP. Her research is focused on the study of neuronal circuitries alterations in sensorimotor cortical areas following CNSinjuriesi.e. spinal cord injury and traumatic brain injury. By manipulating the activity of glial cells (microglia and astrocytes)

and therefore the interaction with neurons, her research aims to control neuronal transmission and synaptic plasticity in order to restore sensory and motor functions usually lost after traumatic injuries. In this symposium, Dr.Rosa will show the latest discovery of a regional-dependent astrocyte function controllingcorticocortical excitability and behavior output.

- Abstract:

ON THE ROAD MAP OF ASTROCYTE FUNCTIONAL HETEROGENEITY: IMPLICATIONS IN SENSORY PROCESSING AND SPONTANEOUS ACTIVITY

Juliana M. Rosa
Experimental Neurophysiology and Neuronal Circuits Group, Hospital Nacional de Parapléjicos, SESCAM. Toledo. Spain.

Astrocytes, the main glial cell type in the central nervous system, are now recognized as integral participants of every major aspect of brain development, function and disease. Traditionally viewed as a largely homogenous population, recent genetic and molecular data mostly obtained from different cortical layers, indicate astrocytes as heterogenous cells in terms of morphology, physiology and gene expression patterns. If such heterogeneity plays a role in the unique patterns of neuronal activity engaged during both spontaneous and sensory-driven responses across different cortical layers is still unknown. By using genetic and pharmacogenetic tools combined with calcium imaging, in vivo electrophysiology and behavior assessment in mice, here we will present new data regarding two unanswered questions: 1) are astrocytes functionally heterogenous across distinct layers from the same cortical column? and 2) does astrocyte heterogeneity interfere in the control of spontaneous and evoked layering excitability? Our data shows, for the first time, that astrocyte activity is inherently distinct across layers of the somatosensory cortex. Such diversity seems to play a role in the fine-tuning of evoked neuronal responses by modulating stimulus sensitivity in a layer-dependent manner with consequences to sensory discrimination and behavior output. Regarding spontaneous activity, astrocytic activation seems to desynchronize neuronal activity within slow wave oscillations with subsequent elongation of up-states. In addition, astrocyte activation awakens a subset of inhibitory neurons whose spiking activity is mostly found during down-states. This effect was only evident in layer 5 neurons, showing a layer-specific control of astrocytes in the layer considered as the main generator of spontaneous cortical activity. Therefore, our overall data demonstrate that astrocytes are functionally heterogenic across cortical layers and that such layer-specific differences play important roles in the regulation of sensory processing as well as the neuronal network controlling slow wave oscillations.

SYMPOSIUM S03
INHIBITORY CELLS THROUGHOUT BRAIN CIRCUITS

TITLE OF THE SYMPOSIUM: Inhibitory cells throughout brain circuits

Brief description of the relevance and timeliness of the topic, including the issues to be discussed by each speaker.

One of the biggest challenges to resolve brain networks is the diversity of the inhibitory interneurons. As a matter of facts, some of the largest community efforts in neuroscience –Allen Institute for Brain Science, Human Brain Project and The Brain Initiative -share the common goal of disentangling the heterogeneity and motifs of the inhibition in the brain.

This symposium will presentemerging work from outstanding laboratories in the field of inhibition, from cells to circuits. Itwill cover the last advances on the classification of the cortical GABAergic cell types, the development and differentiation of inhibitory circuits, and the role of the specific inhibitory interneurons in coordinating brain oscillations and behavior.

We consider that the proposed symposium will be of broad interest for SENC members. The different speaker expertise will give a broad but complementary perspective to the symposium. Dr. Laura Modol (KCL, London) has revealed the functional assemblies that GABAergic neurons form in the developing cortex, and sheaims todecipherhow cellular diversity integrates into the developing brain. Dr. Manuel Valero (NYU, New York) disclosed the inhibitory circuits underlaying the columnar organization of the hippocampal CA1, and he currently studies its contribution in the hippocampal coding. Dr. Sara Mederos (SWC, London) has unraveled unexpectedroles of astrocytes in the inhibitory circuits,and she is focusing on inhibitory hubs in the thalamus with important relevance for the control of innate behaviors. Finally, findings of Dr. Bernardo Rudy (NYU, New York) havesignificantly contributed toestablishthe current state-of-art notions of the inhibitory interneurons diversity in the neocortex, from genes expression and physiology to behavior.

We want to emphasize the gender balance and the junior but recognized stage of 3 out of 4 speakers of this proposal.

SPEAKER #1

-Full Name: **Laura Modol**. Marín LabKing's College London
-Enter three recent publications of the Speaker related to the topic of the symposium:

1. Modol L,Bollmann Y, Tressard T, Baude A, Che A, Duan ZRS, Babij R, De Marco García NV, Cossart R.Assemblies of Perisomatic GABAergic Neurons in the Developing Barrel Cortex.Neuron. 2020 Jan 8;105(1):93-105.e4. doi: 10.1016/j.neuron.2019.10.007. Epub 2019 Nov 25.
PMID: 31780328

2. Duan ZRS, Che A, Chu P, Modol L, Bollmann Y, Babij R, Fetcho RN, Otsuka T, Fuccillo MV, Liston C, Pisapia DJ, Cossart R, De Marco García NV.GABAergic Restriction of Network Dynamics Regulates Interneuron Survival in the Developing Cortex.
Neuron. 2020 Jan 8;105(1):75-92.e5. doi: 10.1016/j.neuron.2019.10.008. Epub 2019 Nov 25.
PMID: 31780329

3.Mòdol L, Sousa VH, Malvache A, Tressard T, Baude A, Cossart R.Spatial Embryonic Origin Delineates GABAergic Hub Neurons Driving Network Dynamics in the Developing Entorhinal Cortex.Cereb Cortex. 2017 Sep 1;27(9):4649-4661. doi: 10.1093/cercor/bhx198. PMID: 28922859

-Relevance for the symposium: Modol's research is focused on investigating the inhibitory cells from a developmental perspective. As postdoctoral scientist she has conducted pioneer studies on the developing brain focusing on inhibition at the lab of R. Cossart in Marseille (France). Since 2019, she is at the Marín lab studying how interneuron cellular diversity integrates into the developing brain and into functional adult circuits.

- Abstract:

Spontaneous coordinated neuronal activity is a hallmark of the developing brain and plays a pivotal role in the formation of neuronal circuits. GABAergic interneurons critically contribute to cortical development starting from neurogenesis and migration, to activity-dependent circuit refinement. In contrast, the dynamics of GABAergic interneurons during postnatal development remain poorly understood. Here I will discuss recent advances made in the study of specific GABAergic subpopulations in the generation of coordinated activity patterns in the mouse barrel cortex and their specific role as functional regulators of developmental dynamics in vivo.

SPEAKER #2

-Full Name: **Manuel Valero**. Buzsaki Lab. NYU Neuroscience Institute
-Enter three recent publications of the Speaker related to thetopic of the symposium:

1. Sleep DOWN state-active ID2/Nkx2.1 interneurons in the neocortexValero M, Viney TJ, Machold R, Mederos S, Zutshi I, Schuman B, Senzai Y, Rudy B and Buzsaki G.. Nature Neuroscience (accepted)
2. Mechanisms for selective single-cell reactivation during offline sharp-wave ripples and their distortion by fast ripplesValero M, Averkin RG, Fernandez-Lamo I, Aguilar J, Lopez-Pigozzi D, Brotons-Mas JR, Cid E, Tamas G, Menendez de la Prida, L.. Neuron, 94 (6), 1234–1247 (2017).
3. Determinants of different deep/superficial CA1 pyramidal cell dynamics during sharp-wave-ripples.Valero M, Cid E, Averkin RG, Aguilar J, Sanchez-Aguilera A, Viney T, Gomez-Dominguez D, Bellistri E and Menendez de la Prida L. Nature Neuroscience 18, 1281–1290 (2015).

-Relevance for the symposium:Manuel Valero provides a circuit and system neuroscience standpoint to the symposium. During his PhDat de la Prida lab (Cajal Institute), he unveiled the inhibitory motifs shaping the columnar organization ofCA1, as well as their failure in the epileptic brain. As postdoc,he moved to the lab of Gyorgy Buzsaki (New York), where he is conducting several projects on the inhibitory circuits in the cortex and the hippocampus.

- Abstract:

THE ROLE OF INHIBITION IN HIPPOCAMPAL PLACE CELLS

Valero M
NYU Neuroscience Institute

The fundamental computation a single neuron performs is to integrate incoming excitatory and inhibitory inputs to decide whether to fire an action potential and feedback its activity into the network. Investigation of this synaptic computation requires access to the neuron subthreshold dynamics, whose state-of-art methodologies have remained unaltered for decades and are unrealistic for cell assemblies and behaving animals.
Instead of intracellular recording, we propose a method to optogenetically probe the membrane polarization of the cells with short depolarizing pulses using chronically implanted µLED probes (4 shanks with 3 µLED/shank). Light-sensitive neurons responded to one or more µLEDs and the spike numbers were used as a proxy for estimating relative changes of the membrane potential dynamics. Strikingly, induced spike responses gain sharply increased several-folds inside the preferred position (place fields) of the responsive cells. These results are compatible with a tuning curve model in the CA1 pyramidal cells where excitation and the inhibition display a concerted and reciprocal relationship. When optogenetic stimulation was probed on non-place cells, majority of the putative non-place cells showed place-related activity. Finally, we optically probed neurons around sharp wave-ripples (SPW-Rs), hippocampal events considered a key mechanism for memory consolidation and action planning. In the short time window of SPW-R, excitatory and inhibitory neurons increased in parallel. As expected, optogenetic probing of the CA1 networks showed that, in contrast to the large gain within place fields, responsivity of pyramidal cells showed a robust decrease during SPW-Rs.
We have developed a method for studying subthreshold dynamics of individual cells in chronic recordings using novel high-resolution optical stimulation as a proxy for the membrane polarization. These experiments disclosed a reciprocal interaction between inhibition and excitation along the place fields of CA1 and demonstrated that the same exact perturbation can bring about opposite responses during exploration and transient SPW-Rs.

SPEAKER #3

-Full Name: **Sara Mederos**. Hofer Lab. Sainsbury Wellcome Centre (UCL) London
-Enter three recent publications of the Speaker related to the topic of the symposium:

1. Mederos S , Sanchez-Puelles C , Esparza J, Valero M , Ponomarenko A, Perea G.GABAergic signaling toastrocytes in prefrontal cortex sustains goal-directed behaviorsNature Neuroscience. Accepted 2Nov2020.

2.Mederos S,Hernández-Vivanco A, Ramírez-Franco J, Martín-Fernández M, Navarrete M, Yang A, Boyden ES, Perea G.Melanopsin for precise optogenetic activation of astrocyte-neuron networks.
Glia. 2019 May;67(5):915-934. doi: 10.1002/glia.23580. Epub 2019 Jan 11.
PMID: 30632636

3. Mederos S, Perea G.GABAergic-astrocyte signaling: A refinement of inhibitory brain networks.
Glia. 2019 Oct;67(10):1842-1851. doi: 10.1002/glia.23644. Epub 2019 May 30.
PMID: 31145508 Free PMC article. Review.

-Relevance for the symposium: Sara Mederos's is focused on how modulation of particular inhibitory cells and specific circuits shapesbrain activity and behaviors. During her PhD at Perea Lab (Cajal Institute) she interrogated the function of astrocytes on shaping the activity of inhibitory circuits. As postdoctoral fellow at Hofer Lab (SWC, London), she is investigating the cortical modulation of innate behaviors, through specific cortical projections to the ventral lateral geniculate nucleus (vLGN) that provides inhibitory control of visually-guided behaviors.

- Abstract:

PATHWAYS FOR REGULATING ESCAPE DECISIONS THROUGH THE VENTRAL LATERAL GENICULATE NUCLEUS

To make decisions, humans and animals have to select appropriate actions after evaluating all available information. This is also true for instinctive, defensive decisions in response to imminent threats, for instance when deciding whether to escape or not. These reactions are adaptive and can be shaped by experience and the internal state, allowing animals to react differently to the same environmental stimuli depending on circumstance. However, how sensory information, previous knowledge and internal state are integrated by neural circuits to allow flexible behavioural decisions is still unknown.

Inhibition is thought to play a key role in enabling flexible control of behaviour, by suppressing inappropriate reactions and – through disinhibition – selecting appropriate actions. Long-range inhibition from subthalamic nuclei has recently been identified to play an important role in the regulation of instinctive behaviours, and these subthalamic circuits have been suggested to integrate external and internal signals.

The ventral lateral geniculate nucleus (vLGN) is an inhibitory, subthalamic nucleus that has been shown to provide strong, bidirectional inhibitory control over visual threat-evoked escape behaviour. Moreover, vLGN activity is modulated by previous threat experience and the anxiety state of the animal.

We have identify visual cortex and ventromedial hypothalamus provide inputs to the vLGN during visual threat signals. Furthermore, using electrophysiological recordings, calcium imaging, and optogenetic manipulations, we have tested the influence of these inputs to vLGN over instinctive defensive reactions on paradigms that allow to test the animal's behaviour depending previous knowledge and internal state.

Further characterization of these outcomes will elucidate important pathways and mechanisms by which the brain can control the generation of flexible behavioural responses to environmental stimuli depending on the behavioural context. Understanding the brain mechanisms of behavioural control is fundamental for identifying causes for inappropriate or maladaptive responses in psychiatric disorders.

SPEAKER #4
-Full Name: **Bernardo Rudy**. Rudy Lab NYU Neuroscience Institute
-Enter three recent publications of the Speaker related to the topic of the symposium:
1. Schuman B,Rudy B.Dynamics of Neuronal Activity in the Cortical Column during Behavior: Both Neuron Type and Layers Matter.
Neuron. 2019 Oct 23;104(2):186-188. doi: 10.1016/j.neuron.2019.10.005.
PMID: 31647892 Free article.

2. Schuman B, Machold RP, Hashikawa Y, Fuzik J, Fishell GJ, Rudy B..
Four Unique Interneuron Populations Reside in Neocortical Layer 1.
J Neurosci. 2019 Jan 2;39(1):125-139. doi: 10.1523/JNEUROSCI.1613-18.2018. Epub 2018 Nov 9.
PMID: 30413647

3. Muñoz W, Tremblay R, Levenstein D, Rudy B.
Layer-specific modulation of neocortical dendritic inhibition during active wakefulness.
Science. 2017 Mar3;355(6328):954-959. doi: 10.1126/science.aag2599.
PMID: 28254942

-Relevance for the symposium:Rudy's work on inhibition has been key for setting the bases of important interneuronclassification and functions. The Rudy lab is interested in how neuronal activityspecificallyin the cerebral cortex regulates behavior. Toward this goal, they study how brain function depends on propertiesof individual neurons (i.e inhibitory cells) and the circuits they make with other neurons. This interrogationaims to unravel cellular and circuit mechanisms that mediate cortical function.

- Abstract:

TWO TUNABLE INHIBITORY SYSTEMS GOVERN THE ACTIVITY OF NEOCORTICAL LAYER 1 (L1).

Bernardo Rudy, Ben Schuman, Rob Machold, Darpan Chakraborty, Shlomo Dellal, Hector Zurita, Illya Kruglikov, Chiung-Yin Chung.
Neuroscience Institute, NYU Grossman School of Medicine, New York, NY, USA

Neocortical 1ayer 1 (L1), the main cortical layer receiving contextual information carried by corticocortical and thalamocortical "feedback" connections, contains the distal "tuft" dendrites of pyramidal cells located in deeper layers. All resident L1 neurons are GABAergic interneurons (IN). In the adult mouse, neuron derived neurotrophic factor (NDNF) expression is largely limited to L1, where it is found in about 70% of L1 INs. The availability of mouse lines expressing Cre recombinase under control of the NDNF promoter has allowed studies exploring the role of these cells on a variety of cortical functions. Studies using up and/or down regulation of NDNF cell activity have suggested, among others, that these neurons terminate neocortical Up states and thus mediate the Down state. On the other hand, another study found that the activity of these INs increases with arousal. How do we reconcile these, and other, seemingly contradictory results?
Inhibition in L1 is thought to mediate the impact of long-range projections on PC tuft dendrites, powerfully controlling PC output. There are two types of NDNF-expressing INs in L1, neurogliaform and canopy cells, that differ in morphology, intrinsic electrophysiological properties, input and output connectivity, and their response to neuromodulators. Furthermore, L1 contains two types of non-NDNF-expressing INs and the dendrites of several IN subtypes with somata in L2/3, which have access to the axonal projections arriving in L1. An additional inhibitory influence arises from ascending axons of SST-expressing Martinotti cells in deeper layers that project to L1. Our studies investigate how these various inhibitory sources and neuromodulation regulate arousal, attention, sensory perception and learning.

SYMPOSIUM S04
UNDERSTANDING QUIESCENCE IN ADULT NEUROGENIC NICHES

TITLE OF THE SYMPOSIUM: Understanding quiescence in adult neurogenic niches

ORGANIZER'S NAME: AIXA V. MORALES AND HELENA MIRA
ORGANIZER'S EMAIL: aixamorales@cajal.csic.es; hmira@ibv.csic.es
CHAIR: Aixa V. Morales and Helena Mira

Adult neurogenesis generates functionally integrated neurons throughout life in both invertebrate and vertebrate brains. The generation of new neurons relies on a pool of neural stem cells (NSCs),that are mostly in a state of reversible quiescence. Quiescent NSCs present a low rate of metabolic activity, a high responsiveness to local signals, and they can be activated by diverse physiological and pathological stimuli. The equilibrium between cell quiescence and activity determines not only the rate of neuronal production but also the long-term maintenance of the stem cell pool and the neurogenic capacity of the ageing brain. In this Symposium, we will present and discuss about signaling pathways and intrinsic programs controlling quiescence both in adult and ageing brain of vertebrate and invertebrate organisms. Understanding the molecular mechanism underlying adult neural stem cell in vivo quiescence will open new approaches towards harnessing quiescent NSCs for regenerative medicine in the treatment of brain disorders.

SPEAKER #1

-Full Name: **François Guillemot**
Neural Stem Cell Biology Laboratory, The Francis Crick Institute, London, UK

-Enter threerecent publications of the Speaker related to the topic of the symposium:

Quiescence of Adult Mammalian Neural Stem Cells: A Highly Regulated Rest.
Urbán N, Blomfield IM, Guillemot F. Neuron. (2019)104(5):834-848.

Nipbl Interacts with Zfp609 and the Integrator Complex to Regulate Cortical Neuron Migration.van den Berg DLC, Azzarelli R, Oishi K, Martynoga B, Urbán N, Dekkers DHW, Demmers JA,Guillemot F.Neuron(2017); 93(2):348-361.

Return to quiescence of mouse neural stem cells by degradation of a proactivation protein.Urbán N, van den Berg DL, Forget A, Andersen J, Demmers JA, Hunt C, Ayrault O, Guillemot F.Science. (2016); 353(6296):292-5.

-Relevance for the symposium:

F. Guillemot's lab explores the fundamental mechanisms that underlie the generation of the main cellular components of the nervous systemby common progenitors or stem cellsfound throughout the embryonic brainand in restricted locations in the adult brain.They are interested in how stem cells integrate multiple signals to select appropriate behaviors.They have identified keytranscription factors that allow stem cells to adopta particular fate, and study the mechanisms that control the precise timing of their expression and activity, including epigenetic mechanisms that regulate the accessibility of regulatory elements to transcription factors.

- Abstract:

PROGRESSIVE CHANGES IN HIPPOCAMPAL STEM CELL PROPERTIES ENSURE LIFELONG NEUROGENESIS

Lachlan Harris, Piero Rigo, Francois Guillemot
The Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK

Neuronal production occurs during embryonic development and ceases around birth in most regions of the mammalian brain, but it continues throughout life in two brain regions, the hippocampus and the subventricular zone. In the hippocampus, the transition from developmental to adult neurogenesis occurs in juvenile mice and involves a rapid reduction of the neural stem cell pool, reaching small but stable stem cell numbers during adulthood. We found that the transition from developmental to adult neurogenesis involves multiple coordinated changes in stem cell behaviour. In particular, while active neural stem cells differentiate rapidly in juveniles, they increasingly return to a state of shallow quiescence, and undergo additional self-renewing divisions in adults. These changes in stem cell behaviour result from a progressive reduction in expression of the pro-activation protein ASCL1 due to increased post-translational degradation. These findings help reconcile current contradictory models of hippocampal stem cell dynamics and may contribute to the different timings of transition from developmental to adult neurogenesis and different rates of decline of hippocampal neurogenesis in mammalian species including humans.

SPEAKER #2

-Full Name: **Christa Rhiner**
Champalimaud Foundation, Champalimaud Centre for the Unknown, Lisbon, Portugal

-Enter three recent publications of the Speaker related to the topic of the symposium:

Culling Less Fit Neurons Protects against Amyloid-β-Induced Brain Damage and Cognitive and Motor Decline.Coelho DS, Schwartz S, Merino MM, Hauert B, Topfel B, Tieche C, Rhiner C*, Moreno E*. Cell Rep (2018);25(13):3661-3673

A Cold-Blooded View on Adult Neurogenesis.Simões AR, Rhiner C.Front Neurosci. (2017);11:327.

Brain regeneration in Drosophila involves comparison of neuronal fitness.Moreno E, Fernandez-Marrero Y, Meyer P, Rhiner C. Curr Biol. (2015); 25(7):955-63.

-Relevance for the symposium:

C. Rhiner’s group is interested in isolating the factors that bring about activation of adult stem cells during tissue regeneration after injury or during the misregulated proliferation of stem cellsthat leads to tumor formation. To that end, the team studies the molecular mechanisms through which neural stem cells are activated in the adult fruit fly brain. Recent work from the lab has resulted in the discovery of damage-responsive stem cells and the identification of several candidate genes that are thought to underlie this process. They also characterize theelimination of abnormal cells based on their fitness status, a process known as cell competition, a quality control mechanism that protects against developmental malformations, tumorigenesis and aging.

- Abstract:

INJURY-INDUCED ACTIVATION OF QUIESCENT NEURAL PROGENITORS IN THE ADULT FLY BRAIN.

Christa Rhiner,
Champalimaud Center of the Unknown, Lisbon.

Abstract:
Numerous adult tissues contain quiescent stem cells residing in a reversible state of dormancy. Injury is a major factor known to trigger plasticity and recruitment of dormant stem cells to tissue repair. We have previously found that the adult brain of Drosophila, similar to humans, harbors quiescent neural stem cells (qNSCs), which - following acute injury - proliferate and give rise to new neurons.
Nevertheless, the mechanisms that activate qNSCs in response to brain injury remain unknown.
In our research we use stab lesions to model traumatic brain injury in adult flies and study injury-dependent control of stem cell activation to gain a mechanistic understanding of the damage-induced dynamic processes, which turn dormant qNSCs into dividing progenitors.
By using a combination of whole genome transcriptome profiling and in vivo functional genetic assays, we have identified several secreted factors that promote injury-induced proliferation of quiescent neural progenitors in the fly. Our results reveal an important role of a neuro-glial signaling relay able to activate distant qNSCs by fostering an extensive, but transient stem cell-activating milieu in the injured brain area.

SPEAKER #3

-Full Name: **Helena Mira**
Stem Cells and Aging Unit, Instituto de Biomedicina de Valencia (IBV), CSIC, Valencia, Spain

-Enter three recent publications of the Speaker related to the topic of the symposium:

Neurogenesis From Embryo to Adult -Lessons From Flies and Mice.Mira H*, Morante J*.Front Cell Dev Biol. (2020);8:533.

Circadian glucocorticoid oscillations preserve a population of adult hippocampal neural stem cells in the aging brain.Schouten M, Bielefeld P, Garcia-Corzo L, Passchier EMJ, Gradari S, Jungenitz T, Pons-Espinal M, Gebara E, Martín-Suárez S, Lucassen PJ, De Vries HE, Trejo JL, Schwarzacher SW, De Pietri Tonelli D, Toni N, Mira H, Encinas JM, Fitzsimons CP.Mol Psychiatry.(2020);25(7):1382-1405.

Noggin rescues age-related stem cell loss in the brain of senescent mice with neurodegenerative pathology.Díaz-Moreno M, Armenteros T, Gradari S, Hortigüela R, García-Corzo L, Fontán-Lozano Á, Trejo JL, Mira H*.Proc Natl Acad Sci U S A. (2018);115(45):11625-11630.

-Relevance for the symposium:H. Mira’s lab is interested in the molecular and cellular mechanisms that regulate neural stem cells in the adult mammalian brain. Their current emphasis is on the aging perspective. They explore both cell intrinsic and cell extrinsic (niche) contributions to the ageing phenotype. Their main goal is to understand: 1) How stem cells are activated from their quiescent state to give rise to new neurons in the adult and aged brain; 2) How fate choices of the stem cell

progeny are determined in the adult and aged brain; 3)Whether such processes can be used to improve brain function in age-related neurodegenerative diseases. They have also characterizedthe function of certain stem cell-related mechanisms in the de-regulation of glioma cancer stem cells.

- Abstract:

COMING FROM WITHIN: CELL INTRINSIC MODULATORS OF QUIESCENT NEURAL STEM CELLS

Helena Mira.
Instituto de Biomedicina de Valencia (IBV), CSIC, Valencia, Spain.

ABSTRACT: Tissue homeostasis and repair in a variety of mammalian organs relies on the persistence of somatic stem cell reservoirs. Throughout adulthood, these resident stem cells remain predominantly in a reversible resting state known as quiescence. Within the brain, a prominent quiescent neural stem cell (NSC) reservoir is maintained in the subgranular zone of the hippocampal dentate gyrus. The development of this stem cell niche ends during the postnatal period, coinciding with the transition of NSCs from the proliferative state into the definitive quiescent state. Thereafter, only a minor fraction of the adult hippocampal radial glia-like NSCs becomes active and engages in a neurogenic cascade that leads to the production of new functional granule neurons involved in learning and memory tasks. Recent studies suggest that quiescence is more than just a passive latency condition developed to protect NSCs from the drawbacks of hyperproliferation. Despite being metabolically less active than their proliferating counterparts, quiescent NSCs are held in a flexible and highly dynamic state that allows them to respond to changes in their microenvironment. This state requires a tight and complex regulation of gene expression. We previously reported that the equilibrium between NSC quiescence and NSC activation leading to productive neurogenesis depends on the interplay of a variety of local extrinsic niche signals, the BMP and Wnt family being of utmost importance. We now provide evidence for the fine-tuning of BMP and Wnt signalling by cell intrinsic post-transcriptional mechanisms, including mRNA splicing, microRNA-mediated events and proteostasis.

SPEAKER #4

-Full Name: **Aixa V. Morales**
Molecular control of Neurogenesis lab, Instituto Cajal (CSIC), Madrid (Spain)

-Enter three recent publications of the Speaker related to the topic of the symposium:

Benzothiazole-based LRRK2 inhibitors as WNT enhancers and promoters of oligodendrocytic fateZaldivar-DiezJ, Li L, García AM, Zhao W, Medina-Menendez C, Haggarty SJ, Gil C, Morales AV*and Martinez, A*. (*corresponding)Journal of Medicinal Chemistry(2020);63:2638-2655.

Adult neural stem cells: born to last. MoralesAV,and MiraH. Front. Cell Dev. Biol.(2019); 7: 96

Brain insulin-like growth factor-I directs the transition from stem cells to mature neurons during postnatal/adult hippocampal neurogenesis. Nieto-Estevez V, Oueslati-Morales CO, Li L, Pickel J, Morales AV, and Vicario-Abejon C.Stem Cells(2016)34(8):2194-209

-Relevance for the symposium:

AV Morales laboratory is interested in understanding the molecular basis behind the generation of new neurons both during the development of the nervous system and in the adult brain. The generation of new neurons is retained to some extent in niches in the adult nervous system throughout lifetime under physiological conditions and constitutes an important source of neural plasticity. However, adult neurogenesis is very restricted, both in number and subtypes of neural cells generated. Herlab is interested in exploring how differences in cell production rates and in the temporal extent of neurogenesis can be attained in the adult brain in comparison with developmental neurogenesis.

- Abstract:

FROM QUIESCENCE TO PROLIFERATION AND BACK: THE ACTIVE LIFE OF NEURAL STEM CELLS

Aixa V. Morales.
Instituto Cajal, (CSIC), Madrid, Spain

The adult neurogenic niche in the hippocampus dentate gyrus (DG) is maintained through the proliferation of neural stem cells with radial glia-like morphology (RGL), which are mostly in a reversible state of quiescence. Signals from the local niche promote quiescent RGLs to enter cell cycle (or "activate") and through several rounds of cell division, to generate new DG granular neurons that participate in learning and memory processes. However, quiescence is not a fixed state, as it involves distinct intermediate sub-states with sets of differentially expressed transcripts (reviewed, in Mira and Morales, 2019). Thus, whereas most of RGLs in the DG will remain in a dormant state of deep quiescence throughout life, the transitions back and forward from an active state to a temporal shallow quiescence or resting state, will ensure the lifelong maintenance of the stem cell population.
The fundamental questions that our laboratory is trying to understand are: i) how RGLs transit from the different sub-states of quiescence to activation and ii) how quiescence is acquire in the first place during the development of the DG. In relation to the first aspect, we have recently determined that SoxD transcription factors (Sox5 and Sox6) are enriched in activated RGLs. Using inducible conditional inactivation of Sox5 and/or Sox6 in adult mice, we have demostrated that SoxD factors transcriptionally activate Ascl1 in RGLs, and are consequently required for RGL activation and new neuron generation in the DG. In relation to the second question, we have determined that the developmental loss of Sox5 expression (Sox5Nestin mice) causes by P30 an enhancement of RGLs proliferation, a reduction in RGL deep quiescence and an increase in neurogenesis, which leads to a reduction of the RGL pool at later stages. These results reveal define windows during postnatal DG development when the balance between dormant and resting quiescence ensures the life-long maintenance of the adult neurogenic niche.

SYMPOSIUM S05
MODELING BEHAVIOR DURING DECISION MAKING

TITLE OF THE SYMPOSIUM: Modeling behavior during decision making.

Organizers:
- Alfonso Renart (Champalimaud Neuroscience Program, Lisbon)
- Jaime de la Rocha (IDIBAPS, Barcelona, *SENC member)

SENC Areas: 3) Neurociencia de sistemas, 4) Neurociencia cognitiva y conductual, 5) Neurociencia teórica y computacional

Brief Description: Systems neuroscience is a fast-changing field heavily influenced in the last years by the development of new methodologies both experimental (e.g. optogenetics, Calcium imaging) and analytical/theoretical (computational models, machine learning methods). More recently there has been a growing emphasis in investigating the brain "in action", i.e. during behavior, and to use a more quantitative approach by developing computational models, establishing fundamental theoretical principles or making use of statistically sound methods to interrogate the data. In addition, the study of behavior is undergoing great transformations becoming a much more quantitative discipline and giving rise to the growing subfield of behavioral modeling. Behavioral tasks are currently designed under the optics of physiology assays, implying having a fine temporal control of as many variables as possible (e.g. precise timing of responses, movements, eye pupil, etc.) and levering behavioral tasks with as many repetitions as possible to power statistical significance (i.e. high throughput trial based tasks). This approach has opened the door to using quantitative behavioral models as a first important step in studies trying to characterize the neural bases of the relevant variables at play.
In the current symposium sitting in the interface between Systems Neuroscience, Cognitive neuroscience and Computational Modeling, four researchers will present their work with a common approach to quantify and model behavior during decision making. They study different types of decisions (social decisions, perceptual decisions, memory guided decisions, etc.) and cover three different species (human, rat and mouse). Each of them will present their recent findings in their corresponding subdisciplines and together showcase of the importance of establishing a quantitative understanding of behavior in order to characterize brain mechanisms underlying cognition.

SPEAKER #1

- Full Name: **Cindy Poo**. Champalimaud Research. Champalimaud Foundation. Lisbon, Portugal.

- Enter three recent publications of the Speaker related to the topic of the symposium:

Poo, C., Agarwal, G., Bonacchi, N. and Mainen, Z., 2021. Spatial maps in piriform cortex during olfactory navigation. bioRxiv, pp.2020-02.

Uchida, N., Poo, C. and Haddad, R., 2014. Coding and transformations in the olfactory system. Annual review of neuroscience, 37, pp.363-385.

- Relevance for the symposium:

Cindy Poo studies the neural mechanisms and computations underlying how olfactory information is used to guide behavior. In particular she focuses on the interplay between olfactory and spatial information in the piriform cortex and on cortico-hippocampal interactions.

- Abstract:

SPATIAL MAPS IN PIRIFORM CORTEX DURING OLFACTORY NAVIGATION

Cindy Poo[1]*, Gautam Agarwal[2], Niccolò Bonacchi[1], Zachary Mainen[1]
[1]Champalimaud Foundation, Lisbon, Portugal.
[2]W. M. Keck Science Center, The Claremont Colleges, Claremont, CA, USA.

Abstract:

Odors are a fundamental part of the sensory environment used by animals to guide behaviors such as foraging and navigation. Animals instinctively use odor memories to guide spatial choices9 and odors are widely used in the study of spatial memory and navigation10. Cortical structures for odor perception and spatial memory are evolutionarily and developmentally linked, together forming an allocortex (consisting of olfactory, hippocampal, and entorhinal cortices).

Olfaction and spatial memory systems are therefore intimately related, as reflected by animal behavior, evolution, and circuit anatomy. Moreover, while the primary olfactory (piriform) cortex receives direct sensory input via olfactory bulb projection neurons, its three-layered circuit architecture shares striking resemblances to the hippocampus, with broadly distributed and unstructured recurrent connections that are highly plastic. This has prompted conjectures that these structures implement similar learning functions. Here, using neural ensemble recordings in freely moving rats performing an odor-cued spatial choice task, we show that posterior piriform cortex neurons carry a robust spatial representation of the environment. Piriform spatial representations have features of a learned cognitive map, being most prominent near odor ports, stable across behavioral contexts, and independent of olfactory drive or reward availability. The accuracy of spatial information carried by individual piriform neurons was predicted by the strength of their functional coupling to the hippocampal theta rhythm. Ensembles of piriform neurons concurrently represented odor identity as well as spatial locations of animals, forming an odor-place map. Our results reveal a previously unknown function for piriform cortex in spatial cognition and suggest that it is well-suited to form odor-place associations and guide olfactory-cued spatial navigation.

SPEAKER #2

- Full Name: **Cristina Márquez**, Instituto de Neurociencias de Alicante, Alicante, Spain

- Enter three recent publications of the Speaker related to the topic of the symposium:

Costa, DF , Moita MA, Márquez C. (2020) Novel Competition test for food rewards reveals stable dominance status in rats. BiorXiv . doi: , - 10.1101/2020.09.24.312033

Tzanoulinou S , Gantelet E, Sandi C, Márquez C. (2020) Programming effects of peripubertal stress on spatial learning. Neurobiology of Stress . in press

Andreia Cruz , Mirjam Heinemans, Cristina Márquez and Marta A. Moita. (2020) Freezing Displayed by Others Is a Learned Cue of Danger Resulting from Co-experiencing Own Freezing and Shock. Current Biology . 30 , 1 – 8.

- Relevance for the symposium:

Cristina Márquez investigates the way we perceive and learn about the world, and despite its importance for social species, we still know very little about how the brain computes social information. She studies the mechanisms of how social behavior shapes the brain with a focus on cooperative social interactions in rodents. She has recently shown that rats can exhibit prosocial behaviors and she aims to identify the neural circuits responsible for this social decision-making, using a combination of behavioral, anatomical, pharmacological, imaging and optogenetic tools in rodents.

- Abstract:

SOCIAL DECISION-MAKING IN RODENTS

C. Márquez
Instituto de Neurociencias de Alicante (CSIC-UMH), Alicante, Spain.

No man is an island, and all behaviors are modulated by our social experience. How we take decisions in social contexts is a fundamental aspect of our daily lives, however, the underlying mechanisms are only starting to be addressed. We previously showed that rats display prosocial behaviors by providing food to conspecifics in the absence of added self-benefit in foraging contexts. In this talk, we will focus on how rats flexibly adapt their choices to social context, in order to gain mechanistic understanding on how decision-makers learn that their choices have consequences on others. Leveraging on the study of non-canonical body-language, through pose estimation of the interacting individuals, and the study of agent-assigned ultrasonic vocalizations, we propose a model of how decision-makers learn social contingencies of prosociality. Moreover, using fiber photometry in socially behaving animals and wireless real-time closed loop optogenetic manipulations, we provide evidences of the neural circuits underlying the perception of emotional states of others and how animals integrate these behaviors into social decision-making processes.

SPEAKER #3

- Full Name: **Alfonso Renart**, Champalimaud Neuroscience Program, Lisbon

- Enter three recent publications of the Speaker related to the topic of the symposium:

F Cazettes, D Reato, JP Morais, A Renart, ZF Mainen. (2020) Phasic Activation of Dorsal Raphe Serotonergic Neurons Increases Pupil Size. Current Biology

Pardo-Vazquez JL, Castiñeiras-de Saa JR, Valente M, Damião I, Costa T, Vicente MI, Mendonça AG, Mainen ZF, Renart A (2019) The mechanistic foundation of Weber's law Nat. Neurosci. 22 (9) (doi:10.1038/s41593-019-0439-7)

D Kobak, JL Pardo-Vazquez, M Valente, CK Machens, A Renart. State-dependent geometry of population activity in rat auditory cortex. Elife 8, e44526 (2019)

- Relevance for the symposium: Alfonso studies the computations underlying decision-making, perception and working memory.

Alfonso Renart investigates the computations underlying decision-making during perception and working memory. He uses a combined approach including psychophysical experiments in humans and rodents, electrophysiology and theoretical models that aim to describe the underlying principles

guiding behavior. He has recently shown that the Weber law is the result of a theoretical invariance between stimulus intensity and time, a theoretical principle which could describe psychophysical data from both rats and humans. He also uses sophisticated analytic tools to quantify population data describing sensory encoding in cortical circuits across brain states both during rest and during decision making.

- Abstract:

MECHANISMS UNDERLYING SIMPLE PERCEPTUAL CHOICES

Alfonso Renart,
Champalimaud Research, Lisbon.
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Email: alfonso.renart@neuro.fchampalimaud.org

In perceptual decision-making, subjects are typically asked to make a binary categorical judgement about the magnitude of a perceptual feature across two stimuli. The accuracy of such judgements can be probed by measuring the smallest intensity difference that can be reliably discriminated, or just-noticeable-difference (JND). A long tradition in psychophysics has demonstrated certain regularities in these kinds of discriminations. The oldest and most general, noticed by Weber in 1836, states that the JND is not a difference, but a certain ratio between the two stimulus intensities which is constant across a wide range of absolute stimulus magnitudes. Although Weber's law is the most firmly established regularity in sensation, no principled way has been identified to choose between its many proposed explanations. We studied how accurately rats could discriminate the lateralization of sounds, which relies on a comparison of intensity across the two ears, at various absolute levels. These experiments revealed the existence of a novel psychophysical regularity – which we term time-intensity equivalence in discrimination (TIED) – describing how reaction times change as a function of absolute intensity while intensity-ratios, and thus discrimination-accuracy, are kept fixed. The relationship between absolute intensity and reaction time is so stringent that it allows us to mathematically specify the computational basis of the sensory discrimination process, placing strict requirements on how stimulus intensity is encoded in the stochastic activity of sensory neurons, and revealing that discriminative choices must be based on bounded exact temporal accumulation of sensory evidence. This mechanism is not only necessary for the TIED to hold, it is also sufficient to provide a virtually complete quantitative description of the rats' behavior. For this reasons, it provides a unique opportunity to guide the search for the neural basis of simple perceptual choices, a task we are currently pursuing.

SPEAKER #4

- Full Name: **Jaime de la Rocha**, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona

- Enter three recent publications of the Speaker related to the topic of the symposium:

Genís Prat-Ortega, Klaus Wimmer, Alex Roxin* and Jaime de la Rocha*. Flexible Categorization in perceptual decision making. Nature Communicatons (in press), 2021 (*equal contribution)

Ainhoa Hermoso-Mendizabal*, Alexandre Hyafil*, Pavel E. Rueda-Orozco, Santiago Jaramillo, David Robbe, Jaime de la Rocha. Response outcomes gate the impact of expectations on perceptual decisions. Nature Communications 11, 1057, 202. (*equal contribution)

Lluís Hernández-Navarro, Ainhoa Hermoso-Mendizabal, Daniel Duque, Jaime de la Rocha*, Alexandre Hyafil*. Sensory evidence integration and action initiation occur in parallel during perceptual decisions. PsyArXiv, 4 Feb. 2020. (* equal contribution)

- Relevance for the symposium:

Jaime de la Rocha studies perceptual decision making in rats and mice and uses computational models of behavior together with electrophysiology and optogenetics to understand the circuitry underlying simple decisions. In particular he is interested in how recent experiences shape the way we make decisions and how choice biases of different types can explain to a large extent why our decisions can often be very suboptimal. Starting from the behavioral model of the task under study, the approach tries to identify the brain areas at play inactivation experiments of cortical and striatal regions using pharmacology and optogenetics. Moreover, seeking for a description of the dynamics of neural circuits underlying decision making, he uses population neural recordings during behavior together with fine statistical analysis of these signals.

- Abstract:

THE DYNAMICS OF EVIDENCE ACCUMULATION IN EXPECTATION-GUIDED PERCEPTUAL DECISIONS

Perceptual decisions require the integration of multiple sources of information over multiple time-scales. Ambiguous stimuli which vary in time require for instance fast accumulation of evidence, a process largely studied but whose underlying mechanisms remain largely debated. Prior experiences on the other hand, also impact the way we perceive the world by creating expectations, a reference frame that can guide future decisions. Where in the brain these expectations are generated and where they are projected onto the space of possible decisions, it still unknown. In this talk I will present data from rats performing two-alternative categorization tasks, which require temporal integration within or/and across trials. First, I will show that rats can leverage in across-trial correlations and consistently develop a tendency to repeat or alternate their previous response using an internal estimate of the sequence repeating probability. Second, I will show that the timing and the orienting trajectory of their responses can be qualitatively explained by a novel model which, building on the standard accumulation to evidence models, incorporates proactive responses whose trajectories can be updated as the stimulus information reaches the decision bounds. Finally, using this behavioral model, pharmacological and optogenetic manipulations as well as electrophysiological recordings, I will characterize the critical role of the associative striatum in this behavior. Together, these results reveal that the accumulation of evidence across trials can exhibit complex dynamics and that the striatum plays a critical role in encoding this evidence and the choice biases it causes.

SYMPOSIUM S06
TRACING CELL LINEAGES IN THE BRAIN

TITLE OF THE SYMPOSIUM: Tracing cell lineages in the brain

Co-organizers: Isabel Espinosa-Medina and Jorge García-Marqués

SENC scientific topics: Developmental Neurobiology, New Methods and Technologies

Relevance and timeliness of the topic:

Reconstructing the genealogy of every cell that makes up an organism remains a long-standing challenge in developmental biology. Besides its relevance for understanding the mechanisms underlying normal and pathological development, resolving the lineage origin of cell types will be crucial to create these types on-demand.

Recent advances in single-cell technologies have made it possible to map cell types and track cell lineages simultaneously by single-cell RNA sequencing (scRNA-seq). These exciting technologies have prompted neuroscientists to profile neuron diversity as well as track neural development by sequencing. However, such sequencing-based methods provide little cellular context. In parallel, other sophisticated genetic methods have been recently developed for mapping neuron morphology and tracking neuronal lineages in situ by microscopy. This symposium will focus on the available strategies for lineage tracing in the brain and how these technologies can help us to understand developmental processes.

SPEAKER #1

- Full Name: **Simon Hippenmeyer**

- Affiliation: IST Austria

- Issues to be discussed: Dissecting the role of genomic imprinting by applying lineage tracing

- Three recent publications related to the topic of the symposium:

Laukoter, S., Pauler, F. M., Beattie, R., Amberg, N., Hansen, A. H., Streicher, C., Penz, T., Bock, C., & Hippenmeyer, S. (2020). Cell-Type Specificity of Genomic Imprinting in Cerebral Cortex. Neuron, 107(6), 1160-1179.e9. https://doi.org/10.1016/j.neuron.2020.06.031

Beattie, R., Postiglione, M. P., Burnett, L. E., Laukoter, S., Streicher, C., Pauler, F. M., Xiao, G., Klezovitch, O., Vasioukhin, V., Ghashghaei, T. H., & Hippenmeyer, S. (2017). Mosaic Analysis with Double Markers Reveals Distinct Sequential Functions of Lgl1 in Neural Stem Cells. Neuron, 94(3), 517-533.e3. https://doi.org/10.1016/j.neuron.2017.04.012

Liu, C., Sage, J. C., Miller, M. R., Verhaak, R. G. W., Hippenmeyer, S., Vogel, H., Foreman, O., Bronson, R. T., Nishiyama, A., Luo, L., & Zong, H. (2011). Mosaic Analysis with Double Markers Reveals Tumor Cell of Origin in Glioma. Cell, 146(2), 209–221. https://doi.org/10.1016/j.cell.2011.06.014

- Relevance for the symposium:

Simon has laid the foundation of analyzing cell lineage progression in the mouse brain, particularly in the context of how genomic imprinting determines cell fate. By applying MADM, one of the most effective tools to trace cell lineages in the mouse brain, his group has contributed to the study of corticogenesis with an unprecedented vision of cell lineages at single-cell resolution.

- Abstract:

MECHANISMS OF NEURAL STEM CELL LINEAGE PROGRESSION IN THE DEVELOPING CEREBRAL CORTEX

Simon Hippenmeyer
Institute of Science and Technology Austria

The concerted production of the correct number and diversity of neurons and glia by neural stem cells is essential for intricate neural circuit assembly. In the developing cerebral cortex, radial glia progenitors (RGPs) are responsible for producing all neocortical neurons and certain glia lineages. We recently performed a quantitative clonal analysis by exploiting the unprecedented resolution of the genetic MADM (Mosaic Analysis with Double Markers) technology and discovered a high degree of non-stochasticity and thus deterministic mode of RGP behavior. However, the cellular and molecular mechanisms controlling RGP lineage progression through proliferation, neurogenesis and gliogenesis remain unknown. To this end we use quantitative MADM-based experimental paradigms at single RGP resolution to define the cell-autonomous functions of candidate genes and signaling pathways controlling RGP-mediated cortical neuron and glia genesis and postnatal stem cell behavior. Ultimately, our results shall translate into a deeper understanding of brain function and why human brain development is so sensitive to the disruption of particular signaling pathways in pathological neurodevelopmental and psychiatric disorders.

SPEAKER #2

- Full Name: **Isabel Espinosa-Medina**

- Affiliation: Janelia Research Campus, Howard Hughes Medical Institute

- Issues to be discussed: A new tool to genetically manipulate cells temporally in vertebrates.

- Three recent publications related to the topic of the symposium:

Espinosa-Medina I, Garcia-Marques J, Cepko C. and Lee T. (2019) Meeting Review: High-Throughput Dense. Reconstruction of Cell Lineages. Open Biology. 9: 190229. http://dx.doi.org/10.1098/rsob.190229

Espinosa-Medina I, Jevans B, Boismoreau F, Chettouh Z, Enomoto H, Birchmeier C, Burns A, Brunet JF (2017). Dual origin of enteric neurons in vagal Schwann cell precursors and the sympathetic neural crest. PNAS. Nov 7;114(45):11980-11985

Espinosa-Medina I, E. Outin, C. A. Picard, Z. Chettouh, S. Dymecki, G. G. Consalez, E. Coppola, J.-F.Brunet (2014). Parasympathetic ganglia derive from Schwann cell precursors. Science 345, 87-90.

- Relevance for the symposium:
Isabel has developed a new tool based on CRISPR/Cas9 that allows temporal cell manipulation in vertebrates. Using zebrafish and mice as model systems, she uses this tool to unravel the temporal mechanisms involved in the development of the nervous system. During her previous work, she discovered that autonomic neurons including a population of enteric neurons share a common origin with Schwann cells.

- Abstract:

TEMPORAL ENCODING AND MANIPULATION OF VERTEBRATE CELL HISTORIES WITH A NEW CRISPR/CAS9 SYSTEM

Espinosa-Medina I. [1*], Feliciano D.[1], Belmonte-Mateos C.[2], Lemon W.C.[1], Garcia-Marques J.[3], Foster B.1, Keller P.J.[1], Koyama M.1, Lee T.[1*]
[1]Janelia Research Campus, Howard Hughes Medical Institute; Ashburn, VA, USA. [2]Universitat Pompeu Fabra; Barcelona, Spain. [3] Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas; Madrid, Spain.

*Correspondence to: espinosamedinai@janelia.hhmi.org, leet@janelia.hhmi.org

Defining the cellular origins and interactions regulating the assembly of functional tissues and organs requires genetic labelling, manipulation and cell-lineage reconstruction strategies. Current imaging-based techniques for cell labelling and manipulation in complex vertebrate organisms lack temporal resolution. Here we present TEMPO (Temporal Encoding and Manipulation in a Predefined Order), a new tool based on CRISPR/Cas9 and transgene frame switches driven by an ordered gRNA cascade. The system allows in vivo labelling and manipulation of cells in a predefined order, preserving both spatial and temporal information. In zebrafish and mouse, we demonstrate that TEMPO recapitulates known developmental sequences of neuron formation. By introducing temporal perturbations in cell cycle regulators in mouse cortex progenitors, we show that TEMPO can be used to manipulate the proportion and distribution of neurons and glia of a given cell generation and reveal their interactions with other cell generations in a single sample. Thus, TEMPO provides a powerful resource to manipulate temporal factors required for cell-type specification and study their influence on vertebrate tissue and organ morphogenesis.

SPEAKER #3

- Full Name: **Karine Loulier**

- Affiliation: Institut des Neurosciences de Montpellier, INSERM

- Issues to be discussed: Corticogenesis: diversity and plasticity of neural stem cells during brain development, in healthy and pathological settings.

- Three recent publications related to the topic of the symposium:

Clavreul S, Abdeladim L, Hernández-Garzón E, Niculescu D, Durand J, Ieng SH, Barry R, Bonvento G, Beaurepaire E, Livet J, Loulier K. (2019) Cortical astrocytes develop in a plastic

manner at both clonal and cellular levels. Nat Commun.10(1):4884. doi: 10.1038/s41467-019-12791-5. PMID: 31653848; PMCID: PMC6814723.

Ros O, Zagar Y, Ribes S, Baudet S, Loulier K, Couvet S, Ladarre D, Aghaie A, Louail A, Petit C, Mechulam Y, Lenkei Z, Nicol X. (2019) SponGee: A Genetic Tool for Subcellular and Cell-Specific cGMP Manipulation. Cell Rep. 27(13):4003-4012.e6. doi: 10.1016/j.celrep.2019.05.102. PMID: 31242429.

Loulier K, Barry R, Mahou P, Le Franc Y, Supatto W, Matho KS, Ieng S, Fouquet S, Dupin E, Benosman R, Chédotal A, Beaurepaire E, Morin X, Livet J. (2014) Multiplex cell and lineage tracking with combinatorial labels. Neuron. 81(3):505-20. doi: 10.1016/j.neuron.2013.12.016. PMID: 24507188.

- Relevance for the symposium:

Karine developed MAGIC Markers, a technology for multiplexed cell labelling essential for the study of cell diversity within complex brains. Using this technology, she has contributed important findings on the development of astrocytes in the mammalian cortex. Now she continues using those tools to study early brain development in mouse models of psychiatric disorders such as schizophrenia. In time, she hopes to identify the origin of certain psychiatric disorders and discover the factors linked to the developmental abnormalities thought to be responsible for them.

- Abstract:

DELVING INTO THE GENERATION OF CORTICAL ASTROCYTE DIVERSITY AND PLASTICITY USING MULTICOLOR LINEAGE TRACING TOOLS

Topic: Developmental Neurobiology

Abstract: Mammalian cerebral cortex functions rely on the cooperation of distinct cell types including neurons and glial cells that must be produced in defined proportions and whose imbalance can lead to severe neurodevelopmental disorders. During cerebral cortex development, neurons and glial cells, including astrocytes, are produced sequentially by neural progenitors and ensure together proper synaptic functions. While neurons have been extensively studied in the context of physiological and pathological development, cellular and molecular players responsible for the emergence of cortical diversity, and in particular astrocyte generation, remain poorly described. Astrocytes constitute an heterogeneous population in terms of morphology, molecular marker expression and function, within and among brain regions in mammals. Using combinatorial genetic markers and multicolor imaging techniques, we marked adjacent cortical progenitors with rare color markers prior to gliogenesis and tracked their descent over long periods of time to study astrocyte development. We showed that cortical astrocyte clones display extensive variability in terms of structural organization, location, number and subtype of generated cells. Furthermore, we demonstrated that cortical astrocyte network is generated through two developmental stages that comprise a dynamic phase of proliferation and spatial dispersion followed by a maturation phase where morphology complexity and volume increase at the single cell level. In addition, we determined that astrocyte network is supplied both pre- and perinatally by cortical progenitors. Altogether, our results highlighted the plasticity of astrocytes that probably acquire their subtype features through interactions with their environment. To better understand the emergence of cortical astrocyte diversity, we are now studying in more detail the embryonic origin of astrocytes during brain development. Our work will allow to better understand

cortical astrogliogenesis and its critical cellular and molecular components that could be altered in neurodevelopmental pathologies.

SPEAKER #4

- Full Name: **Jorge García-Marqués**

- Affiliation: Centro Nacional de Biotecnología, CSIC

- Issues to be discussed: New technology to trace lineages based on genetic multi-intersections.

- Three recent publications related to the topic of the symposium:

Garcia-Marques, J., Espinosa-Medina, I., Ku, K., Yang, C., Koyama, M., Yu, H., & Lee, T. (2020). A programmable sequence of reporters for lineage analysis. Nature Neuroscience. https://doi.org/10.1038/s41593-020-0676-9

Garcia-Marques, J., Yang, C. P., Espinosa-Medina, I., Mok, K., Koyama, M., & Lee, T. (2019). Unlimited Genetic Switches for Cell-Type-Specific Manipulation. Neuron, 104(2), 227-238.e7. https://doi.org/10.1016/j.neuron.2019.07.005

García-Marqués, J., & López-Mascaraque, L. (2012). Clonal Identity Determines Astrocyte Cortical Heterogeneity. Cerebral Cortex (New York, N.Y. : 1991). https://doi.org/10.1093/cercor/bhs134

- Relevance for the symposium:

From the beginning of his career, Jorge has developed cutting-edge technology for analyzing cell lineages in the brain. Some of these tools include Star Track, CaSSA or CLADES. Besides his efforts developing technology, Jorge has contributed with important findings on the heterogeneity of glial populations and their role in neuronal migration.

- Abstract:

CELL LINEAGES IN THE VERTEBRATE BRAIN: LESSONS FROM DROSOPHILA

Garcia-Marques, Jorge
Janelia Research Campus, Howard Hughes Medical Institute, Ashburn VA, USA
Current address: National Center for Biotechnology - CSIC, Madrid, Spain

The brain contains an extensive catalog of distinct neuronal types. Such diversity emerges through cell specification processes in which progenitors proliferate while navigating a labyrinth of cell fate decisions. Cell lineage plays a pivotal role in neuronal specification, with sibling neurons exhibiting common traits. Decoding the relation between cell lineage and neuronal identity allows for the dissection of the exact progenitor cell and timing in which cell fate decisions occur. In Drosophila, fate-restricted progenitors change over the course of development to produce distinct neuronal cohorts. Our understanding of neuronal specification in vertebrates remains limited, mainly due to the lack of tools to consistently trace and manipulate specific lineages by targeting the same progenitor type in multiple experiments. Recently, we have developed CaSSA and CLADES, new tools based

on CRISPR/Cas9 to trace and manipulate neuronal lineages. Here I will discuss how this technology helped us to understand neuronal specification in fly, and how this knowledge could guide us on how to tackle the problem in vertebrates.

SYMPOSIUM S07
MOTOR CIRCUITS AND CONTROL FROM A BASIC AND CLINICAL VIEW

TITLE OF THE SYMPOSIUM: Motor circuits and control from a basic and clinical view

Brief description of the relevance and timeliness of the topic, including the issues to be discussed by each speaker.

This symposium proposal aims to give novel and relevant insights into the complex physiology and function of basal ganglia, brainstem and cortical motor circuits reaching from animal models to neurological disease in humans and covering a wide range of advanced and innovative technical approaches. Despite the steady and laborious investigations performed to comprehend the underlying mechanisms of locomotion and movement disorders, there are still huge knowledge gaps that involve the mentioned brain areas. In this symposium we want to fill some of these gaps and present remarkable findings, some of them published in prestigious journals (i.e. Nature Neuroscience, Current Biology, Brain), that will help to disentangle properties and function of different motor areas, which in the end will contribute to the understanding of normal and dysfunctional motor output.

The symposium will especially focus on the following investigations:

1. The basal ganglia are of major importance for normal motor control and behavior; being the striatum the main input structure of this region. The work of Reig et al. explored the functional circuitry of the rodent dorsal striatum. Despite the anatomically homogeneous appearance of this structure, they showed that the dorsolateral and dorsomedial striatum in mice represent two non-overlapping functional circuits, analogous to the anatomical and functional division of the primate striatum in caudate and putamen. Their group applied in vivo optopatch-clamp (a highly challenging and cutting-edge technique) and extracellular recordings of spontaneous slow wave oscillations, using a specific algorithm for analysis. This novel approach represents a sensible strategy that can provide new insights between anatomy and function with the possibility to study any brain circuit.

2. The dorsal striatum has been implicated in the storage of procedural memories (expressed through movement) and the control of movement kinematics. One critical difficulty is that it is nearly impossible to separate whether impaired performance arises from an inability to implement a preserved procedural memory into actions or from a direct alteration of the stored procedural memory with preserved motor control. To address this performance confound, Jurado-Parras et al. (Current Biology, 2020) explored the impact of dorsal striatum lesion in rats executing a well-learned locomotion-based routine. Surprisingly, dorsal striatum lesion did not impair the rats' ability to remember the procedural steps to successfully perform the learned routine, but movement kinematics were irreversibly altered. This work underlies a primary role of the dorsal striatum in modulating kinematics of reward-oriented actions, a function that may be related to the optimization of the energetic costs of moving.

3. Arrest of ongoing movement is essential for successfully executing all motor programs. The motor arrest may occur as a termination of a goal-directed movement, in context of fear, or upon detection of new environmental signals. The neuronal control of motor arrest related to the detection of new sensory cues is not well understood. Leiras and colleagues will show (article in preparation) that optogenetic activation of a subpopulation of glutamatergic pedunculopontine neurons (Chx10 neurons) leads to a global immobilization in mice. The motor arrest affects any ongoing motor behavior (e.g. locomotion, grooming or rearing), which resumes right after the end of stimulation. The immobilization bouts are similar to those performed during exploratory behavior, when mice detect

novel visual stimuli. The evoked motor arrest is different from the fear induced freezing. This study defines a pedunculopontine glutamatergic subpopulation essential for initiating transient global motor immobilization.

4. Finally, Ammann et al. (Brain, 2020) will make the transition to human movement disorders discussing the functional state of inhibitory circuits of the motor cortex in patients with Parkinson's disease (PD). These patients express a dysfunctional motor output due to striatal dopamine depletion. A key regulator of motor output is the balance between excitation and inhibition in the primary motor cortex, which they measured indirectly through paired-pulse transcranial magnetic stimulation in a large sample of PD patients and healthy controls. They found a significant bilateral cortical disinhibition in PD patients. However, the striking result is that this loss of cortical inhibition was already present in newly diagnosed PD patients in whom the less affected side was still minimally symptomatic. This result suggests that cortical disinhibition is a very early, possibly prodromal feature of PD.

SPEAKER #1

- Full Name: **Ramón Reig**

- Enter three recent publications of the Speaker related to the topic of the symposium:

Alegre-Cortés J, Sáez M, Montanari R, Reig R. Medium spiny neurons spontaneous activity reveal the discrete segregation of mouse dorsal striatum. bioRxiv, Jul 2020

Reig R, Silberberg G. Distinct Corticostriatal and Intracortical Pathways Mediate Bilateral Sensory Responses in the Striatum. Cereb Cortex, Dec 2016

Reig R, Silberberg G. Multisensory integration in the mouse striatum. Multisensory integration in the mouse striatum. Neuron, Sep 2014

- Relevance for the symposium:

Defining the functional circuits of the mouse dorsal striatum implicated in motor control.

- Abstract:

SENSORIMOTOR INTEGRATION BY DORSAL STRIATAL CIRCUITS

The basal ganglia (BG) are involve in a wide range of function, such as planning, motor learning or selection of movements. Malfunctions of the BG can lead severe motor problems, as Parkinson’s and Huntington’s disease or Tourette syndrome. The striatum is the input layer of the BG and receives cortical projections from sensory, motor and associative areas and thalamus. The 95% of the striatal neurons are GABAergic projection neurons called MSNs. They are divided into two subpopulations according to their axonal projections and their different dopamine receptor expression, defining the direct and indirect pathway. In addition, the striatum is massively innervated by dopaminergic axons from the substantia nigra pars compacta. Dopamine is known to play a role in processes leading to corticostriatal synapses, modulating and inducing changes in synaptic transmission and plasticity. Axons from cortical sensory areas target MSNs and interneurons, some of these projections overlap

along dorsal striatum, when transmitting; tactile, auditory and visual information. Therefore, in order to understand the circuits evolved, we first study the axonal innervation and the functional properties of the dorsal striatum. To asses our objectives, we combined in vivo optopatch-clamp recordings in identified MSNs along dorsal striatum with simultaneous local field potential recordings in several cortical areas in anesthetized mice. We found that dorsal striatum is composed by two non-overlapping functional circuits, with particular corticostriatal connectivity and local synaptic attributes of each region (Alegre-Cortés et al, 2020). We stablished that only MSNs located in the dorsomedial striatum are able to integrate information of different sensory modalities, such as visual and tactile. Then, we also investigated the impact of dopamine modulating multisensory integration in MSNs of the dorsomedial striatum. Our data shows that dopamine release modify the integration of visual and tactile inputs and selectively synchronizes multisensory information in a specific subpopulation of MSNs.

SPEAKER #2

- Full Name: **Teresa Jurado-Parras**

- Enter three recent publications of the Speaker related to the topic of the symposium:

Jurado-Parras MT, Safaie M, Sarno S, Louis J, Karoutchi C, Berret B, Robbe D. Dorsal striatum eneregizes motor rutines. Curr Biol, Nov 2020

Safaie M, Jurado-Parras MT, Sarno S, Louis J, Karoutchi C, Petit LF, Pasquet MO, Eloy C, Robbe D. Turning the body into a clock: Accurate timing is facilitated by simple stereotyped interactions with the environment. Proc Natl Acad Sci U S A, Jun 2020

Lalla L, Rueda Orozco PE, Jurado-Parras MT, Brovelli A, Robbe D. Local or Not Local: Investigating the nature of striatal theta oscillations in behaving rats. eNeuro, Sep 2017

- Relevance for the symposium:

Describing the role of the dorsal striatum modulating the vigor of movement

- Abstract:

MOTOR CONTROL IN THE DORSAL STRIATUM
María Teresa Jurado Parras

The dorsal striatum (dS) has been involved in storing and retrieving procedural memories and linked to the control of movements kinematics. Delineating the dS function is challenging because movements are the readout of procedural memories. To disentangle this conundrum, we train rats in a set of original and complementary task. We found that dS lesions spared procedural memories but altered the kinematics of their expression in motor routines. Further behavioral analysis and theoretical simulations demonstrated that dS lesion did not affect animals' motivation, their ability to perform routines nor it modulated their running speed. Quite in contrast, dS lesion increased the animal sensitivity to energy expenditure. We propose that the dS computes an effort signal that influences the

kinematics of purposive actions. By setting the sensitivity to effort, the dS contributes to the optimization of the energy invested into voluntary movements. Such an elementary function of the dS might explain its implication in both procedural decisions and the control of movement vigor.

SPEAKER #3

- Full Name: **Roberto Leiras**

- Enter three recent publications of the Speaker related to the topic of the symposium:

Cregg JM, Leiras R, Montalant A, Wanken P, Wickersham IR, Kiehn O. Brainstem neurons that command mammalian locomotor asymmetries. Nat Neurosci, Jun 2020.

Caggiano V, Leiras R, Goñi-Erro H, Masini D, Bellardita C, Bouvier J, Caldeira V, Fisone G, Kiehn O. Midbrain circuits that set locomotor speed and gait selection. Nature, Jan 2018.

Bouvier J, Caggiano V, Leiras R, Caldeira V, Bellardita C, Balueva K, Fuchs A, Kiehn O. Descending command neurons in the brainstem that halt locomotion. Cell, Nov 2015

- Relevance for the symposium:

Identifying a glutamatergic midbrain subpopulation as responsible for a global non-fear-related immobilization.

- Abstract:

CHX10 PEDUNCULOPONTINE NEURONS, A MIDBRAIN GLUTAMATERGIC SUBPOPULATION THAT CAUSES GLOBAL MOTOR ARREST

Roberto Leiras
Department of Neuroscience, University of Copenhagen

Arrest of ongoing movements is essential for successfully executing all motor behaviors. Motor arrest may occur in different contexts such as termination of a goal directed movement, fear, or upon detection of environmental signals. The neural control of behavioral arrest in response to novel sensory cues is not well understood. Here we report that excitatory Chx10+ neurons in the pedunculopontine nucleus (PPN) induce global motor arrest in mice. We find that a subpopulation of glutamatergic neurons preferentially located in the rostral PPN expresses the transcription factor Chx10. Optogenetic activation of Chx10+-PPN neurons in freely-moving mice arrests all ongoing movements, including forward locomotion, grooming or rearing. The arrest is characterized by a "pause-and-play" pattern, where upon light activation mice abruptly hold the ongoing movement at any phase of its execution, and after stimulus offset mice resume the activity they were previously engaged on. Chronic recordings of hindlimb muscle activity show that the locomotor arrest is not due to flexor-extensor coactivation. Simultaneous whole-body plethysmography and electrocardiography reveal that the motor arrest is accompanied by apnea and bradycardia. Moreover, we observe that mice naturally perform the same combination of motor and autonomic features when presented with non-threatening visual stimuli. The Chx10+-PPN induced motor, respiratory, and cardiac phenotype differs from the ventrolateral periaqueductal gray (vlPAG) induced freezing. Furthermore, Chx10+-PPN neurons broadly project to the pontomedullary reticular formation. Our study defines a group of

excitatory midbrain neurons that induce a global non-fear-related arrest which may be recruited to facilitate attentiveness to salient environmental cues.

SPEAKER #4

- Full Name: **Claudia Ammann**

- Enter three recent publications of the Speaker related to the topic of the symposium:

Ammann C, Dileone M, Pagge C, Catanzaro V, Mata-Marín D, Hernández-Fernández F, Monje MHG, Sánchez-Ferro Á, Fernández-Rodríguez B, Gasca-Salas C, Máñez-Miró JU, Martínez-Fernández R, Vela-Desojo L, Alonso-Frech F, Oliviero A, Obeso JA, Foffani G. Cortical disinhibition in Parkinson's disease. Brain, Nov 2020

Ammann C, Guida P, Caballero-Insaurriaga J, Pineda-Pardo JA, Oliviero A, Foffani G. A framework to assess the impact of number of trials on the amplitude of motor evoked potential. Sci Rep, 2020 (in press)

Ammann C, Márquez-Ruiz J, Gómez-Climent MÁ, Delgado-García JM, Gruart A. The motor cortex is involved in the generation of classically conditioned eyelid responses in behaving rabbits. J Neurosci, Jun 2016

- Relevance for the symposium:

Providing new insights into the functional state of the primary motor cortex in movement disorders.

- Abstract:

DISINHIBITION OF THE MOTOR CORTEX IN PARKINSON'S DISEASE

Claudia Ammann
HM CINAC. Centro Integral de Neurociencias AC. HM Hospitales. Móstoles (Madrid, Spain)

Parkinson's disease is defined by a dysfunctional motor output due to striatal dopamine depletion. A key
regulator of motor output is the balance between excitation and inhibition in the primary motor cortex. In humans, motor cortex excitability can be measured indirectly through transcranial magnetic stimulation (TMS) and a common measure to explore inhibitory mechanisms is the short-interval intracortical inhibition (SICI) induced by paired-pulse TMS stimuli. The disagreement between previously published studies raised doubts about the actual role of SICI in Parkinson's disease. Thus, our objective was to clarify whether SICI is altered in Parkinson's disease and whether and how the possible loss of inhibition, if any, may reflect the clinical state of patients and the evolution of the disease. Moreover, since motor symptoms, such as tremor, bradykinesia and rigidity, are lateralized in most patients with Parkinson's disease, we also wanted to examine the relationship between motor cortex excitability and lateralization of motor symptoms. To address all these issues, we studied a relatively large sample of patients with Parkinson's disease, using paired-pulse TMS protocols. Our results show that SICI is decreased in Parkinson’s disease compared to age-matched healthy controls. Moreover, cortical inhibition was similarly reduced in levodopa-naïve, non-dyskinetic and dyskinetic

patients. The striking result was that this loss of cortical inhibition was already present in newly diagnosed patients in whom the less affected side was still minimally symptomatic. This result suggests that cortical disinhibition is a very early, possibly prodromal feature of Parkinson's disease.

SYMPOSIUM S08
ORIGIN AND EXPANSION OF THE NEOCORTEX

TITLE OF THE SYMPOSIUM: ORIGIN AND EXPANSION OF THE NEOCORTEX

Relevance, timeliness and adequacy to the symposium call:
The neocortex is the largest part of the human brain and the seat of our cognitive abilities. Unique to mammals and dramatically expanded in primates, this outstandingly complex structure emerged during evolution from the simple brains of amphibians. Renewed efforts worldwide are currently dedicated to understanding the genomic, cellular and developmental mechanisms involved in the emergence and expansion of the neocortex during evolution, and how defects in those fundamental mechanisms are causative of brain malformations and disease in the modern human. This symposium will bring together world leaders in this topic, with very different and complementary conceptual and experimental approaches, to present an update on our current understanding of this fundamental question, covering a wide range of different aspects around the main topic. The symposium will be composed of two national and two international speakers, coming from three different countries and two different continents, with a perfect balance of gender (two female, two male) and seniority (two young and two established PIs).

SPEAKER #1

Dr. Nerea Moreno (SENC member)
Universidad Complutense (Madrid, Spain)

- Jiménez et al., 2020. Analysis of pallial/cortical interneurons in key vertebrate models of Testudines, Anurans and Polypteriform fishes. Brain Struct Funct. 225:2239-2269
- Moreno, González, 2017. Pattern of Neurogenesis and Identification of Neuronal Progenitor Subtypes during Pallial Development in Xenopus laevis. Front Neuroanat. 11:24
- Moreno et al., 2014. Conserved localization of Pax6 and Pax7 transcripts in the brain of representatives of sarcopterygian vertebrates during development supports homologous brain regionalization. Front Neuroanat. 8:75

Dr. Moreno research is focused on the evolutionary analysis of the pallial region of vertebrates, the histogenic domain that gives rise to the cerebral cortex. In particular, through the evolutionary study of the genoarchitectonic patterns underlying pallial organization, and its analysis during development. Using amphibians as the main model, she identified the existence of intermediate progenitors in the amniotic pallium and recently her laboratory described the conserved interneuron arrangement found in the pallium of distant evolutionary models. In her talk, Dr. Moreno will present the latest findings of her team about the patterning of the pallium subdivisions in vertebrates, and will discuss about which are conserved in evolution, representing the stem amniote, and which regions are unique amniote acquisitions.

- Abstract:

EVOLUTION OF THE PALLIUM: NEW INSIGHTS FROM STUDIES IN AMPHIBIANS.

NEREA MORENO
Department of Cell Biology. Faculty of Biology. University Complutense of Madrid.

The dorsal telencephalon constitutes the pallium in all vertebrates, and it is the territory that in mammals gives rise to the cortex, the region that shows the greatest functional and organizational complexity during evolution. Despite the differences found in the different vertebrates studied comparable conserved pallial regions have been identified. Our aim is to analyze the genoarchitectonic pattern of these pallial subdivisions particularly at the anamniote-amniote transition, which will allow us to evaluate which regions are conserved at this evolutionary point, likely recapitulating the situation found in the stem amniote, and which are unique amniote acquisitions. Based on the expression of the mammalian pallial domain markers, we have analyzed their expression patterns in the amphibian anuran Xenopus laevis, the only anamiote tetrapod. We have identified different pallial subdivisions, where neither layered nor nuclear structures are formed. Two different domains have been identified in the Xenopus medial pallium based on the Prox1/Er81 expressions, likely homologous to the amniote medial cortex, the hippocampal-like region. Similarly, the dorsal pallium of Xenopus was subdivided in dorsal and dorsolateral parts showing distinct EGFR, Er81, Cux2, Lmo4 and Ctip2 expression. The lateral pallium derivatives have been rostrocaudally identified by the Satb2 and Reln expressions. And finally, the ventral pallium of amphibians was rostrocaudally subdivided by the distinct Tbr1, FeZ2 and Lhx2/9 expressions. Our results suggest that a common bauplan in the pallial organization is present in the tetrapod ancestor and that the differences could be evolutionary divergences and adaptations.

SPEAKER #2

Fernando García-Moreno
Achucarro Basque Center for Neuroscience, Bilbao, Spain

- Rueda-Alaña and García-Moreno, 2021. Time in Neurogenesis: Conservation of the Developmental Formation of the Cerebellar Circuitry. Brain, Behavior and Evolution. In press. DOI: 10.1159/000519068
- García-Moreno and Molnár, 2020. Variations of telencephalic development that paved the way for neocortical evolution. Progress in Neurobiology, 194, 101865.
- García-Moreno F, et al., 2018. Absence of Tangentially Migrating Glutamatergic Neurons in the Developing Avian Brain. Cell Reports 22:96-109.

Dr. García-Moreno focuses his research on the divergent developmental programs that were responsible for the generation of the structurally different vertebrate brains. Since evolution mainly depends on the variation and fixation of developmental events, the concept of homology truly establishes upon a conservation of developmental programs. Dr. García-Moreno researches the chronological neurogenesis and construction of brain circuits that have been proposed as homologous. By means of experimental embryology and single cell RNA sequencing on three selected amniote species (chick, gecko, mouse), he will show how some circuits, although similar in structure and functionality, are built through divergent developmental programs. On the contrary, other brain

circuits are generated following a tightly conserved developmental plan throughout vertebrates. Dr. García-Moreno will present a provocative hypothesis on the mosaic evolution of brain structures, on which divergent, homologous and convergent circuits evolved simultaneously on different regions of the vertebrate brain.

- Abstract:

MOSAIC EVOLUTIONARY HISTORY OF BRAIN CIRCUITS THROUGH THE LENS OF NEURODEVELOPMENT

F. García-Moreno
Achucarro Basque Center for Neuroscience, Bilbao, Spain; Faculty of Medicine of UPV/EHU, Bilbao, Spain

Vertebrate brains display an astonishing variability at structural and functional level, supported by the greatest diversity of cellular types across nature. However, during early embryonic stages all vertebrate brains reflect the same morphological pattern, a unique template that unifies every brain. **How this embryonic brain, repeated across species, is capable of producing so many different adult brains?**

In the lab, we try to understand the divergent developmental processes in the common embryonic brain that produce brain diversity. Our lab enjoys the innovative merge of tools and theoretical frameworks from neurobiology, developmental biology and evolutionary biology.

Considering both the *homogeneity* of the early embryonic brain and the contrasting *diversity* of the mature brains, we hypothesize that the vertebrate brain circuits evolved as a mosaic: tightly preserving essential circuits and allowing creative divergence on association circuits. For this project, we aim to reveal the developmental program giving rise to several known circuits in the vertebrate brain, such as the cerebellum of the sensory pallium. By means of experimental embryology and single cell RNA sequencing on three selected amniote species (chick, gecko, mouse), I will show some brain circuits that are generated following a tightly conserved developmental plan throughout vertebrates. The neurogenic program responsible for the formation of the cerebellar circuit follows the same timing and rules in amniotes. But I will also show how other circuits, similar in structure and functionality across species, are built through divergent developmental programs. Neurogenic rules are not conserved in these circuits, a difference also reflected at the transcriptomic level of their neuronal components. I will present a provocative hypothesis on the mosaic evolution of brain structures, on which divergent, homologous and convergent circuits evolved simultaneously on different regions of the vertebrate brain.

SPEAKER #3

Dr. Víctor Borrell (Organizer and SENC Member)
Instituto de Neurociencias (Alicante, Spain)

- Fernández et al., 2020. Repression of Irs2 by let-7 miRNAs is essential for homeostasis of the telencephalic neuroepithelium EMBO J 39:e105479
- Llinares-Benadero and Borrell, 2019. Deconstructing cortical folding: genetic, cellular and mechanical determinants. Nature Reviews Neuroscience 20:161–176

- Cárdenas et al., 2018. Evolution of Cortical Neurogenesis in Amniotes Controlled by Robo Signaling Levels. Cell 174:590-606.e21

Dr. Borrell investigates cellular and genetic mechanisms that regulate cortical expansion and folding in development, and how these emerged during evolution. Pioneering the use of ferret as animal model, his team discovered a new type of neural progenitor cell that is critical in the expansion and folding of the mammalian neocortex, including human. Then he identified the existence of unique transcriptional signatures along cortical germinal layers that map the prospective location of folds and fissures, including genes mutated in human cortical malformations. More recently, they identified a genetic mechanism conserved across phylogeny that defines the mode of neurogenesis, and the regulation of which determines the size of their cerebral cortex in amniotes. In his talk, Dr. Borrell will present his latest findings on the molecular mechanisms and the diversity of progenitor cell types involved in the evolutionary expansion and folding of the mammalian neocortex.

- Abstract:

EVOLUTION OF CORTICAL PROGENITOR CELLS: MUCH TO GAIN, MUCH TO LOOSE

Víctor Borrell
Instituto de Neurociencias, CSIC-UMH, Alicante, Spain

The evolutionary expansion and folding of the mammalian cerebral cortex resulted from amplification of progenitor cells during embryonic development, but the cellular and genetic mechanisms behind this key process remain poorly understood. Here I will discuss our latest results on this fascinating question, first showing the increased complexity of progenitor cell types in ferret and human compared to mouse. Our ferret scRNAseq data from specific germinal layers and developmental stages reveals an unprecedented heterogeneity of progenitor cell subtypes that are related by parallel cellular lineages, including different self-amplificative activity, which converge on an otherwise common neurogenic path. Intriguingly, the amplification of cortical progenitor cells during mammalian evolution was reversed in the rodent lineage after splitting from primates, leading to smaller and smooth brains. Genetic mechanisms underlying this secondary loss in rodent evolution remain unknown. In the second part of my presentation, I will discuss our most recent results identifying for the first time a gene (miR-3607) selected for secondary loss during mammalian evolution to limit progenitor cell amplification and, potentially, cortex size in rodents. This microRNA is expressed embryonically in the large cortex of primates and ferret, distant from the primate-rodent lineage, but not in mouse. Experimental expression of miR-3607 in embryonic mouse cortex led to increased Wnt/β–Catenin signaling, amplification of Radial Glia Cells (RGCs) and expansion of the Ventricular Zone (VZ), via blocking the β–Catenin inhibitor APC. In summary, I will illustrate how the evolution of the mammalian neocortex involved both the gain and loss of cellular and molecular players. Funding: ERC StG (309633), Agencia Estatal de Investigación (PGC2018-102172-B-I00, SEV2017-0723), Fundación Santiago Grisolía

SPEAKER #4

Dr. Katie Long

Centre for Developmental Neurobiology – King´s College London (London, United Kingdom)

- Long et al., 2019. How the extracellular matrix shapes neural development. Open Biology doi.org/10.1098/rsob.180216
- Long et al., 2018. Extracellular matrix components HAPLN1, lumican and collagen I cause hyaluronic acid-dependent folding of the developing human neocortex. Neuro 99:702-719.e6
- Long et al., 2016. Integrin signaling regulates the expansion of neuroepithelial progenitors and neurogenesis via Wnt7a and Decorin. Nature Communications 7:10354

Dr. Long research focuses on how the human neocortex develops with the correct size, shape and organization, and the role of the extracellular matrix in these processes. The extracellular matrix is very abundant in the embryonic human neocortex, but its function in neocortex development is not fully understood. Dr. Long will present her findings following an interdisciplinary approach to learn about the cellular and mechanical mechanisms by which the extracellular matrix drives the development of the human neocortex, and how dysregulation of these functions may contribute to neurodevelopmental disorders. She will particularly focus on the formation of the folds and fissures, and how changes in extracellular matrix composition contributed to the diversity and degree of cortical folding during evolution.

- Abstract:

HOW DOES THE HUMAN BRAIN DEVELOP TO THE RIGHT SIZE AND SHAPE? THE ROLE OF THE EXTRACELLULAR MATRIX.

Katie Long
Centre for Developmental Neurobiology and MRC Centre for Neurodevelopmental Developmental Disorders, King's College London

Understanding how our own brains develop is a vital step in understanding how they perform the many higher cognitive functions that make us human, such as our memory, speech and advanced learning. Although these are clearly important functions, we still understand very little about how the neocortex controls them. Evidence from patients with neurodevelopmental disorders suggests that this relies on the correct development of the neocortex, as defects in the size, shape or organisation of the neocortex can lead to cognitive impairment. In spite of this importance, how these processes are regulated during development remains elusive. This is especially true for certain aspects, such as the formation of the folds on the surface of the neocortex. A better understanding of these processes will broaden our perspective of and offer new insights into the neurodevelopmental disorders that affect neocortex function.

Our research focuses on how the human neocortex develops with the correct size, shape and organisation, and the role of the extracellular matrix in these processes. Extracellular matrix is highly abundant in the developing human neocortex, but its function in neocortex development is not yet fully understood. To address this we use an interdisciplinary approach to look at the cellular and mechanical mechanisms by which the extracellular matrix drives the development of the human neocortex, including the formation of the folds present on the surface of the neocortex, and how dysregulation of these functions can lead to neurodevelopmental disorders. We have shown that

manipulating the extracellular matrix can both induce and reverse cortical folding in human fetal neocortex slice cultures, providing a novel model in which to study the mechanisms that drive folding in the human brain.

SYMPOSIUM S09
UNDERSTANDING THE NEUROIMMUNE AXIS IN HEALTH AND DISEASE: MYELOID AND LYMPHOID CELLS IN THE CENTRAL NERVOUS SYSTEM

SYMPOSIUM TITLE: "Understanding the neuroimmune axis in health and disease: myeloid and lymphoid cells in the Central Nervous System"

SYMPOSIUM SUMMARY: Enriching the old dogma of the central nervous system as an immune privileged organ, recent observations have highlighted that the CNS parenchyma and its interfaces host a large diversity of immune cells that serve essential roles not only in diseases but also in basic brain physiological processes. In this symposium, we bring together four expert neuroimmunologists in different stages of their career to discuss recent advances in our understanding of how resident and invading immune cells shape brain's function in health and disease. From microglia to macrophages and T cells, this symposium will cover their role in brain development, adult neurogenesis and diseases such as multiple sclerosis and Alzheimer´s disease.

SPEAKER #1:

Full Name (affiliation):
Amanda Sierra (Achucarro Basque Center for Neuroscience)

Tentative title:
Immunometabolic rewiring of microglia during phagocytosis

Recent publications related to the topic of the symposium (3)

1. Diaz-Aparicio I, Paris I, Sierra-Torre V, Plaza-Zabala A, Rodriguez-Iglesias N, Marquez-Ropero M, Beccari S, Huguet P, Abiega O, Alberdi E, Matute C, Bernales I, Schulz A, Sperlagh B, Lemke G, Maletic-Savatic M, Valero J, Sierra A. Microglia actively remodel adult hippocampal neurogenesis through the phagocytosis secretome, J Neurosci 2020, 40 (7) 1453-1482.
2. Sierra A, Paolicelli RC, Kettenmann H. Cien años de microglía: milestones in a century of microglial research. Trends in Neurosciences 2019, 42(11):778-792.
3. Sierra-Torre V, Plaza-Zabala A, Bonifaci P, Abiega O, Diaz-Aparicio I, Tejelberg S, Lehesjoki AE, Valero J, Sierra A. Microglial phagocytosis dysfunction is related to local neuronal activity in a genetic model of epilepsy (Epilepsia, in press).

Relevance for the symposium
Phagocytosis of apoptotic cells is a classical immune function that also plays important roles in brain physiology and pathology. Apoptosis is a widespread phenomenon in the brain from early embryonic development to adult neurogenic niches, neurodegenerative diseases and aging. Apoptotic cells must be swiftly removed from the parenchyma to prevent the spillover of toxic intracellular compounds through phagocytosis by microglia. In this talk, we will discuss that the impact of phagocytosis goes beyond debris clearance. We will show unpublished data from in vitro and in vivo models supporting that the engulfment and degradation of apoptotic debris triggers a series of epigenetic, transcriptional and metabolic adaptations in microglia that result in long-term functional changes. We will conclude that phagocytosis of cell debris may be a key to understanding how microglia impact surrounding surviving neurons.

- Abstract:

NOT JUST CORPSE REMOVAL: HOW MICROGLIAL PHAGOCYTOSIS MAINTAINS TISSUE HOMEOSTASIS

Amanda Sierra
Achucarro Basque Center for Neuroscience, Leioa, Spain

Microglia are the brain professional macrophages and they efficiently remove dead cells and other forms of cell debris, both during development and in pathological conditions. But what happens to microglia after engulfing and degrading apoptotic cells? In this talk, I will argue that phagocytosis is not a dead-end process but rather the begging of a new life for microglia. I will discuss that the events triggered in microglia by phagocytosis have an impact on the surrounding tissue, using as a model the adult neurogenic cascade, where microglia engulfs the excess of newborn cells. We are currently learning how phagocytosis affects microglial metabolism, transcription, and cell function, with the goal of developing pharmacological approaches to harness microglial phagocytosis in the diseased brain.

SPEAKER #2:

Full Name (affiliation):
Diego Gómez Nicola (School of Biological Sciences, University of Southampton, UK)

Tentative title:
Altered population dynamics determines the microglial phenotypic specification associated with Alzheimer's disease

Recent publications related to the topic of the symposium (3)

1. Olmos-Alonso A, Schetters STT, Sri S, Askew K, Mancuso R, Vargas-Caballero M, Holscher C, Perry VH, Gomez-Nicola D. Pharmacological targeting of CSF1R inhibits microglial proliferation and prevents the progression of Alzheimer's-like pathology. Brain. 2016 Mar;139(Pt 3):891-907.
2. Askew KE, Li K, Olmos-Alonso A, Garcia-Moreno F, Liang Y, Richardson P, Tipton T, Chapman M, Riecken K, Molnár Z, Cragg MS, Garaschuk O, Perry VH, Gomez-Nicola D. Coupled proliferation and apoptosis maintain the rapid turnover of microglia in the adult brain. Cell Reports. 2017 Jan 10;18(2):391-405.
3. Mancuso R, Fryatt G, Cleal M, Obst J, Pipi E, Monzón-Sandoval J, Ribe E, Winchester L, Webber C, Nevado-Holgado A, Jacobs T, Austin N, Theunis C, Grauwen K, Ruiz ED, Mudher A, Vicente Rodriguez M, Parker C, Simmons C, Cash D, Richardson J, Jones D, Lovestone S, Gomez-Nicola D, Perry VH. Blockade of microglial proliferation by CSF1R inhibitor JNJ-40346527 in mouse models of neuroinflammation and tau pathology impacts tau aggregation and prevents neurodegeneration. Brain. 2019 Oct 1;142(10):3243-3264.

Relevance for the symposium
The progression of chronic neurodegeneration seen in diseases such as Alzheimer's disease (AD) is characterized by a strong activation of the innate immune response, driven by microglia. In AD, one

of the earliest changes observed in microglia is a rapid and localized proliferation, in response to nascent amyloid pathology. Over recent years we have characterized this response, and in this talk we will present evidence supporting that the accumulated proliferation of microglia drives the onset of replicative senescence in these cells. This is a physiological state characterized by shortening of telomeres and a dysfunctional inflammatory response. Our data supports that replicative senescence characterizes the main disease-associated microglial phenotype observed in AD, known as DAM, and is responsible for the initial accumulation of amyloid pathology characteristic of preclinical AD. Overall, we provide a comprehensive picture of the dynamics of the microglial population, and how this defines key roles of these cells.

- Abstract:

UNDERSTANDING MICROGLIAL DYNAMICS FROM DEVELOPMENT TO AGE-RELATED NEUROLOGICAL DISORDERS

D Gomez-Nicola
School of Biological Sciences, Faculty of Environmental and Life Sciences, University of Southampton, United Kingdom

Microglia play a critical role in the development and maintenance of the inflammatory response characteristic of several neurodegenerative disorders, showing enhanced proliferation and activation. Here we will discuss recent findings describing the mechanisms driving the expansion and maintenance of the microglial population from early development to age-related neurological disorders. These support the rationale for studying and targeting neuroinflammation in Dementia, and allow the identification and validation of key therapeutic targets. We will discuss data derived from a multidisciplinary approach combining the study of laboratory models of chronic neurodegeneration with the study of post-mortem samples from patients, to describe the time-course and regulation of microglial proliferation. Our results demonstrate that microglial proliferation is an important feature of the evolution of chronic neurodegenerative disease, with direct implications for the specification of disease-associated phenotypes through engagement of replicative senescence.

SPEAKER #3:

Full Name (affiliation):
Giuseppe Locatelli (Theodor Kocher Institute, University of Bern, CH)

Tentative title:
Dwellers and trespassers: myeloid cell dynamics within the central nervous system

Recent publications related to the topic of the symposium (3)

1. Locatelli G, Theodorou D, Jordao MJ, Staszewski O, Dagkalis A, Bessis A, Prinz M, and Kerschensteiner M, Mononuclear phagocytes locally specify and adapt their phenotypes in the inflamed central nervous system, (2018), Nature Neuroscience, 21(9):1196-1208;
2. Ivan D, Walthert S, Locatelli G, Monocyte recruitment to the inflamed central nervous system: migration pathways and distinct functional polarization, (2020), BioRxiv, https://doi.org/10.1101/2020.04.04.025395;

3. Jordão MJC, Sankowski R, Brendecke SM, Sagar, Locatelli G, Tai YH, Tay TL, Schramm E, Armbruster S, Hagemeyer N, Groß O, Mai D, Çiçek Ö, Falk T, Kerschensteiner M, Grün D, Prinz M, Single-cell profiling identifies myeloid cell subsets with distinct fates during neuroinflammation, (2019), Science, 363(6425).

Relevance for the symposium

The central nervous system (CNS) parenchyma is enclosed by multilayered functional borders populated by dynamic myeloid observers known as barrier-associated macrophages. Upon autoimmune inflammation as observed in Multiple Sclerosis (MS) and in its animal model Experimental autoimmune encephalomyelitis (EAE), these phagocytes start playing additional roles as immune regulators contributing to disease evolution. At the same time, pathological conditions drive the recruitment of blood-borne monocyte-derived cells across distinct CNS gateways. This invasion process drastically increases border complexity and can lead to parenchymal infiltration of blood-borne myeloid cells playing a direct role both in damage and in tissue repair. Using a combination of 2-photon imaging in transgenic mice and experimental in vitro models, we have recently highlighted the extreme heterogeneity of these myeloid CNS cells and investigated their functional polarizations at CNS interfaces and within the parenchyma. We have shown that differentially-activated myeloid cells traffic to the CNS in a barrier-specific fashion acquiring their functional specifications in a complex molecular interplay with other CNS cells. Furthermore, our study highlights the choroid plexus as a key access gateway for activated macrophages during neuroinflammation.

- Abstract:

DYNAMICS OF CNS INVADING MYELOID CELLS AND PHENOTYPE ACQUISITION DURING NEUROINFLAMMATION

Daniela C. Ivan, Sabrina Walthert, Giuseppe Locatelli
Theodor Kocher Institute, University Bern, Bern, Switzerland

Correspondence: Giuseppe Locatelli, Giuseppe.locatelli@tki.unibe.ch

In multiple sclerosis (MS) and in general upon neuroinflammation, monocyte-derived cells (MoCs) traffic through central nervous system (CNS) barriers exerting detrimental or beneficial functions. How and where these cells acquire their different functional commitments remains however unclear.
To clarify this issue, we investigated the distribution of iNOS+ pro-inflammatory and arginase-1+ anti-inflammatory MoCs at the distinct CNS barriers in a mouse model of MS. Interestingly, MoCs within perivascular parenchymal spaces displayed a predominant pro-inflammatory phenotype compared to MoCs accumulating at the leptomeninges and at the choroid plexus (ChP). Furthermore, we could observe the general ability of polarized MoCs to migrate through the ChP epithelial barrier, together indicating the ChP as a CNS entry and polarization site for MoCs. Thus, pro- and anti-inflammatory MoCs differentially accumulate at distinct CNS barriers before reaching the parenchyma, but the mechanism for their phenotype acquisition remains unclear.
Shedding light on this process, we observed that endothelial (BBB) and epithelial (ChP) CNS barrier cells regulate transcription of Nos2 (coding for iNOS) and Arg1 (coding for arginase-1) in interacting

MoCs. More specifically, while IFN-□ stimulated BBB cells induced Nos2, IL-1β driven activation of BBB cells significantly upregulated Arg1 in MoCs. Supporting this latter finding, the distribution of IL1R1 in CNS vessels correlated to a different phenotype of invading macrophages during neuroinflammation.
Our data indicate differential distribution of pro- and anti-inflammatory MoCs at CNS borders and highlight how the interaction of MoCs with CNS barriers can significantly affect the functional activation of these CNS-invading MoCs during autoimmune inflammation.

SPEAKER #4:

Full Name (affiliation):
Emanuela Pasciuto (KU Leuven- VIB Center for Brain and Disease Research, University of Leuven (Belgium.)

Tentative title:
Role of CD4 T cells in brain development

Recent publications related to the topic of the symposium (3)
1. Pasciuto E*, Burton OT*, Roca CP*, Lagou V, Rajan WD, Theys T, Mancuso R, Tito RY, Kouser L, Callaerts-Vegh Z, de la Fuente AG, Prezzemolo T, Mascali LG, Brajic A, Whyte CE, Yshii L, MartinezMuriana A, Naughton M, Young A, Moudra A, Lemaitre P, Poovathingal S, Raes J, De Strooper B, Fitzgerald DC, Dooley J, Liston A. Microglia Require CD4 T Cells to Complete the Fetal-to-Adult Transition. Cell. 2020 Aug 6;182(3):625-640.e24. *Equal contribution
2. Briz, V*., Restivo, L.*, Pasciuto, E.*, Juczewski, K., Mercaldo, V., Lo, A. C,… Bagni, C. The non-coding RNA BC1 regulates experience-dependent structural plasticity and learning. Nature communications 2017, 8, 16. *Equal contribution.
3. Pasciuto E, Almheid T, Wahle T, Gardoni F, Tassone F, Balschun D, Dotti CG, Muller U, Di Luca U, De Strooper B and Bagni C. Dysregulated ADAM10-Mediated Processing of APP during a Critical Time Window Leads to Synaptic Deficits in Fragile X Syndrome. Neuron 2015, 87:1-17

Relevance for the symposium
The brain is a site of relative immune privilege. While CD4 T cells have been reported in the central nervous system, their presence in the healthy brain remains controversial, and their function largely unknown. Using a combination of imaging, single cell and surgical approaches we identified a CD69+ CD4 T cell population in both the mouse and human brain, distinct from circulating CD4 T cells. The brain-resident population is derived through in situ differentiation from activated circulatory cells, and shaped by self-antigen and the peripheral microbiome. Single cell sequencing revealed that in the absence of murine CD4 T cells, resident microglia remained suspended between the fetal and adult states. This maturation defect resulted in excess immature neuronal synapses and behavioral abnormalities. Our findings illuminate a role for CD4 T cells in brain development, and a potential interconnected dynamic between the evolution of the immunological and neurological systems.

- Abstract:

ROLE OF CD4 T CELLS IN BRAIN DEVELOPMENT

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The brain is a site of relative immune privilege. While CD4 T cells have been reported in the central nervous system, their presence in the healthy brain remains controversial, and their function largely unknown. Using a combination of imaging, single cell and surgical approaches we identified a $CD69^{+}$ CD4 T cell population in both the mouse and human brain, distinct from circulating CD4 T cells. The brain-resident population is derived through *in situ* differentiation from activated circulatory cells, and shaped by self-antigen and the peripheral microbiome. Single cell sequencing revealed that in the absence of murine CD4 T cells, resident microglia remained suspended between the fetal and adult states. This maturation defect resulted in excess immature neuronal synapses and behavioral abnormalities. Our findings illuminate a role for CD4 T cells in brain development, and a potential interconnected dynamic between the evolution of the immunological and neurological systems.

SYMPOSIUM S10
RETHINKING THE EPIGENETIC LANDSCAPE IN INTELLECTUAL DISABILITY

TITLE OF THE SYMPOSIUM: Rethinking the epigenetic landscape in intellectual disability

Organizers: Angel Barco and Mara Dierssen

Intellectual disability (ID) disorders (IDDs) are characterized by impaired cognitive abilities, and severe deficits in the capability to adapt to the environment and social milieu. The development and function of the brain require tight control of gene expression. Many chromatin-modifying enzymes and other epigenetic regulators have been genetically associated with ID disorders. This symposium will explore how alterations in the function of histone modifiers, chromatin remodelers, and methyl-DNA binding proteins contribute to neurodevelopmental defects and altered brain plasticity. We will also discuss how progress in 3D genome architecture is opening new perspectives to understand the mechanisms governing genome architecture in the brain and the contribution of altered chromatin architecture to IDDs.

SPEAKER #1

- Full Name: **Ana Pombo**

- Enter three recent publications of the Speaker related to the topic of the symposium:

1. Sparks TM, Harabula I, Pombo A. (2020) Evolving methodologies and concepts in 4D nucleome research. Curr Opin Cell Biol. 64:105-111.
2. Beagrie RA, … Pombo A. (2017) Complex multi-enhancer contacts captured by genome architecture mapping. Nature. 543(7646):519-524.
3. Ferrai C, … Pombo A. (2017) RNA polymerase II primes Polycomb-repressed developmental genes throughout terminal neuronal differentiation. Mol Syst Biol. 13(10):946.

- Relevance for the symposium:

Ana Pombo investigates how the 3D folding of chromosomes influences gene expression in mammalian development and disease, and epigenetic mechanisms that prime genes for future activation. She has developed Genome Architecture Mapping (GAM), an exquisite technology to map the 3D structure of chromosomes genome-wide which has unique advantages. Her lab has shown that active genes and enhancers form the most specific chromatin contacts, including previously unappreciated complex three-way contacts between super-enhancers, which span the length of whole chromosomes. GAM is uniquely powerful to quantify the higher-order complexity of 3D genome and the study of rare cell types directly from tissue, including precious human biopsies. These developments open a huge field of potential applications to identify the genes affected by disease-associated genetic variants present in non-coding parts of the genome, through long-range chromatin contacts. She is applying this technique to Down syndrome, the most common form of intellectual disability

- Abstract:

SPECIALIZATION OF BRAIN CELL TYPES IS ENCODED BY SPECIFIC 3D GENOME STRUCTURES

Ana Pombo
Berlin Institute for Medical Systems Biology, Max Delbrück Centre for Molecular Medicine, Berlin, Germany
Humboldt University of Berlin, Berlin, Germany

During lineage commitment, cells sustain cascades of gene activation and repression to generate specific cell types that execute specialized functions. To investigate the variability of the 3D conformation of the genome in different cell types and their relation with cell-type specific patterns of gene expression, we applied Genome Architecture Mapping is specific brain cell types from the adult murine brain, without disturbing their native tissue environment: dopaminergic neurons (DNs) from the midbrain, pyramidal glutamatergic neurons (PGNs) from the hippocampus, and oligodendrocyte lineage cells (OLGs) from the cortex. We discover extensive reorganization of genome topology, which the reorganization of topological domains, chromatin compartments and specific long-range contacts. We also discover that long neuronal genes undergo extensive decondensation, or 'melting', when highly transcribed, many of which are associated with neurodevelopmental disorders or neurodegeneration. Finally, by exploring neuronal-specific chromatin contacts, we identify hubs of synaptic genes in PGNs and of addiction pathway genes in DNs. Our work shows that the 3D organization of the genome is highly specific of cell type and strongly related with gene expression programs.

SPEAKER #2

- Full Name: **Andre Fischer**

- Enter three recent publications of the Speaker related to the topic of the symposium:

1. Jain G, … Fischer A (2019). A combined miRNA-piRNA signature to detect Alzheimer's disease. Transl Psychiatry 9(1):250.

2. Benito E, … Fischer A (2018). RNA-Dependent Intergenerational Inheritance of Enhanced Synaptic Plasticity after Environmental Enrichment. Cell Rep. 23(2):546-554.

3. Kerimoglu C, … Fischer A (2017). KMT2A and KMT2B Mediate Memory Function by Affecting Distinct Genomic Regions. Cell Rep. 20(3):538-548.

- Relevance for the symposium:
The long-term goal of Andre Fisher research is to understand the cellular and molecular mechanisms underlying brain diseases, including both congenital intellectual disability disorders and neurodegenerative conditions such as Alzheimer's disease, and to develop neuroprotective and neuroregenerative therapeutic approaches. His work has provided evidence that health or disease critically depends on the interaction between genes and environment. Epigenetic mechanisms such as histone-modification, DNA-methylation and non-coding RNA-mediated processes are key-regulators

of gene-environment interactions and have recently been implicated with the pathogenesis of neurodegenerative and neurodevelopmental disorders diseases.

- Abstract:

EPIGENETIC AND EPITRANSCRIPTOMIC PROCESSES IN COGNITIVE DISEASES

Andre Fischer[1, 2, 3]
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[2]*Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC), University of Göttingen, Germany*
[3]*Department of Psychiatry and Psychotherapy, University Medical Center Göttingen, Von Siebold Str 5, 37075 Göttingen, Germany*

Epigenetic processes play a key role in the pathogenesis of neurodevelopmental and adult onset cognitive diseases. Of particular interest is the methylation of Histone 3 at lysine 4 (H3K4me) that is mediated by six different lysine methyltransferases. Mutations in all of these enzymes are found in neurodevelopmental intellectual disability disorders and de-regulated H3K4me is also observed in Alzheimer's disease. I will discuss recent work in which we compare the role of H3K4 methyltransferases in neuronal gene-expression and cognition. Specifically, we will show novel data on SET domain containing 1b (SETD1B) that has been linked to syndromic intellectual disability but its role in the postnatal brain has not been studied yet. In addition to the role of histone-methylation I will discuss the methylation of RNA, an epitranscriptomic process that is emerging as an important player in cognitive disease. I will present novel data in which we compare the RNA methylation in the healthy and diseased human brain as well as in corresponding animal models and summarize our findings on the functional consequences related to synaptic plasticity.

SPEAKER #3

- Full Name: **Angel Barco**

- Enter three recent publications of the Speaker related to the topic of the symposium:

1. Lipinski M, … Barco A (2020). KAT3-dependent acetylation maintains neuronal identity in the adult mouse brain. Nat Comm. 11(1):2588.

2. Fernandez-Albert J, … Barco A (2019). Immediate and deferred epigenomic signatures of in vivo neuronal activation in mouse hippocampus. Nat Neurosci. 22, 1718-30.

3. Scandaglia M, … Barco A (2017). Loss of Kdm5c causes spurious transcription and prevents the fine-tuning of activity-regulated enhancers in neurons. Cell Reports 21(1):47-59.

- Relevance for the symposium:
Angel Barco investigates the role of activity-driven gene expression and chromatin modifications in neuronal plasticity, learning and memory, and intellectual disability disorders. Over the last decade, his group made important contributions to the understanding of the relationship between epigenetic

marks in the chromatin, gene expression and neuronal plasticity both in physiological conditions and in the context of intellectual disability and neurodegenerative disorders. Particularly relevant in the context of this symposium are their investigation on the etiopathology of rare neurodevelopmental disorders such as Rubinstein-Taybi syndrome and Claes-Jensen X-linked intellectual disability which are both caused by mutations in genes encoding important epigenetic regulators of neuronal gene expression.

- Abstract:

ID-LINKED KDMS PREVENT SPURIOUS TRANSCRIPTION IN NEURONS

Angel Barco, Ana Martin-Gonzalez, Sergio Niñerola and Beatriz del Blanco
Instituto de Neurociencias (UMH-CSIC), San Juan de Alicante, Alicante, Spain. abarco@umh.es

Lysine demethylases (KDMs) play a pivotal role in the establishment of cell fate and their loss or reduction is frequently linked to intellectual disability (ID). Our recent research underscores the relevance of ID-linked H3K4 demethylases in cell-type specific gene silencing, suggesting that the disruption of the neuronal transcriptome and chromatin organization that result from the loss of these epigenetic regulators critically contributes to ID etiopathology.

SPEAKER #4

- Full Name: **Mara Dierssen**

- Enter three recent publications of the Speaker related to the topic of the symposium:

1. De Toma I, … Dierssen M. (2020) Re-establishment of the epigenetic state and rescue of kinome deregulation in Ts65Dn mice upon treatment with green tea extract and environmental enrichment.
1. Sci Rep. 10(1):16023.
2. Martínez Cué C, Dierssen M (2020). Plasticity as a therapeutic target for improving cognition and behavior in Down syndrome. Prog Brain Res. 251:269-302.
3. De Toma I, Manubens-Gil L, Ossowski S, Dierssen M. (2016) Where Environment Meets Cognition: A Focus on Two Developmental Intellectual Disability Disorders. Neural Plast.2016:4235898.

- Relevance for the symposium:
Mara Dierssen investigates the disturbance of learning and memory processes in intellectual disability. Pioneering studies of her lab have demonstrated activity-dependent plasticity deficits and lack of structural plasticity in a Down syndrome mouse model, the Ts65Dn mice, that are regulated by histone acetylation. She has identified HSA21 genes, such as the dual-specificity tyrosine (Y) phosphorylation regulated kinase 1A (DYRK1A) that are involved in epigenetic regulation. DYRK1A can promote both histone deacetylation by phosphorylating SIRT1 and histone acetylation and also interferes with chromatin remodeling. Unpublished data from Dierssen lab have shown histone hypoacetylation in

the hippocampus of Ts65Dn mice and restoring acetylation levels using the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA), recovers recognition memory in Ts65Dn mice.

- Abstract:

EMERGING ROLES FOR LONG NONCODING RNAS IN DOWN SYNDROME HIPPOCAMPUS

Sierra C, Dierssen M
Center for Genomic Regulation, The Barcelona Institute for Science and Technology, 08003 Barcelona, Spain

Down syndrome (DS) is the most common genetic cause of intellectual disability. Even though great advances in the last decades have allowed better delineation of its pathogenetic mechanisms, its cellular and molecular bases are still poorly understood. Particularly, the contribution of epigenetic mechanisms to the disordered gene expression in DS remains largely unexplored, mainly due to their high cell-type specificity, which limits their study in bulk analyses. This is the case of long noncoding RNAs (lncRNAs), which show a refined cellular and region specificity. Despite their high abundance in the brain and that specific lncRNAs have been associated to specific brain functions such as learning and memory, the role of the vast majority of them in health and memory related disorders has yet to be described. Here, the single-cell transcriptome of the hippocampus of a DS mouse model, the Ts65Dn, has allowed us to identify specific lncRNAs deregulated in DS. We will discuss their involvement in learning and memory and their contribution to DS-specific neuropathology.

This research was funded by the Agencia Estatal de Investigación (PID2019-110755RB-I00/AEI/10.13039/501100011033), the European Union’s Horizon 2020 re-search and innovation programme under grant agreement No 848077, Jerôme Lejeune Foundation (Grant number 2002), NIH (Grant Number: 1R01EB 028159-01), Marató TV3 (#2016/20-30), and JPND (Heroes). C. S. received the FI grant from Agència de Gestió d’Ajuts Universitaris i de Recerca (AGAUR) de la Generalitat de Catalunya

Posters

Topics

Posters

Topic 1

Developmental Neurobiology

Posters

A RIBO-TAG BASED SCREEN IDENTIFIES A COHORT OF PROTEINS LOCALLY TRANSLATED AT THE AXONS DURING AXONAL NAVIGATION

Dr. Veronica Murcia-Belmonte[1], M Teresa López-Cascales[1], Dr Angel Barco[1], Dr Eloisa Herrera[1]
[1]*Instituto de Neurociencias UMH-CSIC, Alicante, Spain*

Local translation in the growth cone has been proposed as a mechanism for rapidly producing proteins "on demand" during axonal navigation in the developing embryo. The binary decision of crossing or not the midline that visual axons take at the optic chiasm attending to attractive or repulsive signals, is an ideal model to investigate the molecular mechanisms underlying axon pathfinding. By using a cre-dependent Ribo-Tag mouse line (translated ribosome affinity purification) crossed with two cre-lines specific for retinal neurons that cross or not the midline, we have isolated mRNAs locally translated in contralateral or ipsilateral axons respectively. By high resolution FRAP (fluorescence recovery after photobleaching) visualization of Venus-3UTR constructs in axons navigating the optic chiasm, we confirmed axonal translation of the mRNAs identified in our screen. Interestingly, the axonal translatome obtained from these two populations of retinal neurons includes a subset of genes enriched in common elements of cytoplasmic polyadenylation that are known to activate specific mRNAs for local protein translation. These findings confirm for the first time the existence of local protein synthesis in the cone of growing axons in vivo. In addition, they reveal the translatome of contralateral and ipsilateral axons and provide a deeper understanding of the molecular machinery involved in axon guidance decisions.

ADAMTS2 AND POLY (I:C): GENETIC AND ENVIROMENTAL MOUSE MODELS OF SCHIZOPHRENIA DISORDER

Miss Celia Martín-Cuevas[1,2], Mr Víctor Darío Ramos-Herrero[2], Dr. Susana García-Cerro[1,2], Dr. Manuel Canal-Rivero[1,2,3], Miss Nathalia Garrido-Torres[1,2,3], Mr. Idalino Rocha-González[1,2,3], Dr. Miguel Ruíz-Veguilla[1,2,3,4], Dr. Benedicto Crespo-Facorro[1,2,3,4], Dr. Ana Carmen Sánchez-Hidalgo[1,2]
[1]Spanish Network for Research in Mental Health (CIBERSAM), Madrid, Spain, [2]Seville Biomedical Research Centre (IBiS), Seville, Spain, [3]University Hospital Virgen del Rocío, Seville, Spain, [4]University of Seville, Seville, Spain

Schizophrenia (SCZ) is a neurodevelopmental psychiatric disorder that affects 1% of the world's population. Theories suggest that SCZ is caused by genetic and environmental factors, with strong epidemiological evidence showing an association between maternal infection during pregnancy and development of SCZ in offspring in adult life. In recent years, our group has described a set of six overexpressed genes associated with the appearance of the disease (ADAMTS2, CD177, CNTNAP3, ENTPD2, RFX2 and UNC45B). ADAMTS2 was the most overexpressed gene in independent samples of patients and was moderated by the antipsychotic action in responsive patients. We propose two animal models to reproduce the cerebral and behavioral symptoms of SCZ disorder. We have generated a transgenic mouse model that selectively overexpresses ADAMTS2 in brain regions under CaMKIIα promoter. We have also established a maternal immune activation (MIA) mouse model with Polyinosinic-polycytidilic acid (Poly (I:C)), a synthetic analog of double-stranded RNA that triggers a citokine-associated immune response. Pregnant dams are exposed to viral agent during gestation. In the last described model, we found weight differences and an altered body temperature in injected pregnant females with Poly (I:C). Positive, negative and cognitive symptoms are evaluated in Poly (I:C) model through behavioral tests. Results show defects in social and sensorimotor gating tasks without affected locomotion. All experiments are performed both in female and male mice, since SCZ affects patients of both sexes differently. On the other hand, different brain parameters have also been evaluated, such as cortical thickness and its layers (I-VI) and dendritic spines density. Our results suggest differences in cortical thickness and decreased dendritic spines density in adult offspring. Finally, both models separately and its combination will permit us evaluate genetic and environmental theories and will help us advance in the knowledge and treatment of SCZ disorder.

AMYLOID PRECURSOR PROTEIN (APP) REGULATES CELL FATE SPECIFICATION IN HUMAN NEURAL STEM CELLS

Ms. Raquel Coronel[1,2], Ms Andreea Rosca[1], Ms Rosa González[1,3], Ms Patricia Mateos[1], Dra. Victoria López[3], Dr. Eduardo Arilla-Ferreiro[2], Dra. Isabel Liste[1]

[1]Unidad de Regeneración Neural, Unidad Funcional de Investigación de Enfermedades Crónicas (UFIEC), Instituto de Salud Carlos III (ISCIII), Majadahonda, Madrid, Spain, [2]Unidad de Neurobioquímica, Departamento Biología de Sistemas, Facultad de Medicina, Universidad de Alcalá, Alcalá de Henares, Madrid, Spain, [3]Unidad de Biología Computacional, Unidad Funcional de Investigación de Enfermedades Crónicas (UFIEC), Instituto de Salud Carlos III (ISCIII), Majadahonda, Madrid, Spain

The pathological implication of Amyloid Precursor Protein (APP) in Alzheimer's disease has been widely documented due to its involvement in the generation of amyloid-β (Aβ) peptide. However, the physiological functions of APP are still poorly understood.

APP is a type I transmembrane glycoprotein widely expressed in the central nervous system (CNS) and is encoded by a single gene located on chromosome 21. Due to its role in a wide variety of processes, APP is considered by various authors as a multimodal protein. Specifically, APP seems to be implicated in neural development of CNS, playing a key role in the proliferation, differentiation, cell fate specification and maturation of neural stem cells (NSCs).

We have examined the endogenous APP expression in hNS1 cells, a model cell line of human NSCs, both under proliferation and throughout the differentiation period. Our results show elevated APP-immunoreactivity in hNS1 cells and, to investigate the potential function that APP plays in biology (proliferation, differentiation, cell fate specification and cell death) of human NSCs, we performed a loss-of-function study. To achieve the down-expression of APP, we used a commercial siRNA against human APP and we transfected it into hNS1 cells. Our data indicate that low levels of APP induce hNS1 cell fate towards a neuronal phenotype, while decreasing glial differentiation. Moreover, according our results, these effects could be, in part, mediated by β-catenin protein.

The knowledge of physiological functions of APP, as well as the possible signaling pathways that could be implicated, are essential to advance the understanding of the pathogenesis of AD.

ANALYSIS OF THE ACTIVATION DYNAMICS OF POSTNATAL NEURAL STEM CELLS OF THE SUBVENTRICULAR ZONE USING IN UTERO ELECTROPORATION

Ms. Isabel Mateos-White[1], Mr. Jaime Fabra-Beser[1], Mr. David de Agustín-Durán[1], Dra. Isabel Fariñas[1], Dra. Cristina Gil-Sanz[1]
[1]*BIOTECMED Institute, Universidad de Valencia, Burjassot, España*

The subventricular zone (SVZ) of the lateral ventricles is one of the neurogenic niches of the adult mammalian brain. Neural stem cells (NSCs) populating this niche are pre-specified since early embryonic stages, but they remain quiescent until its postnatal reactivation. At this time, they give rise to different types of interneurons that migrate through the rostral migratory stream (RMS) to populate the olfactory bulb. Although many studies have been carried out in the last years characterizing the particular cellular behaviour of these NSCs, important questions are still open including their activation dynamics throughout the postnatal period. Building on the embryonic specification of NSCs, we can target them using in utero electroporation. This method allows in vivo DNA transferring in order to label, analyze fine cell morphology or conduct functional experiments to perturb the expression of genes of interest, among other approaches. In the present work, we analyze the activation dynamics of NSCs in the SVZ since early ages of postnatal development until adulthood implementing lineage-tracing studies by in utero electroporation in Cre-reporter mice.

APC/C-CDH1 INHIBITION PROMOTES HYPOMYELINATION DURING POSTNATAL DEVELOPMENT

Ms. Silvia Gomila[1,2], Dr. Verónica Bobo-Jiménez[1,2], Dr. Rebeca Lapresa[1,2], Dr. Jesús Agulla[1,2], Dr. Angeles Almeida[1,2]

[1]Instituto de Investigación Biomédica de Salamanca (IBSAL), Hospital Universitario de Salamanca, Salamanca, Spain, [2]Instituto de Biología Funcional y Genómica (IBFG), Universidad de Salamanca, CSIC, Salamanca, Spain

The E3 ubiquitin ligase APC/C-Cdh1 plays a key role in the developing brain, where it regulates the onset of neurogenesis during gestation (Delgado-Esteban et al, 2013). Whereas neurogenesis is complete at birth, synaptogenesis and myelination occurs during the postnatal brain growth. Recently, we described a novel human missense mutation in Cdh1 protein, which results in microcephaly, psychomotor retardation and refractory epilepsy (Rodríguez et al, 2019), alterations directly related to synapse dysfunction and hypomyelination (Repudi et al, 2021). Moreover, the APC/C-Cdh1 target, Rock2 (Bobo-Jiménez et al, 2017), negatively regulates myelination (Muñoz-Esquivel et al, 2019). Here, we study the role of APC/C-Cdh1 activity on oligodendroglial lineage and myelination in the postnatal brain.

We used Nestin-Cre Cdh1 conditional KO model by mating mice harboring a floxed allele of the Cdh1 gene with Nestin-Cre animals, which express Cre recombinase in neural cells from embryonic day 11. Oligodendrocyte, myelination and myelin sheath were analyzed by electron microscopy, immunohistochemistry, and western blotting.

Cdh1 deletion results in brain morphology alterations, mainly including microcephaly, severe hydrocephalus and ventriculomegaly, which becomes more pronounced during postnatal growth. Magnetic resonance T2 value at P21 (21 postnatal days) showed less cellular density and corpus callosum dysgenesis. In fact, Cdh1 cKO mice presented lower oligodendrocyte lineage cells in cerebral cortex, evidencing disorders in myelination process. Immunohistochemical Myelin Basic Protein (MBP) staining confirms corpus callosum agenesis and hypomyelination in P21 cortex. Moreover, P7 Cdh1 cKO mice presented less myelinated pons and midbrain areas, than wild-type mice, suggesting myelination delay. Finally, myelin ultrastructure analysis at P21 revealed decompacted and disrupted myelin sheath, compromising axon integrity.

Then, APC/C-Cdh1 activity regulates postnatal myelination, which highlights the impact of Cdh1 in the pathogenesis of neurodevelopmental myelin disorders.

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APOE GENOTYPE AND POSTNATAL CHLORPYRIFOS EXPOSURE AFFECT MICE CEREBRAL LIPID PROFILE

Dra. Laia Guardia-Escote[1], **Ms. Judit Biosca-Brull[1,2]**, Ms. Mikaela Mladenova-Koleva[1], Dra. Pia Basaure[1], Dr. Jordi Blanco[1,3], Dra. Maria Cabré[1,4]

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The brain is an organ rich in lipids, which requires different lipid molecules for its proper development and maturation. Lipids are involved in both structural and functional roles, including signaling in the brain. Apolipoprotein E (APOE) is important for the distribution of lipids and its different isoforms may lead to differences in lipid content in brain. In addition, the different isoforms of APOE confer different vulnerabilities to the toxic effects of the pesticide chlorpyrifos (CPF) that impact the brain, during development. The aim of this study was to assess the differences in the cerebral lipidic content depending on the APOE genotype and early exposure to CPF. We used C57BL/6, apoE3- and apoE4-TR male mice, which were orally exposed to 1 mg/kg/day of CPF from postnatal day (PND) 10 to 15, whereas the control group was exposed to the vehicle (corn oil). Four hours after treatment, at PND 15, mice were sacrificed and the whole brain dissected to study the cerebral lipidome by means of LC-MS. Our results showed that lactating mice presented differences in the lipid profile of the brain depending on APOE genotype. General screening showed differences between genotypes in some cholesteryl esters, diglycerides, lysophosphocholines, phosphatidylcholines, sphingomyelin and triglycerides. General treatment effects and some genotype x treatment interactions were also observed. Differences in lipid profile present during the developmental period could explain some functional and maturation variances between genotypes, which are present at very early ages. Overall, this study provides more information on the relationship between the APOE genotype and the brain lipid composition.

CALCIUM CHANNELS IN SYNAPSE ELIMINATION DURING NEUROMUSCULAR JUNCTION DEVELOPMENT

Ms. Marta Balanyà Segura[1], Dr. Neus Garcia[1], Mr. Pablo Hernández[1], Dr. Maria A. Lanuza[1], Dr. Marta Tomàs[1], Mr. Víctor Cilleros-Mañé[1], Ms. Laia Just-Borràs[1], Ms. Maria Duran-Vigara[1], Ms. Aleksandra Polishchuk[1], Dr. Josep Tomàs[1]

[1]*Universitat Rovira I Virgili, Reus, Spain*

During the development of the nervous system, synaptogenesis occurs in excess though only the appropriate connections consolidate. At the neuromuscular junction, competition between several motor nerve terminals results in the maturation of a single axon and the elimination of the others. The activity-dependent release of transmitter, and neurotrophic factors allow the direct mutual influence between motor axon terminals through receptors such muscarinic ACh autoreceptors (mAChRs) and the tropomyosin-related kinase B (TrkB). Thus, a multiple metabotropic receptor-driven downstream balance between PKA and PKC isoforms modulate the phosphorylation of targets involved in transmitter release and nerve terminal stability. A similar level of PKA inhibition and PKC potentiation would be required during development to promote synapse loss. We observed in the weakest endings on the polyinnervated NMJ that M1 subtype mAChR receptors reduce ACh release through the PKC pathway coupled to an excess of Ca2+ inflow through P/Q-, N- and L-type voltatge-gated calcium channels (VGCC).

Here, we investigate the involvement of the VGCC in synapse elimination during development. Selective VGCC blockers and activators were applied daily on the Levator auris longus (LAL) muscle surface from P5-P8 transgenic B6.Cg-Tg (Thy1-YFP)16 Jrs/J mice and the axon number and postsynaptic receptor cluster morphologic maturation were evaluated in P9 NMJ. We found that both L- and P/Q-type VGCC (but not the N-type) are equally involved in postnatal axonal competition and synapse elimination. The block of these VGCC or [Ca2+]i sequestration results in the same delay of axonal loss that the cPKCβI isoform block or PKA activation. However, nPKCε block results in a significant greater delay suggesting the involvement in this case of a calcium-independent mechanism. The involvement of the VGCC in the postsynaptic maturation seems more complex and some contribution of the N-type VGCC canot be discarded and merites further investigation.

Funding: PID2019-106332GB-I00, 2017PFR-URV-B2-85,2017SGR704, PRE2020-092084, 2021-FI-B00755, LE1511314-2014PEJ-04, LE1911587-2019PEJ-04.

CB1 RECEPTORS DEFICIENCY IN OLIGODENDROCYTE PRECURSORS DISRUPTS POSTNATAL OLIGODENDROGENESIS AND CAUSES HYPOMYELINATION IN MICE

Aníbal Sánchez-de la Torre[1,2,3], Tania Aguado[1,2,3], Alba Huerga-Gómez[1,2,3], Juan Carlos Chara[4,5], Krisztina Monory[6], Carlos Matute[4,5], Beat Lutz[6], Susana Mato[4,5], Manuel Guzman[1,2,3], Ismael Galve-Roperh[1,2,3], Javier Palazuelos[1,2,3]

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Exogenous and endogenous cannabinoid molecules have been shown to modulate oligodendrogenesis and developmental CNS myelination. However, the cell-autonomous action of these compounds on oligodendrocyte precursor cells (OPC) in vivo has never been explored. Here, by using OPC-specific genetic mouse models we show that selective CB1 cannabinoid receptor depletion in OPC prevented cell differentiation and perturbed oligodendrogenesis and postnatal myelination. Moreover, early postnatal CB1 depletion in OPC caused hypomyelination and motor alterations at adult ages in mice. Conversely, CB1 receptor pharmacological activation promotes oligodendrocyte development and CNS myelination in wild type but not in OPC-CB1-null mice. Overall, this study addresses a cell-autonomous role for CB1 receptors in OPC modulating oligodendrogenesis that may help in understanding the complex network of signaling molecules that drives CNS myelination.

CEREBELLAR ABNORMALITIES IN A CONDITIONAL MOUSE MUTANT OF THE LIS1 GENE

Dr. Abraham Andreu-Cervera[1], Ms. Ana María Jiménez[1], Ms. Mar Azorín[1], Ms. Francisca Almagro-García[1], Dr. Eduardo Puelles[1], Dr. Diego Echevarría[1], Prof. Emilio Geijo-Barrientos[1], Prof. Salvador Martínez[1]

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The cerebellum is related mainly to the coordination and regulation of locomotor activities such as control of fine movements, equilibrium, motor learning, and ocular movements. The formation of cerebellar neuronal circuits and the development of the physiological functions of the cerebellar cortex require a precise cortical sequence of neural differentiation and migration steps.

The Lis1 gene regulates the dynein dependent axonal transport, mediates neuronal migration in the developing brain, and supports synaptic integrity in the mature brain. In human, defects in LIS1 expression produce lissencephaly type I and is probably related to other neurological disorders such as schizophrenia. Lis1 is expressed throughout the entire lifespan of the central nervous system, but little is known about its potential role in the development and mature structure of the cerebellum, and particularly about the consequences of Lis1 dysfunction in specific neuronal types.

Parvalbumin(PV) is highly expressed in cerebellar Purkinje cell layer(PCL) during the formation and maturation of this cerebellar sheet. We have studied the cerebellar alterations caused by the selective inactivation of Lis1 in PV expressing neurons, creating a conditional Lis1 knockout mouse in PV+ neurons (Lis1cKO-PV+).

During prepuberal stages(P15-P21), Lis1cKO-PV+ mice presented a strong ataxic locomotor phenotype, reflecting possible dysfunctions in the cerebellum. At the cellular level, PCL displayed a clear impaired organization. Anatomically, Purkinje cells presented an abnormal soma with a rudimentary and atrophic dendritic arborization. Moreover, V-Glut1, a glutamatergic neuronal terminals marker, showed an abnormal organization of the excitatory basket nets generated by these terminals around the Purkinje soma. Altogether, these data suggest that Lis1 expression in Purkinje cells is not only required for their proper development and therefore the PCL organization but also for the normal synaptogenetic mechanisms leading to the organisation of the glutamatergic presynaptic terminals. These results also indicate the presence of non-cell autonomous effects of Lis1 dysfunction.

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CEREBRAL CORTEX DEVELOPMENT IS COORDINATED BY MITOCHONDRIAL REACTIVE OXYGEN SPECIES

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The nervous system is particularly sensitive to reactive oxygen species (ROS) (1). Under physiological conditions, ROS regulate cell proliferation, neuronal differentiation, and synapse maintenance, indicating a key role of ROS in neuronal function and homeostasis. Moreover, mitochondrial ROS (mROS) generated by astrocytes regulate brain metabolism and behavior. Particularly, the reduction of astrocytic mROS in the adult alters neuronal structure and integrity leading to cognitive decline (2). However, the impact of mROS generation in the developing brain is unknown.

Here, we used mice genetically engineered to constitutively express a mitochondrial tagged enzyme catalase (mCAT) to downmodulate endogenous mitochondrial ROS generation (2).

We found higher levels of neuronal markers, TAU and MAP2, and increased neurite outgrowth in primary cortical neurons from mCAT, in comparison with wild-type (WT) neurons, at 3 days in culture. Then, mROS downregulation accelerated neuronal differentiation in vitro. Next, we evaluated whether the decreased mROS in the brain altered neurogenesis in vivo. Downregulation of mROS altered neurogenesis and layer organization in cerebral cortex from E15 mice. The proportion of cells expressing the progenitor cell marker NESTIN was lower, whereas that expressing neuronal markers TUJ1 and MAP2, was higher in the E15 mCAT cortices, compared to WT. Moreover, number of proliferating cells (BrdU-positive cells) in the ventricular/subventricular zones was lower, whereas immature neurons (TUJ-1 positive cells) were enriched in the interzone layer of E15 mCAT cortices, in comparison to WT. This was accompanied by an altered cortical radial distribution of MAP2 positive neurons in the cortex of E15 mCAT.

Our results suggest a key role of endogenous mROS levels in cell proliferation and neurogenesis onset, which would coordinate layer patterning in the cerebral cortex during brain development.

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CHARACTERIZATION OF DIFFERENT TYPES OF PROGENITOR CELLS IN THE POSTNATAL RETINA OF SHARKS

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Neurogenesis is the process by which progenitor cells persistently generate new neurons from neural progenitor cells (NPCs). Postnatal NPCs reside within well-defined neurogenic niches in the brain, which in mammals contain relatively quiescent radial glia-like progenitor cells (RGCs), transient intermediate progenitor cells (IPCs), and neuroblasts that subsequently differentiate into neurons. The study of the evolution of postnatal NPCs has gained significance, since they amplify the number of generated neurons and allow for the diversification of neuronal cell types. However, much of our understanding in this field is based in studies on mammals and zebrafish, a modern bony fish. The use of the cartilaginous fish Scyliorhinus canicula as a model expands the evolutionary scenario to a representative species of basal gnathostomes that shows significant neurogenic activity in the postnatal nervous system. In particular, the ciliary marginal zone (CMZ) of the retina constitutes an interesting neurogenic niche to identify different types of progenitors, since cells at different stages of commitment are spatially ordered from the most peripheral CMZ (where NPCs lie) to the central retina (comprised of differentiated cells). In this work, we have characterized different types of progenitors in the CMZ of juveniles by analysing the expression pattern of proliferation markers (proliferating cell nuclear antigen and phosphohistone-H3), a stem cell marker (ScSox2), glial markers usually found in RGCs (brain lipid-binding protein and the glial fibrillary acidic protein), the typical marker of IPCs ScTbr2 and markers of neuroblasts (doublecortin). By analysing their combined expression in the peripheral retina of juveniles, we identified the different types of NPCs (RGCs, IPCs and neuroblasts) previously described in mammalian brain neurogenic niches, which contributes to foster our knowledge about the evolution of postnatal neurogenesis in vertebrates.

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CHARACTERIZATION OF THE PARALAMINAR NUCLEUS IN THE MICE: AN AMYGDALAR REGION WITH PROTRACTED MATURATION

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The paralaminar nucleus (PL) is a region located in the ventral amygdala which has been little studied and whose cells in humans and non-human primates show a late maturation profile. Despite its heterogeneous composition, a large population of cells in the PL present simple morphology, dense clustering, and expression of the microtubule-associated protein doublecortin (DCX) and PSA-NCAM, both markers of immature neurons. Since the PL has been described in several species, including the rat, our main hypothesis is the existence of a homologous region to the PL in the mice brain, a species with no evidence in the matter.

In this work, we studied the cellular dynamics of the PL during four stages of postnatal development in C57BL6 mice (P7, P14, P21 and P28). First, we delimited its anatomical localization by Cresyl violet staining in sagittal brain sections and we characterized the PL cell populations by immunohistochemistry against several molecular markers. The ultrastructural characteristics of the PL cells were studied by transmission electron microscopy and pre-embedding immuno-gold for DCX.

Our results indicate that, in mice, the PL is a discrete region of the amygdala and extends in a frontal-posterior direction as dense clusters of small cells around the basolateral amygdala. It is characterized by the presence of neurons in different maturational stages in juvenile individuals. Ultrastructurally most of DCX+ cells located in the PL presented immature morphology, with compacted heterochromatin and reduced cytoplasmatic volume. However, we found low expression of DCX in bigger neurons with a more developed cytoplasm, suggesting an active maturation process of these cells.

In conclusion, the presence of immature DCX+ cells in the PL of mice which mature at juvenile postnatal stages supports the idea that protracted maturation could provide neuronal plasticity at an important time in the development of the amygdala.

CHROMATIN SIGNATURES OF NEURONAL SUBPOPULATIONS WITH DIVERGENT PROJECTION AT THE MIDLINE IDENTIFY NOVEL WIRING REGULATORS

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Therapeutic approaches aimed to restore the function of damaged neuronal circuits will benefit from a better understanding of the mechanisms driving and constraining circuit assembly. The binary decision of crossing or avoiding the midline that retinal ganglion cells (RGCs) axons take at the optic chiasm during embryonic development is essential for binocular vision and represents a simple and robust model to identify novel mechanisms controlling axon guidance decisions during circuits formation. By comparing the transcriptome and chromatin occupancy profiles of crossed and uncrossed RGCs, we identified key differences between these two populations of neurons. Our unbiased screens revealed important differences in the expression of guidance molecules and the binding of transcription factors to regulatory regions, exposing novel transcriptional mechanisms underlying axon guidance decisions. Functional in vivo experiments with candidate genes validate this approach and reveal the implication of several transcription factors, such as the Lhx family, in the navigation of RGC axons. Overall, our study retrieved novel factors controlling axon guidance, thereby contributing to a better understanding of the transcriptional regulatory logic underlying neuronal connectivity.

CONSERVED CELL TYPES IN THE EARLY EMBRYONIC BRAIN ACROSS VERTEBRATES

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The "phylotypic" denomination of pharyngula embryonic stage arises from being considered as the most conserved developmental period across species from a given phylum. The evolutionary similarities in the phylotypic stage have long been observed morphologically and have also been confirmed transcriptionally in the last decade. At this intermediate point in development, segmentation and organogenesis processes stablish a phylum-wide common body-plan or bauplan. This simultaneous early formation of multiple organs is thought to be governed by a common set of phylotypic genes, which are evolutionarily conserved due to their pleiotropy. In the brain, most of these patterning genes are historically known to contribute to primordial, widely conserved neural structures development. Nonetheless, inter-species comparisons beyond individual master genes are still challenging considering the lack of in-depth brain transcriptomic data at cell type level. To overcome this shortage, we performed single cell transcriptomics analysis of the phylotypic brains from representative vertebrata species: mouse (Mus musculus), chicken (Gallus gallus), gecko (Paroedura picta) and zebrafish (Danio rerio). Added to our current neuroanatomical knowledge and in situ hybridization assays, this ground-breaking tool allowed us to map the cell types that comprise the phylotypic brains of these species and compare their gene expression levels. Therefore, these transcriptomic brain atlases from several species not only complement current evolutionary neural cytoarchitectonic knowledge, but also provide deeper insights of cell type homologies across vertebrates. In conclusion, our results confirm the existence of an ancestral phylotypic brain and sheds light on the reasons why its cell types have been barely modified for over 500 million years despite thereafter speciation events in vertebrata.

DEREGULATION OF THE EPITHELIAL-TO-MESENCHYMAL TRANSITION PROCESS UNDERLIES ZIC2-LINKED HOLOPROSENCEPHALY

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Holoprosencephaly (HPE) is a congenital brain malformation resulting from incomplete separation of the two hemispheres. Mutations in the Zic2 gene cause holoprosencephaly type 5, but the mechanisms that translate Zic2 mutations into this devastating pathology remain unclear. Here, we report that Zic2 is expressed in a few epiblast cells during gastrulation to become transiently upregulated in the primitive streak. Later, during neurulation, Zic2 is re-expressed in neural crest cells and downregulated as they delaminate from the neural tube. In combination with transcriptomic data from mutant embryos, chromatin occupancy profiles in gastrula and neural crest cells reveal that Zic2 regulates a large number of genes associated with the Wnt, cadherin and TGF-β pathways. In proliferating cells exposed to Wnt, Zic2 prevents the translocation of β-catenin to the nucleus, subsequently accumulating in the cytoplasm. This blocks activation of the canonical pathway inducing a non-canonical Wnt response necessary to initiate EMT. Our results elucidate the role of Zic2 in early development and provide an explanation for the wide variety of developmental alterations in HPE5 patients that, unlike other HPE patients, include many other mesoderm-derived defects.
Altogether, these analyses identify cell types, signaling cascades, and genomic regions implicated in the etiology of Zic2-linked neurodevelopmental disorders.

DEVELOPMENT OF OTP AND SIM1 CELLS IN THE CHICKEN EXTENDED AMYGDALA

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The amygdala is recognized as the master regulator of the stress response and plays a key role in social behaviour and cognition. Using cell-specific functional mapping, it has been shown that, in the mouse extended amygdala, different types of GABAergic neurons are involved in this regulation. These neuronal subtypes originate in different embryonic divisions of the subpallium. Using an evodevo approach, homologous cells have also been found in chicken. However, the extended amygdala of mouse and chicken also includes glutamatergic neurons. In mouse, some of these glutamatergic cells express the transcription factors Otp or Sim1. The Otp cells mostly populate the medial extended amygdala and were initially thought to originate in an Otp-expressing domain of the alar hypothalamus. However, our laboratory recently showed that most of these Otp glutamatergic cells also coexpress the telencephalic transcription factor Foxg1, and originate in a distinct telencephalon-opto-hypothalamic embryonic domain (TOH), at the border between telencephalon and hypothalamus. In addition, Sim1 cells originate in the alar hypothalamus and migrate to other parts of the amygdala. In this project, we investigated whether similar Otp and Sim1 cells are found in the chicken amygdala. To that aim, we performed double labeling of in situ hybridization for Otp or Sim1, and immunohistochemistry/immunofluorescence for Foxg1 in the chicken brain. Our results demonstrate: 1) The existence of a TOH domain in chicken, coexpressing Otp/Sim1 and Foxg1, that produces cells for the medial extended amygdala. 2) A subset of Sim1 expressing cells that seems to migrate from the alar hypothalamus to the capsular part of the central extended amygdala. These findings open the venue for further studying the connections and functions of these different neuronal subtypes and their relation to the GABAergic cells of the extended amygdala.

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DEVELOPMENTAL ORIGIN OF ADULT NEUROGENESIS: ANALYSIS OF THE POSTNATAL HIPOCAMPAL NEUROGENIC NICHE IN Sox5 CONDITIONAL MUTANTS

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During embryonic and postnatal development of the dentate gyrus (DG), neural stem cells (NSCs) proliferate, migrate and generate mature granule neurons. In sharp contrast to other brain regions, in the DG a subpopulation of NSCs, are set aside in the subgranular zone (SGZ) and continues generating new granular neurons throughout adult life. One of the characteristics that distinguish adult NSCs most clearly from their embryonic counterparts is the acquisition of quiescence, by which adult NSCs remain for long periods out of the cell cycle. While most of NSCs will remain in a dormant state of deep quiescence throughout life, the transitions back and forward from an active /proliferative state to a temporal shallow quiescence or resting state, ensure the lifelong maintenance of the hippocampal stem cell population. However, it is unclear when and how adult NSCs acquire dormant or resting quiescence during development.

We have recently determined that SoxD transcription factors are required for the transition from quiescence to activation in NSCs and for the generation of new neurons in the adult SGZ. Now, we have determined that abolishing Sox5 expression in DG during development (using a Nestin-cre line; Sox5Nestin mice) provokes a drastic decrease in NSCs proliferation during the first postnatal weeks and a decrease in neurogenesis. Surprisingly, by P30, Sox5Nestin mice show an enhancement of RGLs proliferation, a reduction in RGL quiescence and an increase in neurogenesis. Moreover, we have established that at P30 the transitions between a primed /superficial quiescence and active/proliferative state are severely altered in NSCs from Sox5Nestin mice. Finally, by P150, the pool of NSCs and that of new neurons are reduced, indicating that in the absence of Sox5 the life-long maintenance of the adult neurogenic niche is compromised. Thus, our studies reveal a critical time window around P14-P30 when dormant and resting quiescence is established and when Sox5 plays a crucial role.

DEVELOPMENTAL-BASED CLASSIFICATION OF NEURONS IN THE CHICKEN CENTRAL EXTENDED AMYGDALA

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The amygdala is extremely complex and heterogeneous, and contains different neuron subpopulations involved in functions essential for survival. It plays a key role in the stress response in mammals, but very little is known on its role in non-mammals. In order to improve welfare in laying hens, it is crucial to disentangle amygdalar structure and function in these animals. In mammals, two major populations of peptidergic neurons of the central extended amygdala (EAce), containing somatostatin or enkephalin, become active (on) or inactive (off) during stress response. These cell types originate in different embryonic domains and express different region-specific transcription factors during development. The evolutionary developmental biology approach has provided crucial information on how the amygdala develops in different vertebrates, which has become essential to identify homologous cell populations across species. Our goal was to identify neurons of the chicken EAce homologous to those of mammals using this approach. In particular, we investigated the embryonic origin of enkephalin and somatostatin neurons of the EAce in domestic chicks (Gallus gallus domesticus), by studying their co-expression with different region-specific developmental transcription factors. We performed double labelling experiments combining chromogenic or fluorescent in situ hybridization for enkephalin and somatostatin with immunohistochemistry or immunofluorescence for the transcription factors Pax6, Nkx2.1 and Islet1. We found that: (1) enkephalinergic cells of the capsular and peri-interpeduncular parts of the EAce express mainly Pax6, which is specific of cells derived from the dorsal striatal embryonic division; and (2) the somatostatinergic cells express mainly Nkx2.1, typical of cells derived from the pallidal embryonic division. These results support the hypothesis of homology of these neurons between mouse and chicken, and set the basis to study their function during the stress response. The results also contribute to extract general developmental-based principles on the neural architecture of the central extended amygdala of amniotes.

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DIFFERENTIAL EXPRESSION LEVELS OF SOX9 IN EARLY NEOCORTICAL RADIAL GLIAL CELLS REGULATE THE DECISION BETWEEN STEM CELL MAINTENANCE AND DIFFERENTIATION.

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Radial glial progenitor cells (RGCs) in the dorsal telencephalon directly or indirectly produce excitatory projection neurons and macroglia of the neocortex. Recent evidence shows that the pool of RGCs is more heterogeneous than originally thought and that progenitor subpopulations can generate particular neuronal cell types. Using single cell RNA sequencing, we have studied gene expression patterns of RGCs with different neurogenic behavior at early stages of cortical development. At this early age, some RGCs rapidly produce postmitotic neurons, whereas others self-renew and undergo neurogenic divisions at a later age. We have identified candidate genes that are differentially expressed between these early RGCs subpopulations, including the transcription factor Sox9. Using in utero electroporation, we demonstrate that elevated Sox9 expression in progenitors affects RGC cell-cycle duration and leads to the generation of upper-layer cortical neurons. Our data thus reveal molecular differences between progenitor cells with different neurogenic behavior at early stages of corticogenesis and indicates that Sox9 is critical for the maintenance of RGCs to regulate the generation of upper layer neurons.

DISPERSION AND FATE OF PALLIAL PROGENITORS WITHIN THE ADULT FOREBRAIN

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NG2-glia, also named as NG2-cells, is a neural cell type with a particular morphology and function different to neurons, astrocytes, and oligodendrocytes. Classically NG2-glia were considered as precursor of oligodendrocyte cells (OPC) but those cells have many different roles from development to adulthood, besides to be involved in the response to brain injury. In addition, this neural cell population persists in the adult brain, where they comprise about 5-8% of all CNS cells, and distribute homogenously throughout grey and white matter. NG2-glia is a highly proliferative cell population in both during development and adulthood, suggested that they are not just lineage-committed progenitors, but also multipotent neural stem cells. To decipher in vivo the adult cell potential of NG2 progeny of single E16 progenitors, we performed a novel variant of the StarTrack, the NG2-StarTrack, which expression is drived by the mouse NG2 promoter. NG2-StarTrack plasmids and the hyPBase transposase were injected intracerebroventricular lateral of embryos mice and the E16-progenitors of the dorsal wall transfected by electroporation. At P250 we performed a morphological and immunohistochemical analysis of the derived NG2-cell progeny from E16-progenitors, to assess the progenitor cell fate at late adult ages. We identified StarTrack labeled cells with several neural phenotypes in different regions of the forebrain, as interneurons in the granule cell layer of the olfactory bulb. In addition, in the somatosensory cortex labeled cells showed diverse morphologies corresponding to oligodendrocytes, protoplasmic astrocytes, pial astrocytes, NG2-glia, and even neurons. In the corpus callosum, NG2-StarTrack labeled cells displayed cell phenotypes with a morphological and immunohistochemical features of oligodendrocytes, fibrous astrocytes, and NG2-glia. Together, this specific in vivo targeting of embryonic neural progenitors reveals new data on the cell fate of the heterogeneous pool of single E16 dorsal progenitors. Supported by research Grants from MICINN (PID2019-105218RB-I00) and Fundación Ramón Areces (Ref. CIVP9A5928). EAC is supported by CONACyT number 770752.

EFFECTS OF CHLORPYRIFOS ON CELL DEATH AND CELLULAR PHENOTYPIC SPECIFICATION OF HUMAN STEM CELLS

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Chlorpyrifos (CPF) is one of the most widely used organophosphate pesticide in agriculture. Inhibition of acetylcholinesterase is the best described mechanism for CPF neurotoxicity.

Chemical exposure during developmental stages can interfere with the proper development of the nervous system resulting in functional alterations or diseases during the lifespan of the individual and thus, resulting in developmental neurotoxicity (DNT). Despite the large body of results on animals, these studies are costly, time consuming and the results are not always reliable to assess the impact of chemical compounds on the developing human because animal models do not perfectly reflect human physiology. It remains clear that there is a growing necessity for developing alternative methodologies that can better identify and assess chemical substances with the potential to induce neurotoxicity during brain development and maturation.

Human stem cells are currently being a model that promises to be very useful in evaluating this type of toxicity and may be a valuable tool for DNT. In this study, the cell line hNS1 was used to evaluate the effects of CPF on early developmental stages. hNS1 cells were exposed to different concentrations of the pesticide and cell death, proliferation and cell fate specification were analyzed under differentiation conditions. The results showed that this compound induces apoptotic cell death at the highest doses tested. Besides, CPF promoted the differentiation of hNS1 cells into glial cells by increasing the pool of proliferating glia progenitors. This effect may be associated with a protective effect of glia against CPF.

In addition, we used brain organoids derived from human induced pluripotent stem cells (hiPSCs) to see if CPF has the same effects as in hNS1. We found that CPF induced cell death at the high dose tested and a decrease in the number of neurons and glial cells

EFFECTS OF Lis1 GENE LOSS IN PARVALBUMIN EXPRESSING CELLS ON THE MOUSE HIPPOCAMPAL CYTOARCHITECTONICS

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Type I lissencephaly is a severe developmental brain disorder caused by mutations in the Lis1 gene. Despite the recent advances in understanding its functions, it is not fully understood the roles of Lis1 in specific neuronal populations during development and how these roles may modulate the maturation and function of the central nervous system (CNS).

Inhibitory GABAergic interneurons are important players in regulating the correct migration and activity of excitatory pyramidal neurons necessary for a proper functional maturation of the cortex. These interneurons express Lis1 and it is well known that a disruption of the balance and coordination between excitatory pyramidal neurons and inhibitory interneurons result in deep alterations of the brain functions such as epilepsy.

To address the unexplored role of Lis1 in GABAergic interneurons and its contribution to neurological disorders etiology and associated brain malfunctions, we generated a new mouse model specifically targeting the loss of Lis1 at the parvalbumin positive (PV+) GABAergic interneurons (Lis1cKO-PV+).

The hippocampus of postnatal (P15-P21) Lis1cKO-PV+ mice displayed a severe phenotype characterized by the presence of neuronal heterotopia affecting to the CA2/CA3 regions. In this hippocampal area the pyramidal cell layer was disorganized and there was an abnormal placing of pyramidal neurons from the stratum oriens to the stratum radiatum. These histological data highlighted, for the first time, that the specific role of Lis1 on the hippocampal GABAergic PV+ interneurons had a non-cell autonomous impact on the surrounding neural population of this cortical region.

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EFFECTS OF SHORTENING THE HABITUATION PROTOCOL ON EXERCISE CAPACITY DURING ADOLESCENCE OF RATS

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The brain causal mechanisms working on peripheral tissues during a physical activity are poorly understood. In previous studies we developed a model to analyze motor responses based on a habituation program to exercise consisting of a progressive increase of the training load. This initial phase is determinant to improve motor performance during training programs in forced running conditions. Determine if shortening the habituation program modify responses in exercise capacity, and if this can be related with muscle peripheral adaptations.
Sessions of the habituation in forced running wheel were developed throughout 2, 4 and 8 days of training. Once the habituation phases were completed, rats were subjected to an incremental exercise test to determine the physical capacity. Samples of gastrocnemius and soleus were used for qPCR and Western blot analysis of markers involved in muscle adaptation.
The total time of running during the incremental test was of 37.78 ± 2.65 min for 8 days habituated rats but 15.84 ± 1.22 min for non-habituated rats (wheel control). In the case of 4 days habituated rats the total time of running was 36.02 ± 2.67 min with 17.35 ± 2.86 for the control. Finally, we found that after 2 days of habituation, rats run during the incremental test 28.95 ± 3.94 min, but 19.97 ± 2.57 min in the case of the control. We didn´t observed any differences in selected muscle markers in terms of mRNA molecules or protein concentrations justifying peripheral adaptations.
Shortening of habituation period to 4 days produce similar effect in the incremental test than 8-days habituation period. However, 2 days habituation period produce a decreased response during the incremental test compared with 4- and 8-day habituated rats. The differences in running can not be justified in terms of muscle peripheral adaptations, strengthening a central nervous system effect.

EMBRYONIC CANNABINOID CB1 RECEPTOR KNOCKDOWN CONSEQUENCES IN GENE EXPRESSION AND FUNCTIONAL MATURATION OF PYRAMIDAL NEURONS

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Cortical development occurs by a series of proliferation and differentiation waves in a tightly regulated and coordinated process that ensures the appropriate formation of specific neuronal populations. Developing projection neurons migrate to their final layer location and after functional maturation and axon growth integrate in specific circuits. The endocannabinoid system exerts a neurodevelopmental regulatory role. Endocannabinoid production and CB1 receptor signalling regulates different processes including neural progenitor proliferation and identity, neuronal differentiation, and migration. Prenatal transient CB1 receptor knockdown causes alterations in neuronal morphology and interferes with radial migration, leading to greater seizure susceptibility in adulthood. To understand the consequences of defective embryonic CB1 receptor signalling in projection neuron development we performed gene expression analyses (microarray) of fluorescent FACS-sorted neurons at different times after siCB1 and control-siRNA in utero electroporation. In parallel we performed pathclamp electrophysiological analyses of delayed siCB1 neurons. We found that CB1 receptor knockdown induced gene expression changes with many downregulated transcripts involved in biological processes related to neurogenesis, axon growth and guidance, adherens junction, signalling mechanisms (PI3K/AKT/mTor signaling) and cell fate regulation. CB1 receptor knockdown neurons that migrated incorrectly, possess decreased firing frequency and less excitability, compared to control neurons. Ongoing studies aim to elucidate the causative gene expression changes responsible of defective neuronal maturation induced by CB1 signalling blockade. In summary, our findings describe the neuronal adaptations induced by cannabinoid CB1 receptor silencing at gene expression and functional level. The observed alterations of neuronal development have important implications for the understanding of neurodevelopmental disorders and the consequences of prenatal THC exposure.

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EXPRESSION OF GONADAL HORMONES' RECEPTORS IN OTP-RELATED SOCIAL BEHAVIOR NETWORK

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The brain of vertebrates includes a social behavior network, responsible for producing and regulating social interactions in a flexible and dynamic way. Social behaviors, like mating, parental care and aggression, show strong variations between sexes and are regulated by gonadal hormones. Many of the areas of this network express estrogen and androgen receptors. These brain areas include multiple neuron populations with specific connections, but the relation between gonadal receptors and specific neuron populations is poorly understood. In this study, we took advantage of the Otp-eGFP transgenic mice, with permanent labelling of Otp cells and their axonal projections, to study the relation between gonadal hormones' receptors and Otp cells. Otp is a transcription factor critical for the differentiation of neuroendocrine neurons in the hypothalamus and also for the generation of glutamatergic neurons of the medial extended amygdala. We studied the expression of estrogen receptors alpha (Erα) and beta (Erβ) and the androgen receptor (AR), in areas rich in Otp neurons and in Otp neurons' targets. To study receptor expression, we used in situ hybridization method. Otp cells and fibers were visualized using immunohistochemistry to detect GFP. The study was performed in two developmental stages, before and after sexual maturation. The results show that in both stages there is a high expression of receptors in areas rich in Otp cells, like the medial amygdala, especially its posterodorsal subdivision, the BSTM and the paraventricular hypothalamic nucleus. We also observed a high expression of receptors in targets rich in Otp-expressing terminals, such as the lateral septum, the medial preoptic region, the ventromedial hypothalamic nucleus and the periaqueductal gray. The fact that the Otp-related social behavior network expresses gonadal receptors suggests a prominent role of these cells and their connections in regulating sexual and other social behaviors.

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IDENTIFICATION OF CONSERVED NEURON SUBTYPES EXPRESSING OTP AND FOXG1 IN THE EXTENDED AMYGDALA OF A LIZARD

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The amygdala is a very complex structure, essential for emotion and social behavior. In particular, medial extended amygdala is involved in different aspects of social behavior (such as sexual, parental and aggressive behaviors). Research focused on its functional organization is important to understand the biological bases of the normal and pathologic development of social behaviors. The organization of this area is a consequence of two processes which act at different temporal scales: development and evolution. Using an evolutionary developmental neurobiology approach, our group recently identified a new embryonic domain at the transition between telencephalon and hypothalamus, that coexpress Otp and Foxg1, and produces most of the glutamatergic cells of the mouse medial extended amygdala. To investigate if this domain is also present in reptiles, we analyzed the expression of the transcription factors Otp and Foxg1, during brain development in the lacertid lizard Psammodromus algirus. Our results showed an area of Otp/Fogx1 expression overlap, with coexpressing cells that spread into the medial extended amygdala of P. algirus. This suggests the existence of a highly conserved neuron subpopulation in the medial extended amygdala of amniotes, and opens the venue to study its specific function and interaction with other neurons of the social behavior network. Funded by Ministerio de Ciencia e Innovación (PID2019-108725RB-100) and a Collaboration Fellowship in Research of the Ministerio de Educación (Beques de col·laboració universitària per al curs 2019-2020, COLAB 2019).

IN VITRO STUDY OF NEURODEVELOPMENT IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a neurodegenerative disorder that primarily affects the medium spiny neurons (MSNs) of the striatum. Recent evidence indicates that there is a neurodevelopmental component to HD where MSN specification, maturation and cellular homeostasis may be affected. However, the dysregulated cellular mechanisms underlying these impairments remain to be established. To enhance understanding of how neurodevelopment is affected in HD, further study of striatal development in a healthy context is also required as it is a brain region whose development is relatively understudied.

To investigate human striatal development in both a healthy and HD context, we differentiate control and HD human pluripotent stem cells in vitro towards an MSN fate. A range of analyses including bulk RNA-seq, single cell RNA-seq (scRNA-seq), and functional assays are performed during differentiation to evaluate the progression of striatal development and how it is altered in HD.

Bulk RNA-seq analysis indicates that alternative splicing is affected in HD cells. A set of neurodevelopment-related genes display differential expression of specific isoforms that is likely to impact on neuronal development and function.

scRNA-seq analysis has identified two main neuroblast populations. Using machine learning we have mapped the developmental trajectories that neural precursor cells follow to acquire these neuroblast identities. By plotting the expression of known striatal development genes onto these trajectories, we identify additional genes with a similar expression pattern that are also likely to have a role in striatal development.

Analysis of this diverse range of data sets is ongoing as they are integrated to develop a predictive model of striatal development. Using this approach, we anticipate that we will identify genes, signaling pathways and developmental modules whose modification will revert MSNs in HD to a healthy state.

INCREASED GABA LEVELS IN POSTNATAL DEVELOPMENT ALTER CORTICAL INTER-HEMISPHERIC CIRCUITS

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The corpus callosum (CC) is the largest fiber tract of the brain, connecting the two cerebral hemispheres and integrating sensory, motor, and higher-level cognitive information. The excitatory-inhibitory balance is crucial for sculpting cortical networks during the early postnatal period. Defective CC connectivity during this developmental period can correlate with defects in information processing in the adult, such as those observed in autism or schizophrenia. Here, we developed an experimental setup that alters excitatory-inhibitory balance in mice by injecting Diazepam –an agonist of the inhibitory neurotransmitter GABA– at specific postnatal windows. The number and location of callosal neurons were characterized via stereotaxic injections of the retrograde tracer cholera toxin B (CTB) in the CC of the primary somatosensory and visual areas (S1 and V1). We found that intraperitoneal injections of Diazepam result in a reprogramming of the interhemispheric adult circuit. In S1 there is a striking increase in layer 4 callosal neurons, while in V1 both layer 2/3 and 4 are increased. Interestingly, injections during the first postnatal week preferentially altered S1 over V1, while later treatments produce greater changes in V1 compared to S1. Furthermore, immunostaining of GABAergic markers to evaluate the status of the inhibitory circuit revealed a decrease in the total number of somatostatin interneurons and an increase in the parvalbumin population. Overall, our data show that disrupting the excitatory-inhibitory balance during development leads to alterations in both the inter-hemispheric and interneuron networks, perhaps in an attempt to maintain network homeostasis. We show that Diazepam-dependent plasticity is restricted temporally depending on the sensory area, possibly related to each area's critical period of plasticity. Further experiments using chemogenetics and electrophysiology will address whether the observed reprogramming is due to the activity of pyramidal neurons.

INTRAMODAL FUNCTIONAL PLASTICITY IN THE DEVELOPING SOMATOSENSORY SYSTEM

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Sensory systems are represented in the primary sensory areas of the brain, organized in anatomical and functional maps. Understanding how the brain adapts to the sensory loss might help us to better decipher the role of intrinsic and extrinsic mechanisms involved in cortical maps development. A paradigm extensively used to unravel the role of the afferent input during the development of the cortex is the deprivation of one sensory modality, which leads to an adaptive reorganization of the deprived and non-deprived sensory circuits. We are interested in understanding the mechanisms that trigger the establishment of territories during sensory systems development. To this end, we developed a mouse model in which whisker pad is cauterized unilaterally at the embryo stage (embWPC) to induce changes in the territories representing the distinct body sensory maps. These mice showed an intra-modal enlargement of the antero-lateral barrel subfield (ALBSF) area both in the thalamus and primary somatosensory cortex (S1) before the onset of sensory experience. Furthermore, dye tracing studies and in vivo calcium imaging in the embWPC mice showed severe structural changes of the somatosensory point-to-point axonal topography from embryonical stages suggesting that prenatal whiskers deprivation, before experience-dependent activity, induces functional rearrangements within a critical window. Finally, blockade of the patterned spontaneous activity in the thalamus of the embWPC mice revealed that these reorganizations of sensory territories are independent of thalamic activity. In sum, our results showed that the territories and sensory maps designated to distinct peripheral representations within a sensory system rely on prenatal mechanisms that are mainly based on axonal competition rules while patterns of spontaneous activity would play a crucial role in their later refinement.

LINEAGE CELL-POTENTIAL OF SINGLE NEURAL PROGENITOR CELLS

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The assemble of the brain from a pool of Neural Progenitor Cells (NPCs) is a complex process. Increasing evidence supports the heterogeneity of NPCs across and within distinct brain regionsand their importance for the generation of the different neural types. Some studies suggest that progenitor diversity is more restricted to one specific lineage whereas others show a potential cell diversity depending on the spatio-temporal patterning. Neural stem cells give rise to transient progenitors termed neuroepithelial cells that generate Radial Glial Cells (RGC), multipotent progenitors that produce, in overlapping waves, neurons, astrocytes, NG2-glia and oligodendrocytes. However, although RGCs are the most known cortical NPCs, NG2-glia (or NG2-cells) is another remarkable cell type that can also act as a progenitor. In the adult mouse brain NG2-glia is able to generate OLs, Astrocytes or even Neurons. Moreover, our previous works revealed the existence of NG2-progenitors during development, enable to produce different neural cell lineages depending on the embryo stage.
To elucidate the cell potential of single-NPCs, my lab developed the UbC-StarTrack a multicolor genetic tool that allow us to tag single progenitors with stable and heritable labelling.This strategy, based on PiggyBac system, consists of the integration (thanks to a hyperactive transposase PiggyBac -HyPBase) of twelve plasmids that codify up to six different fluorescence proteins (XFs) aim to cytoplasm or nucleus. To target single NG2 or GFAP-progenitor cells, we echanged the CMV-promoter of HyPBase in UbC-StarTrack for NG2 or GFAP-promoter in UbC-(NG2-PB)-StarTrack or UbC-(GFAP-PB)-StarTrack, respectively. After targeting NPCs at either E12, E14, E16 or P0, we performed a clonal analysis of the derived-cell progeny at P30. Data showed that GFAP- and NG2-expressing progenitors produce distinct cell types and whose differentiation potential changes with time and space. Our results provide new data of the lineage cell potential of NG2 and GFAP-progenitors that strengthen the heterogeneity of NPCs during cortical development.

MICROGLIA GRADUALLY ACQUIRE THEIR MATURE PHENOTYPE IN THE DEVELOPING HIPPOCAMPUS

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Microglia originate from yolk sac progenitors and invade the brain at embryonic stages to progressively become integrated in the parenchyma, presenting a brain-specific phenotype that distinguish them from other tissue macrophages. During the first postnatal weeks of development, microglia go over a period of high transcriptional heterogeneity before their mature phenotype is settled. However, the molecular mechanisms by which microglia identity and function are established and maintained are largely unknown. We hypothesize that microglia morphology and function progressively mature once they enter the brain parenchyma. To test this hypothesis, we focused on the hippocampus because it develops its cellular network during the first postnatal weeks, when microglia heterogeneity is at its peak. We analyzed microglial morphology and phagocytosis efficiency by confocal microscopy, and microglial dynamics by 2-photon microscopy. We found that microglia progressively invaded the hippocampal dentate gyrus from postnatal day 2 (P2) to P10. We are currently exploring two possible routes of colonization: one resembling that of neural precursors, based on the co-localization with the reelin scaffold; and another one related to meningeal macrophages, which decrease over time as microglia increase in the parenchyma. Then, microglia progressively acquired a branched morphology and achieved their highest efficiency of phagocytosis at P14. Hence, they invaded the hippocampus in the first postnatal days, and subsequently acquired their characteristic morphology, dynamics, and mature function. The concurrent maturation of microglia and the hippocampal structure in the first postnatal days suggests the intriguing hypothesis of an active role of the brain environment. Deciphering the microglial maturation program is highly relevant because early changes could be genetically imprinted and lead to long-term functional alterations, which could have an impact in many neurodevelopmental and neurodegenerative disorders.

MOTOR NEURONAL CONVERSION OF HUMAN MESENCHYMAL STEM CELLS BY APPLICATION OF SMALL MOLECULES

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Mesenchymal Stem Cells are a good alternative to the induced pluripotent Stem Cells in different areas of developmental biology as well as in translational medicine. Its easy extraction, manipulation and maintenance, no teratoma risk transplantation or beneficial properties (immunomodulatory) after the transplantation are some examples of the advantages of its usage.

Apart from the mentioned properties, mesenchymal stem cells are able to produce non-classical differentiation processes. One important differentiation trajectory observed in mesenchymal stem cells is a general neuronal differentiation which could produce neuron-like cell types.

In this study we try to extend this idea, and see whether the manipulation of the differentiation process of mesenchymal stem cells derived from adipose tissue by the application of cocktail of different small molecules (without genetic manipulation) could open the possibility of formation of motor neuron-like cell types. Furthermore, we analyse whether this non-classical differentiation could be useful to produce a cellular model to study Amyotrophic Lateral Sclerosis.

Our results based on the analysis by immunofluorescence and quantitative PCR of different specific markers point to the idea that: first, the conversion to motor neuronal-like progenitors is possible starting from mesenchymal stem cells by the application of a sequence of cocktails of different small molecules. And second, that further analysis should be made to confirm its application for the production of a cellular model of Amyotrophic Lateral Sclerosis.

NETRIN-1/DCC SIGNALING SYSTEM DIFFERENTIALLY REGULATES THE MIGRATION OF PAX7, NKX6.1, IRX2, OTP, AND OTX2 NEURONAL POPULATIONS IN THE DEVELOPING INTERPEDUNCULAR NUCLEUS

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The interpeduncular nucleus (IPN) is a hindbrain structure highly conserved among vertebrates. It is formed by three main subdivisions, the prodromal (Pro) domain located at the isthmus (Ist), and the rostral and caudal interpeduncular domains (IPR, IPC) within rhombomere 1 (r1). Various cell populations can be detected in the IPN through the expression of the Nkx6.1, Otp, Otx2, Pax7, and/or Irx2 transcription factors. These cell populations follow independent dorsoventral tangential and radial migratory routes targeting the ventral paramedian region of Ist and r1. Here we set out to examine the influence of the Netrin-1/DCC
system on these migrations, since it is known to regulate other processes of neuronal migration in the brain. To this end, we analyzed IPN development in late gestational
wild-type and DCC null mice, using mainly in situ hybridization (ISH) to identify the cells expressing each of the aforementioned genes. We found that the migration of Nkx6.1+ and Irx2+ cells into the Pro domain was strongly disrupted by the loss of DCC, as occurred with the migration of Pax7+, Irx2+, and Otp+ cells that would normally form the
IPR. In addition, there was mild impairment of the migration of the Pax7+ and Otx2+ cells that form the IPC. These results demonstrate that the Netrin-1/DCC signaling pathway is involved in the migration of most of the IPN populations, mainly affecting those of the Pro and IPR domains of this nucleus. There are psychiatric disorders that involve the
medial habenula (mHb)-IPN system, so that this experimental model could provide a basis to study their neurodevelopmental etiology.

NEUROGRANIN-LIKE EXPRESSION IN THE ZEBRAFISH BRAIN DURING EARLY STAGES OF DEVELOPMENT AND CHANGES INDUCED BY Mn2+ EXPOSURE

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Neurogranin (Nrgn) is a small peptide that seems to participate in neuroplasticity through, at least, interactions with calmodulin and Protein Kinase C (PKC) phosphorylation. Altered levels of Nrgn correlate with cognitive decline in aging and neurodegenerative disorders such as Parkinson or Alzheimer's disease. In this study, we determine the distribution of Nrgn in the brain of zebrafish (Danio rerio) embryos and larvae by using a polyclonal antibody (AB5620, Merk-Millipore). As a first approach to understand how Nrgn levels are affected in neurodegenerative diseases, we analysed its expression after exposure to Mn2+, a cation that can lead to Parkinsonism. Immunocytochemistry analysis showed that Nrgn expression is first observed by 2 days post fertilization and is maintained throughout adulthood. By 6dpf, Nrgn expression is observed in the telencephalon (olfactory bulbs and pallium), diencephalon (pineal, preoptic area, posterior tubercle, hypothalamus, and adenohypophysis), optic tectum, medulla oblongata and spinal cord. Zebrafish embryos exposed to a sublethal concentration of manganese dichloride (500 µM) from 2.5 to 120 hours post fertilization (hpf) show a significant decrease in Ngrn expression compared to controls. This includes a very strong reduction in the olfactory bulbs and pallium. Shorter exposure (from 2.5 to 48 hpf) to the same concentration of manganese dichloride rescues by 5dpf Nrgn expression to control levels. These results suggest there is Mn2+ mediated cytotoxicity in the zebrafish olfactory system, as described previously in humans and other vertebrates, affecting Nrgn expression. Further studies will be necessary to characterize in more detail Mn2+ cytoxicity and its direct or indirect role on neurogranin levels.

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OTP EXPRESSION IN CORTICAL NEURONS THROUGHOUT ONTOGENESIS

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Different studies have shown the important role of embryonic origin to understand the mature phenotype of neurons. Recently, we described a new radial telencephalon-opto-hypothalamic embryonic domain, coexpressing the transcription factors Foxg1 and Otp, which produces the vast majority of the glutamatergic cells of the medial extended amygdala, as well as some cells of the pallial amygdala. To determine if there are other pallial populations that co-express both transcription factors, we used Otp-eGFP transgenic mice and double immunofluorescence for Foxg1 and GFP at different embryonic stages and postnatal ages. From middle embryonic stages, we observed very few GFP/Otp expressing cells, with migratory neuroblast morphology, in the primordium of the anterior cingulate cortex, mostly intermingled with fibers of the developing cingulate fascicle. These cells were in continuity with similar cells and fibers found in the septo-preoptic region, resembling a ventro-dorsal tangential migratory stream. The number of cells and fibers in this stream increased during development. Postnatally, some scattered cells were observed in the mantle of the neocortex, mostly in the cingulate cortex and adjacent cortical areas. All of them coexpressed Foxg1. Moreover, other GFP/Otp cells were found in the hippocampal complex. A densely packed group was found in the pre/parasubicullum, which gave rise to a projection to the hippocampal formation. The latter also included some cells, which coexpressed Foxg1. The finding of GFP/Otp cells in the neocortex and hippocampal complex raises questions on their origin, phenotype and function throughout ontogenesis. The location of some GFP/Otp cells in relation to developing septal and cingulate fiber bundles suggest a role as guidepost during fascicle development. Otp may also be important for the migration and differentiation of specific cortical neurons. In addition, as Otp is also expressed in adulthood, it may be playing a regulatory role affecting mature cortical function.

*E.D and L.M contributed equally as supervisors of this work.

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PERTURBATION OF ADHERENS JUNCTIONS ASSOCIATED PROTEINS IN THE NEOCORTEX AFFECTS NEURODEVELOPMENTAL PATHWAYS CAUSING COGNITIVE AND SOCIAL DEFICITS IN MICE

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The neocortex accomplishes a wide range of sophisticated tasks, such as cognition, language, sensory perception and motor integration. In order to conduct them, the neocortex displays an extensive cell diversity arisen from neural stem cells, known as radial glial cells (RGCs), through tightly regulated mechanisms. In particular, RGCs lining the ventricular walls produce different subsets of excitatory projection neurons (PNs) distributed in a laminar manner with specific molecular signatures, morphologies and connection patterns. Among them, cortico-cortical PNs, mostly located within the upper cortical layers, are increased in superior mammals like primates and are described to be more susceptible to dysfunction in psychiatric disorders. Previous studies have shown that genetic inactivation of adherens junction (AJ)-associated proteins Cdh2 and afadin in the dorsal telencephalon, produces dramatic expansion of the neocortex due to the overproduction of PNs expressing upper-layer neurons markers. These features have been detected in certain rodent models of autism spectrum disorder (ASD) as well as in some ASD patients. Here, we aim to understand the molecular mechanisms acting downstream of these AJ-associated proteins involved in the control of neocortical progenitor behaviour and how alterations in these genes could cause cognitive and social deficits like the observed in ASD patients. To this end, we have examined the expression of candidate genes by RT-qPCR in these conditional AJs mutant mice and found changes in some genes whose dysregulation has been also linked with ASD. In order to unveil the existence of cognitive and social alterations in these mice, we have performed a thorough behavioural characterization. Taken together, our data underline the importance of afadin and Cdh2 in the regulation of neocorticogenic mechanisms that, when perturbed, cause behavioural deficits, and might help to provide new insights into ASD pathogenesis.

POSTNATAL REFINEMENT OF INTERHEMISPHERIC CALLOSAL PROJECTIONS: GluN3A-MEDIATED MECHANISMS

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During critical periods of brain development, synaptic and circuit refinement is driven by sensory experience. Both presynaptic and postsynaptic refinements involve loss and strengthening of synaptic contacts but how these processes are coordinated remains obscure. The non-canonical NMDA-receptor subunit GluN3A is thought to determine the timely remodelling of these circuits, but how it operates in specific brain circuits has not been addressed. Here we are investigating the roles of GluN3A in presynaptic and axonal refinement. We analyse interhemispheric callosal projection axons via in utero electroporation of the mouse somatosensory cortex with transgenic labelling. Analysis at different postnatal (P) days showed that initial extension and innervation of the contralateral cortex by callosal axons is not affected in GluN3A knockout (KO) mice with similar patterns to wild-type mice at P4 and P6. At P8, differences begin to arise in axon arbor density in layers 2/3 of the somatosensory S1/S2 border region, relative to other target layers. By P13, when a more mature configuration is reached, GluN3A KOs show a significantly different callosal axon cortical profile with arbors concentrating towards inner positions within L2/3 relative to wild-type that profusely innervate layer 1. This altered distribution of callosal axon arbors persisted at P20-22. Additionally, P13 and P20-22 GluN3A KOs display increased targeting in S2 as shown by the appearance of a second column displaying strong labelling. Ongoing experiments are testing for the cellular locus of GluN3A function within this circuit using specific manipulation in presynaptic or postsynaptic neurons in floxed-GluN3A mice lines. Potential candidate mediators of altered axonal refinement have been previously identified through RNA-seq experiments and some candidates show corresponding changes at the protein level. Future work involving chemogenetic and sensory deprivation approaches aims to determine activity and experience dependent roles within this GluN3A-affected axonal development.

PROLIFERATIVE RATE AND NEUROGENESIS IN HUMAN NEURAL STEM CELLS ARE INCREASED BY Aβ40 PEPTIDE

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Amyloid-β 40 peptide [Aβ1-40 (Aβ40)] is present within the amyloid plaques in brains of patients with Alzheimer's disease (AD). Even though Aβ peptides are considered neurotoxic, they can mediate many biological processes both in adult brain and throughout brain development. However, physiological function of these Aβ peptides remains poorly understood, and existing data are sometimes controversial. Here we analyze and compare the effects of monomeric Aβ40 on the biology of differentiating human Neural Stem Cells (human-NSCs). For that purpose, we have used a model of human NSCs, called hNS1. Our data demonstrate that Aβ40 at high concentrations provokes apoptotic cellular death and damage of DNA in human NSCs while also increases the proliferation and favors neurogenesis in hNS1 cells by raising the percentage of proliferating neuronal precursors. These results provide evidence of how Aβ modulate/regulate human NSCs proliferation and differentiation, suggesting Aβ40 may be a pro-neurogenic factor. These data could contribute to a better understanding of the molecular mechanisms involved in AD's pathology and for the development of human NSC-based therapies for AD treatment, since these results could then be used in diagnosing the disease at early stages and be applied to the development of new treatment options.

ROLE OF BASAL AUTOPHAGY IN THE REGULATION OF HIPPOCAMPAL NEURAL STEM CELL

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Two stem cell niches are still producing new neurons in the adult mammalian brain: the subventricular zone (SVZ) and the subgranular zone (SGZ) of the hippocampal dentate gyrus. Neural stem cells (NSCs) in these adult niches are preserved in a quiescent state. Dentate neural progenitors acquire a radial-glia like morphology and enter quiescence (qNSCs) during the early postnatal period. Later on, they regain proliferation in order to generate new neurons. The first step in neuronal production in the adult neurogenic niches is indeed the transition of qNSCs into an activate state (aNSCs). The implication of constitutive (basal) autophagy in the regulation of this transition in the mature brain and in NSC metabolism and protein quality control are beginning to be addressed.

In this work, we explored the role of the autophagy-lysosomal pathway in the maintenance of quiescence and in the regulation of proteostasis in NSCs from the SGZ. We performed in vitro experiments using NSCs in two different states: active or quiescent (4 days treatment with BMP4). Our results showed that quiescent cells have a higher overall protein content and more cytoplasmic protein aggregates. On the other hand, genome-wide microarray transcriptional analysis and RNAseq analysis showed an increase in the expression of genes related to the autophagy-lysosomal pathway in qNSCs vs. aNSCs. Moreover, not only the levels of autophagic proteins (LC3II, p62) are increased in qNSCs but also, there is a raise in the activity of autophagic-inducing kinases (AMPK, ULK1). Experiments with the autophagy inhibitor (Bacilomycin A1) and activator (Metformin) showed that basal autophagy is required to maintain the quiescent state. In addition, NSCs (Glast+ cells) prospectively isolated from mouse hippocampus at different postnatal ages allowed us to validate the in vitro findings.

In conclusion, our data demonstrate that basal autophagy is increased in qNSCs and that it has a role in the maintenance of quiescence. Currently, genetic and pharmacologic in vivo experiments are ongoing in order to complete this study.

THE CELLULAR EFFECT OF SHH SIGNALING IN OLIGODENDROCYTE PRECURSOR CELLS (OPCS) DEPENDS ON THE MICROENVIRONMENT

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The morphogen Sonic Hedgehog (Shh) controls the proliferation of cells with stem cell properties in several regions of the CNS during embryonic and postnatal development, as well as in the adult[1]. The blockage of hedgehog signaling perinatal and in adult mice results in diminished expression of Gli1, reducing cell proliferation in vivo[2].

More recently the role of Shh signaling has been shown to be a critical pathway in the transition from neurogenesis to oligodendrogenesis in dorsal forebrain progenitors during late embryonic development[3]. Shh also regulates oligodendrocyte production in adulthood in the cortex and corpus callosum (CC). After focal demyelination induced by lysolecithin, the transcription of Shh target genes is increased[3], but Shh-expressing cells are not detected in the CC after chronic demyelination with cuprizone. However, the use of SAG (agonist of both canonical and non-canonical Hedgehog signaling pathways) increases the cell proliferation and enhances remyelination, but Gli1 fate-labelled cells in the CC do not change, which may indicate that is signaling through the non-canonical pathway[4].

Our present work shows the direct effect of Shh signaling on OPC proliferation and myelination. To do this, we crossed the NG2-CreERT2TdT mice with both SmoM2 and Smofl/fl lines and studied the gain and loss of function of smoothened receptors implicated in the canonical and non-canonical pathways. We also provide evidence that Shh synergizes with PDGFAA signaling in the modulation of OPC proliferation in vitro from postnatal and adult cortex.

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THE DEVELOPMENT OF THE CEREBELLO-CORTICAL CONNECTIVITY

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Classically, the cerebellum has been considered a pure motor brain structure. However, mounting evidence has suggested that it also has essential contributions to nonmotor functions, such as cognition and emotion.
The cerebellum is well poised to contribute to these complex behaviors because it is connected with the cerebral cortex which controls these functions. In addition, the cerebellum receives afferent input from the cerebral cortex through the pons. The closed-loop circuits between these two regions is the anatomical substrate by which the cerebellum could modulate the activity pattern of distal cortical regions.
Importantly, early abnormalities of these circuits have been related with different neurodevelopmental disorders, such as autism spectrum disorders. Thus, understanding how the
cerebello-thalamo-cortical pathway develops is the initial step to understanding the cerebellar contribution to high-order neurodevelopmental disorders.
Here we have developed diverse in utero strategies to specifically target the cerebellum and its long-range projections. This approach, together with tissue clearing methods and 3D light-sheet microscopy, has allowed us to study the development of cerebellar connectivity in three-dimensions.
The data shows that cerebellar axons invade the contralateral thalamus as early as embryonic day (E)17. At E18, these axons target multiple motor and non-motor thalamic nuclei. Strikingly, at postnatal stages, the cerebellar axons recross the thalamic midline to innervate the ipsilateral thalamus. This broad innervation suggests a potential bilateral influence of the cerebellum over immature motor and non-motor thalamocortical networks. Hence, we have stablished the anatomical basis by which the cerebellum could impact the development and/or function of cortical circuits.

THE IMPACT OF NMDA RECEPTOR SUBUNIT GluN3A DELETION ON THE BRAIN ACTIVITY OF YOUNG AND ADULT MICE

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The maturation of functional sensory circuits with the capacity to process information is a highly orchestrated process that takes place during postnatal stages. It involves the interplay of progressive and regressive events that will stabilize some synapses and remove others. Problems during this critical period contribute to a wide spectrum of neurological disorders in later life. The pruning of axons and dendrites is controlled by multiple factors, one of them being the activity sensed and transmitted by N-methyl-D-Aspartate receptors (NMDARs). Here we focus on a new class of NMDARs that are characterized by the presence of GluN3A subunits and play an essential role in the activity-dependent refinement of synaptic connections. In mouse primary somatosensory cortex (S1) GluN3A expression peaks around postnatal days 6-9 and declines until reaching very low adult levels, with temporal differences between cortical layers (Murillo et al., Cereb Cortex 2020). Results from our collaborators show that GluN3A-containing NMDARs participate in axonal refinement in such a way that knocking out the gene encoding GluN3A (Grin3a) results in an aberrant pattern of callosal innervation of S1. Therefore, in this study, we investigated the impact of GluN3A deletion on the communication between hemispheres, and analyzed the spontaneous activity and sensory processing in S1 of juvenile (P20) and adult (P40) mice. To that end, we performed multi-site bilateral recordings of spontaneous activity in S1 of awake GluN3A knockout mice (Grin3a-/-). Preliminary results reveal changes in the power of several frequency bands in Grin3a-/- compared to wild-type mice at P20. We are currently characterizing the ipsilateral whisker responses in S1, which are mediated by callosal inputs. Understanding the dynamics of intra- and interhemispheric processing will shed light on the role of the GluN3A-containing NMDARs.

THE SOUND OF SIGHT: MAPPING CROSSMODAL CIRCUITS OF AUDIO-VISUAL CONNECTIVITY IN THE MAMMALIAN BRAIN.

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In the mammalian neocortex, information corresponding to different sensory modalities is processed separately in specialized cortical areas. Nevertheless, brain regions that process the inputs from different senses are interconnected and provide contextual information to each other, allowing multisensory integration. However, how this integrative and crossmodal network is formed and shaped during development is not well understood.

We have recently reported axonal exuberance as a principal cellular strategy for the establishment of cortical circuits. At the early stages of development, neurons from most areas and layers of the neocortex develop transient projections to ipsi- and contralateral cortical territories. Normally, these exploratory branches are not stabilized. However, in the context of loss of sensory inputs or brain insult in young brains, this early plasticity facilitates the rearrangement of crossmodal canonical connections, resulting in non-canonical circuits. Such might be the case of congenitally blind people, where visual cortices respond strongly to auditory inputs.

To investigate how these associative networks are initially formed and later on refined, we reconstructed audio-visual circuits by combining in-utero electroporation (for anterograde labeling) with stereotaxic injections of the Cholera Toxin Subunit-B (for retrograde labeling) in wild type adult and postnatal mice. We observed that both, primary (V1) and secondary (V2) visual areas, receive inputs from distinct auditory areas. Interestingly, the degree of crossmodal connectivity between these regions changes during postnatal development, being secondary areas more represented in the adult circuit. Furthermore, we noticed that the cortical layers involved in this circuit also differed between stages of development. These findings suggest that the audio-visual network undergoes extensive remodeling during the first weeks of life, highlighting the importance of early axonal plasticity for the acquisition of the final configuration of cortical circuits.

THE TRANSCRIPTION FACTOR Zic2 PARTICIPATES IN ADULT NEUROGENESIS AT THE HIPPOCAMPAL SUBGRANULAR ZONE (SGZ)

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Neurogenesis is the process whereby new postmitotic neurons are produced by neural progenitors during the formation and maturation of the nervous system. In the adult mouse, neurogenic process is largely restricted to discrete brain regions, such as the subgranular zone (SGZ) of the hippocampal dentate gyrus, and plays a key role in brain function. Adult neurogenesis in the SGZ generates new cohorts of granular neurons, which contribute to learning and memory events, and a malfunction of this process has been associated to depression or epilepsy. It is known that the Wnt signaling pathway is an important regulator of adult neurogenesis in mice, but the cellular and molecular mechanisms driving neuron generation in adult brain still remain obscure. The transcription factor Zic2, formerly identified as a neuron-intrinsic axon guidance regulator during retinothalamic circuit development, is expressed in neurogenic niches of the adult brain and was recently described to modulate the Wnt-β-catenin signaling pathway. We found that Zic2 is expressed in the radial glia-like (RGL) progenitors of the SGZ. In particular, we uncovered that Zic2 specifically localizes to quiescent RGLs. Following Zic2 overexpression in adult neural progenitors, we observed a decreased proliferation in the hippocampal SGZ, marked with Ki67 and BrdU labelling. Conversely, the downregulation of Zic2 led to an increase in intermediate type2a progenitors in the SGZ, labelled by Ascl1. These results suggest that Zic2 acts as a regulator of the transitions between quiescence and proliferation in the RGL progenitors. Our data support a possible role of Zic2 in the acquisition and maintenance of quiescence in adult neural stem cells.

TRANSCRIPTOMIC CORRELATION OF THE TOPOGRAPHIC AFFERENT INNERVATION DISTRIBUTION IN THE HABENULAR COMPLEX

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The Limbic System is composed by circuits that regulate emotional sensations and self-protective behaviours (i.e. feeding, fighting or reproduction) and circuits that correlate expressive states and feelings of sociability and procreation. Among the several subcortical neuronal components of this system, the Habenula (Hb) seems to play a crucial centered role. The Hb is constituted by two main domains, the medial and the lateral Habenula (mHb, lHb). The afferences to the habenular complex, despite having different origins, are concentrated in a single tract, the stria medullaris (sm). This fascicle can be subdivided into two groups depending on their target (either mHb or lHb domains).
Recently, the subnuclear organization of both domains has been deeply analysed. The mHb subnuclei present a well stereotyped boundaries with differential gene expression profile while the lHb subnuclei are composed of many distinct cell types with a non-predicted molecular heterogeneity.
Our hypothesis is that the transcriptomic subdivision of Hb has a functional role in the limbic system circuits. Thus, we analysed the sm different innervation terminations by means of Hb transcriptomic subdivision. We used the Mouse Brain Connectivity section of the Allen Mouse Brain Atlas (www.allenbrainatlas.org) and selected eleven origin nuclei that project into the Hb complex. Our results suggest that septal nuclei would mainly innervate the ventral portion of the mHb. Meanwhile, pallidal nuclei would innervate the dorsal aspect on the mHb. Finally, the hypothalamic nuclei would innervate the lHb in a less compartmentalized manner. We therefore, may conclude that afferences to the Hb display a topographic-transcriptomic distribution. This distribution may underlay the still poorly understood internal circuitry in the Hb complex.
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UNDERSTANDING THE MECHANISMS INVOLVED IN MIGRATION AND CIRCUIT INTEGRATION OF THALAMIC INTERNEURONS

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GABAergic interneurons (INs) are inhibitory cells necessary to balance excitation and inhibition in the brain. During development, INs migrate from their site of origin towards their final destination, where they build stable neural circuits together with the excitatory cells. Thus, our aim is to unravel the mechanisms that direct INs migration and circuit assembly in the mouse thalamus. We specifically investigate, first, the role of thalamic activity on INs migration and integration, and second, to what extent the abnormal embryonic thalamic activity affects the distribution of cortical interneurons. We have seen changes in the number of dorso lateral geniculate nucleus (dLGN) INs in mice lacking embryonic thalamic spontaneous activity, and strikingly, changes also in the density and morphology of microglia. In addition, in primary sensory cortices, the proportion of Somatostin-positive and Parvalbumin-positive INs varies between mice lacking thalamic spontaneous activity and control littermates. Thus, normal patterns of thalamic spontaneous activity appear to contribute to the circuit assembly both in the thalamus and in the cortex.

UNRAVELLING THE NEURAL CELL PROGENY OF SINGLE SUBPALLIAL PROGENITOR CELLS

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Over the last decades a growing body of studies has provided evidence for the heterogeneity of neural progenitor cells (NPCs). Distinct subsets of progenitor cells are destined to become lineage-restricted while others function as bi/multipotent providing a molecular and functional diversity of their neural lineages. The multi-color genetic lineage tracing system, UbC-StarTrack, allows an independent tracking of sister clones derived from a common targeted progenitor under in vivo conditions. As a result, we designed different StarTrack strategies to permanently label individual progenitor cells and their progeny accordingly with the identity of the NPCs (Sánchez-Gonzalez et al 2020a). In particular, we used the UbC-(Gsh-2-hyPB)-StarTrack, with a PiggyBac transposase under the expression of Gsh-2 promoter (Sánchez-González et al., 2020b), now enabling to specifically target progenitor cells located in the ventricular surface of the mouse embryonic subpallium (ventral area of the telencephalon). The derived-cell progeny of those early embryonic subpallial progenitors produced the different neural types: astrocytes, oligodendrocytes, NG2 and even neurons. In addition, this strategy allowed us to perform an accurate analyses of the clonal relationships between the derived-cell progeny of those targeted individual subpallial progenitors, that could generate daughter cells of different lineages.

Our findings illustrate the degree of progenitor heterogeneity, particularly considering the molecular and functional diversity of their cell-derived progeny. Moreover, data revealed the lineage relationships of individual subpallial progenitors and their daughter cells, that might help to gain new insights into their behavior, complexity and functionality.

Wnt1 EFFECT ON THE FASCICULUS RETROFLEXUS AXONAL NAVIGATION

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During development of the neuronal system, immature neurons must generate their dendritic arborizations and axonal neurites properly. The developing axons follow a stereotypic map between short and long range signals. The haptotaxis mechanism involves short range signals that require cell-cell contact and they produce permissive or avoidance territories for the growing axons. Meanwhile, the quimiotaxis process is related to long range signals that are able to attract or repeal the growth of the axons towards the sources. One clear example are the fasciculus retroflexus axons, that, as they reach the diencephalic floor plate vicinity, bend caudalwards. Then, they navigate crossing the midbrain until their target in the rostral hindbrain. They must cross the Isthmic territory, a well-known secondary organizer, that contains several morphogens such as Fibroblast growth factor 8 (Fgf8) and Wingless (Wnt1). Our aim in this work is to unveil the Wnt1 role in the determination of the mid-hindbrain territory crossed by the fasciculus retroflexus and how it affects the guidance mechanism needed by this tract. We used a Wnt1 lack of function transgenic mice and analyzed its phenotype by immunohistochemistry, axonal tracing dyes and iDisco techniques. The results obtained showed that the mesencephalic basal territory was severely affected. The axons were able to travel caudally but displayed strong abnormalities in their direction. The isthmic territory in the absence of Wnt1 was not able to properly induce the surrounded territories triggering a dramatic alteration of the correct pathway cues needed for the fasciculus retroflexus axons.
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Wnt1 ROLE IN THE SPECIFICATION AND DIFFERENTIATION OF THE HABENULAR COMPLEX

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The neuroblasts of the developing neural tube requires the activity of precise regions (secondary organizers; SO) to direct their specification and differentiation programs. Along the antero-posterior axis, it has been described three SO: the isthmic organizer, the zona limitans and he anterior neural ridge. They produce secreted signaling molecules named morphogens. These mainly include Fibroblast growth factor 8 (FGF8), Bone morphogenetic proteins (BMP), Wingless (WNT1) and Sonic Hedgehog (SHH). In fact, some of them organize more precisely the dorso-ventral patterning of the neural tube. Thus, SHH is produced mainly in the floor/basal plate and BMP with WNT1 on the roof plate.

Based on our interest in the limbic system development, we studied the WNT1 role in the specification and differentiation of the habenular complex. This neuronal structure is located at the dorsal aspect of the thalamic prosomere, central part of the diencephalic territory. We analyzed in a Wnt1 loss of function murine model the embryonic development of this neuronal complex. This study has included different approaches that has included proliferation, immunohistochemistry and iDisco techniques. The lack of function of this morphogen produced a severe alteration in the proliferation rates and a dramatic extension in the anteroposterior dimension of the habenular complex. The subnuclei subdivision in the components of the lateral and medial habenula maintained a similar distribution. Therefore, Wnt1 is necessary for the correct habenular complex growth but not for their specification and differentiation.

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Topic 2

Neuronal excitability, Synapses and Glia: Cellular mechanisms

Posters

A MOUSE GENETIC STRATEGY TO INVESTIGATE THE ROLE OF CSP ALPHA/DNAJC5 IN GLUTAMATERGIC SYNAPTIC FUNCTION AND MAINTENANCE

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Cysteine String Protein (CSPα/DNAJC5) is a synaptic co-chaperone that prevents activity-dependent degeneration of synapses. CSPα/DNAJC5 knock-out mice suffer from a neurological phenotype and early postnatal lethality soon after the first month of age. CSPα/DNAJC5 is critical to maintain the levels of the SNARE protein SNAP25, especially in highly active synapses. Indeed, the decrease in SNAP25 levels is thought to be a key event leading to presynaptic neurodegeneration. We are interested in studying the synaptic effects of removing CSPα/DNAJC5 from adult glutamatergic neurons that operate at a low activity regime. Based on a mouse line bearing a Dnajc5 floxed allele (Nieto Gonzalez et al., Proc. Natl. Acad Sci. USA, 2019) we have used the CamKIIαCreERT2 mice (Erdmann et al. BMC Neurosci. 2007) to conditionally target Dnajc5 in adult hippocampal glutamatergic neurons (CaMKCreERT2:Ai27D:Dnajc5flox mice). This line also expresses channelrhodopsin2 fused to the fluorescent reporter td-tomato (Ai27D) in targeted neurons. CSPα/DNAJC5 conditional knock-out mice survive and do not develop an evident neurological phenotype. We have analyzed synaptic transmission in two hippocampal synapses: (1) synapses formed by mossy fibers at CA3 pyramidal neurons (mf-CA3 synapse) and (2) synapses formed by Schaffer collaterals at CA1 pyramidal neurons (sc-CA1 synapse). Interestingly, those synapses show different phenotypes in the absence of CSPα/DNAJC5. We are investigating the molecular mechanisms of such a phenotype to understand why those apparently similar synapses have different requirement of CSPα/DNAJC5 and how those differences might be related to SNAP25.

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ACTIVITY-DEPENDENT RECONNECTION OF ADULT-BORN DENTATE GRANULE CELLS IN A MOUSE MODEL OF FRONTOTEMPORAL DEMENTIA

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Frontotemporal dementia (FTD) is characterized by remarkable neuronal loss in both the frontal and temporal lobes of the brain. FTD-Tau is a variant of this disease that belongs to the family of tauopathies. Our laboratory generated a mouse model which carries three familial mutations, namely G272V (V), P301L (L), and R406W (W), on MAPT, the gene encoding tau. This mouse model (named TauVLW) exhibits behavioural impairments as well as hippocampal anatomic alterations similar to those described in patients with FTD. The hippocampus is involved in learning, memory, and mood regulation. Moreover, it hosts the generation of new hippocampal dentate granule cells (DGCs) throughout life. This process, named adult hippocampal neurogenesis (AHN) is impaired in patients and animal models of neurodegenerative diseases.

We used a novel combination of retroviral approaches to in-depth characterize the morphological and functional maturation of adult-born DGCs in TauVLW mice. First, we used Red-Green-Blue (RGB) retroviruses to address the temporary course of the morphological alterations observed in these neurons. Retroviruses encoding either PSD95: GFP or Syn: GFP revealed dramatic impairments in the afferent and efferent connectivity of newborn DGCs in TauVLW mice. Monosynaptic retrograde rabies virus tracing showed that these cells are disconnected from distal brain regions and local sources of excitatory innervation, and subjected to increased inhibitory innervation by local interneurons. Similarly, increased levels of markers of inhibitory neurotransmission were found in the DG of FTD patients.

Finally, we used a retrovirus that encodes the excitatory Designer Receptor Exclusively Activated by Designer Drugs (DREADDs) H3Dmq and achieved a complete reversion of the morphological alterations exhibited by newborn DGCs of TauVLW mice. Moreover, functional impairments were also partially reversed. Our results suggest that chemoactivation may be explored as a future therapeutic target for the treatment of distinct neurodegenerative diseases in which neuronal connectivity is compromised.

ACUTE GENETIC ELIMINATION OF A SYNAPTIC CO-CHAPERONE IN ADULTHOOD TO STUDY PROTEIN STABILITY IN NEURODEGENERATION

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Neurons and synapses operate during decades to sustain brain function throughout life. Synaptic proteins last only for several days or weeks. The mechanisms by which synaptic proteins are maintained are not well understood yet. We are interested in the mechanisms of action of a trimeric chaperone complex (CSPα/DNAJC5-SGTA-HSC70) involved in that process and to identify key client proteins. Conventional knockout mice lacking CSPα/DNAJC5 develop presynaptic degeneration and die soon after birth, making extremely difficult studies in adulthood. We have bred mice bearing the Dnajc5 floxed allele (Nieto Gonzalez et al. Proc. Natl. Acad. Sci. USA. 2019) against UBC-Cre-ERT2 mice (Ruzankina et al. Cell Stem Cell. 2007) to target Dnajc5 ubiquitously in a time-controlled manner. CSPα/DNAJC5 is almost undetected in hippocampal cultures of UBC-Cre-ERT2:Dnajc5flox/flox mice after 13 days in tamoxifen. Next, to investigate the function of CSPα/DNAJC5 in adulthood we fed 2 months old UBC-Cre-ERT2:Dnajc5flox/flox mice with tamoxifen during 15 days. Two weeks after ending tamoxifen administration, mice exhibited weight loss and a neurological phenotype characterized by loss of spontaneous activity, strength and motor coordination. This lead, two weeks later, to early death. We focused on hippocampus to detect, as expected, a general decay in the levels of CSPα/DNAJC5 transcript (RNAscope) and protein (western-blot). However, using immunofluorescence, we detected that the level of decay of CSPα/DNAJC5 was not the same in different hippocampal synaptic layers. This suggests that CSPα/DNAJC5 lifetime is not only determined by its amino acid sequence but also by environmental influences such as the specific neuronal type and/or the network activity. This novel mouse model will be used to investigate changes in protein stability related to neurodegeneration.

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ADAPTATIVE MYELIN PLASTICITY LINKED TO INCREASED NEURONAL EXCITABILITY IN THE SOMATOSENSORY CORTEX

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The neocortex is organized in vertical columns responsible to integrate and compute signal information. In addition to that, distinct layers within the vertical organization project horizontally to neighbouring columns in order to distribute activity between cortical areas. Interestingly, recent anatomical data shows that horizontal axonal projections, specially the long-ones in L2/3, exhibit increased myelin segments elongation following monocular deprivation. Such adaptive myelin plasticity may improve synaptic efficacy of corticocortical connections with a possible role in mediating the reorganization of cortical maps after sensory deprivation. Here, we explored the relationship between axonal myelination and the level of cortical reorganization in distinct layers of the somatosensory cortex following central sensory deprivation. Furthermore, we also investigated the ability of astrocytes, known to provide trophic factors for myelinating glia, to influence the adaptive myelin plasticity. Complete thoracic spinal cord was used to induce sensory deprivation of the cortical areas receiving information from hindlimbs. Fifteen-to-thirty days later, injured and control animals were subjected to electrophysiological recordings using a vertical array lowered into the hindlimb cortex to record evoked potentials in response to contralateral forelimb stimulation. Our experimental design aimed to determine the strength of the synaptic connectivity between the deprived and intact cortex. Our data showed that sensory deprivation enhanced L2/3 corticocortical connectivity observed as increased magnitude and slope of deprived neurons. Next, we explored whether the increased L2/3 synaptic efficacy was associated to myelin remodelling. While immunostaining against the neurotrophic factor-oligo2 showed no overall changes in both deprived and intact cortices, myelin basic protein staining showed increased intersections and longitudinal myelination patterns. These changes were not observed in IP3R2-/- mice exhibiting deficient astrocyte activity, suggesting that astrocytes directly impact myelination. Overall, our data indicate a positive correlation among neuronal excitability and adaptive myelin plasticity that may mediate cortical reorganization through increased L2/3 corticocortical connectivity.

ADENOSINE RECEPTOR-MEDIATED DEVELOPMENTAL LOSS OF SPIKE TIMING-DEPENDENT DEPRESSION IN LAYER 4 TO LAYER 2/3 SYNAPSES OF SOMATOSENSORY CORTEX

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Spike timing-dependent plasticity (STDP) is a Hebbian learning rule important for synaptic refinement during development and for learning and memory in adults. Presynaptic spike timing-dependent long-term depression (t-LTD) exists in layer 4 to layer 2/3 synapse of somatosensory cortex, which is present until the 4th postnatal week in mice, disappearing at the end of this 4th (P13-P27) week of development. We were interested in the mechanisms underlying this developmental loss of t-LTD. We have found that t-LTD is recovered when adenosine A1Rs are antagonized at P28-P37, whereas it´s induction is prevented at P13-P27 by activating A1Rs. Furthermore, we found that the adenosine that mediated the loss of t-LTD is supplied by astrocytes, as when astrocytes are treated with the Ca2+ chelator BAPTA, t-LTD is recovered at P28-P35. Similarly to STDP, pairing the stimulation of astrocytes with EPSPs at P13-P27 induces LTD, but not at P28-P37. These results provide direct evidence for the mechanism that closes the window of plasticity associated with t-LTD, revealing novel events probably involved in synaptic remodeling during cortical development.

AFFERENT SYNAPTIC TERMINALS ON SPINAL CORD MOTOR NEURONS ARE ACUTELY DISRUPTED AFTER PERIPHERAL NERVE TRANSECTION: INVOLVEMENT OF NECROPTOTIC PATHWAY AND MICROGLIAL PIECEMEAL PHAGOCYTOSIS

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The disconnection of motor neurons (MN) from their skeletal muscle targets, as occurs after a peripheral nerve section, leads to a rapid recruitment of reactive microglia in the affected regions of the spinal cord. We reported that the degeneration of synaptic inputs on axotomized MNs occurs closely associated with activated recruited microglia. Synaptic terminals undergo disrupted and fragmented leading to extracellular vesicles before they are "ingested" by microglia. These observations are in contrast with the classical concept of synaptic stripping in which afferent terminals are detached from the surface of axotomyzed MNs by microglia without degeneration signs.

To explore the interactions between recruited microglia and presynaptic terminals on lesioned MN somata we have performed electron and confocal microscope analysis of axotomized MNs between 1 - 15 days after sciatic nerve transection in mice.

Between 1 and 3 days post-injury, microglial cells surrounding the injured MNs were highly phagocytic showing an increased expression of the lysosomal marker CD68. After ultrastructural examination we did not observe bulk engulfment of synaptic boutons by microglia. Instead, microglia internalized small membranous-vesicular fragments originated from the disruption of synaptic terminals. Abundant extracellular vesicles in the perineuronal space after axotomy were seen together with the expression of the necroptosis effector protein p-MLKL, and later, with the appearance of exosomal markers. Moreover, activated microglia and synaptic boutons displayed C1q immunoreactivity, suggesting a contribution of the complement to the microglial-mediated synaptic elimination.

Overall, our data reveals new mechanisms by which afferent synapses are removed from acutely injured MNs. Microglia is actively involved in eliminating fragments of damaged presynaptic terminals. Furthermore, our data is relevant in the context of neuroinflammation and MN disease as well as for understanding the functional recovery after peripheral nerve injury.

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AGING ENTAILS MOTONEURON DEAFFERENTATION AND NEUROINFLAMMATION IN THE MOUSE SPINAL CORD

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Aging is accompanied by functional and structural alterations in the neuromuscular system. The causative factors of sarcopenia associated with aging are controversial and poorly understood, hampering the development of effective therapeutic interventions.

In the present study, we simultaneously analyzed changes in distinct components of the neuromuscular system of young, adult, middle-aged and old C57BL/6J mice, including: motoneurons (MNs), glia, and motor nerves.

We found that aging was not accompanied by a significant loss of spinal MNs, although a proportion of them in old mice exhibited an abnormally dark appearance. Morphological alterations in motor axons were already noticed in adulthood but substantially increased with age. We observed prominent microgliosis and astrogliosis around MNs, with significant increased density of pro-inflammatory M1 microglial and A1 astroglial phenotypes, and reduced proportion neuroprotective M2 microglia and A2 astroglia. Microglial cells exhibited an activated state in old mice, as we found a reduction in the length of branching and an increase in phagocytic markers inside these cells. Moreover, aged MNs were depleted of cholinergic and glutamatergic afferent terminals, the density of V0c interneurons was reduced and dorsal root ganglia cell populations were affected in terms of size and number, all of this suggestive of age-associated alterations in MN excitability and firing.

Overall, these results provide a global view of age-associated changes in the neuromuscular system of mice. Further work is necessary to examine the relevance of gliosis in MN deafferentation occurring with aging and the impact of both processes in motor-activity defects found in the elderly.

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ASTROCYTE-MEDIATED SWITCH IN SPIKE TIMING DEPENDENT PLASTICITY DURING HIPPOCAMPAL DEVELOPMENT

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Presynaptic spike timing-dependent long-term depression (t-LTD) at hippocampal CA3-CA1 synapses is evident until the 3rd postnatal week in mice, disappearing during the 4th week. At more mature stages, we found that the protocol that induced t-LTD induced t-LTP. We characterized this form of t-LTP and the mechanisms involved in its induction, as well as that driving this switch from t-LTD to t-LTP. We found that this t-LTP is expressed presynaptically at CA3-CA1 synapses, as witnessed by coefficient of variation, number of failures, pairedpulse ratio and miniature responses analysis. Additionally, this form of presynaptic t-LTP does not require NMDARs but the activation of mGluRs and the entry of Ca2+ into the postsynaptic neuron through L-type voltage-dependent Ca2+ channels and the release of Ca2+ from intracellular stores. Nitric oxide is also required as a messenger from the postsynaptic neuron. Crucially, the release of adenosine and glutamate by astrocytes is required for t-LTP induction and for the switch from t-LTD to t-LTP. Thus, we have discovered a developmental switch of synaptic transmission from t-LTD to t-LTP at hippocampal CA3-CA1 synapses in which astrocytes play a central role and revealed a new form of presynaptic LTP and the rules for its induction

ASTROCYTES EXERT NEGATIVE MODULATION ON HIPPOCAMPAL NEURON EXCITABILITY

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Neuronal firing is the essential element of neuronal networks as action potentials are the end product of synaptic integration. Therefore, brain neurons adjust their intrinsic membrane excitability to maintain the firing rate within their own optimal operational range. A principal homeostatic factor of neuronal excitability in the mammalian hippocampus is the postburst afterhyperpolarization (AHP). AHP exerts a negative control predominantly through a Ca2+-dependant K+-current that contributes to the slow AHP (sIAHP) responsible for the spike-frequency adaptation, and can be dynamically influenced by neuronal modulators. Accumulating evidence indicates that astrocytes respond to neurotransmitters released by synaptic terminals and- modulate neuronal activity and synaptic transmission through the release of gliotransmitters. However, little is known about the direct effect of astrocyte signalling on neuronal intrinsic properties and the excitability of neuronal networks beyond synapses. To address this issue, we used electrophysiological and Ca2+ imaging techniques in mouse hippocampal slices, as well as chemogenetic, electric and optogenetic stimulation of astrocytes and/or GABAergic interneurons. We saw that chemogenetic stimulation of astrocytes with Clozapine-N-oxide (CNO) in CA1 hippocampal region decreases pyramidal neuron excitability through increasing sIAHP and the reduction of action potential firing. Both effects were blocked by using specific adenosine 1 receptor (A1R) antagonist. We next hypothesized that GABAergic interneurons may play an important role regulating ATP/adenosine release from astrocytes. Therefore, we used high frequency stimulation and optogenetic protocols to specifically stimulate hippocampal interneurons. In these experimental conditions, we found that GABA released from interneurons activates astrocytic GABAB receptors. Consequently, astrocytes release ATP/adenosine, which acts on pyramidal neurons A1 receptors increasing sIAHP and reducing neuronal excitability. Present results uncover the role of astrocytes in the regulation of neuronal intrinsic properties and reveal a novel mechanism involved in network dysfunctions and brain disorders related with neuronal hyper-excitability.

ASTROCYTES GATE SPIKE TIMING DEPENDENT PLASTICITY IN THE NUCLEUS ACCUMBENS

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The nucleus accumbens (NAc) is a pivotal locus for reward-related behaviours and addiction circuitry. It is mainly composed by medium spiny neurons that receive dopaminergic and glutamatergic afferents. The glutamate homeostasis in the NAc is regulated by the glutamate transporter 1 (GLT-1), a highly specific glutamate electrogenic transporter that is expressed on astrocytes. GLT-1 controls signal transmission uptaking glutamate from the synaptic cleft and thus controlling the time course of excitatory postsynaptic currents and potentials. Drugs of abuse causes glutamatergic dysregulation in the NAc and a downregulation of GLT-1 after prolonged drug-withdrawal. Although numerous studies show that GLT-1 regulate the synaptic plasticity triggered by different cell conditioning paradigms like spike timing-dependent plasticity (STDP). In STDP, the temporal coincidence of pre- and postsynaptic spiking activity leads to long-term potentiation or depression. However, the role of astrocytes in the regulation of NAc plasticity induced by drugs of abuse is poorly understood.
In this study, we combined cell biology and electrophysiological approaches in brain slices, to test the hypothesis that dopaminergic activity alters the expression of astrocytic GLT1 and regulates the time course of glutamatergic synaptic transmission in the NAc. Astrocytic activation with opto-stimulation of dopaminergic axons, or with different drug abuse or with selective stimulation via DREADDs decreases GLT-1 functionality and glutamate synaptic currents in astrocytes. Furthermore, we found an increase in time and space of glutamate in the synaptic cleft modifying the kinetic of excitatory postsynaptic potentials. This phenomenon allowed us to find a temporal window between presynaptic activity and postsynaptic spikes in STDP paradigm in which the synaptic weigh was modified after drugs of abuse, adjusting the computational ability of the system during addiction.

ASTROCYTIC NETWORK HETEROGENEITY IN THE NUCLEUS ACCUMBENS

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Astrocytes have been traditionally studied as a homogeneous group, however, recent research has started to evidence their heterogeneity between different brain areas and within the same region. Our hypothesis is that specialized astrocyte subsets are responsible for the modulation of specific neuronal circuits. In the NAc converge different glutamatergic signals coming primarily from the medial prefrontal cortex (mPFC), basolateral amygdala (Amyg), and ventral hippocampus (vHip), providing us the perfect structure to study the presence of specialized astrocytic networks.
In this work, we analyze whether astrocytes establish segregated populations in the NAc with intrinsic properties and functional consequences for the circuit. To this end, we have used optogenetic manipulations to perform afferent-specific synaptic stimulation to the NAc, combined with a new adapted technique (CaMPARIGFAP, calcium-modulated photoactivatable ratiometric integrator under GFAP promoter) to specifically dissect the active astrocyte circuits with spatio-temporal precision.
We demonstrate that NAc astrocytes show pathway-specific interactions with the glutamatergic afferents coming from the mPFC, Amyg, and vHip, and that this activity unexpectedly does not correlate with glutamatergic innervation patterns, suggesting astrocytic connectivity, i.e. activation of a precise astrocytic population in response to specific glutamatergic inputs. Moreover, the activation of these defined spatial astrocytic networks are not influenced by alterations in astrocyte density or by uneven expression of mGluR5. Finally, we show that different sub-populations of astrocytes in both NAc regions receive and integrate signals arising from all the excitatory afferents.
This work reveals astrocytic functional heterogeneity in the NAc regarding glutamatergic signaling, showing pathway-specific astrocytic responses mediated by mGluR5. Also, all these observations provide a potential explanation for comprehension of how NAc integrates information from multiple glutamatergic regions.

ASTROGLIAL CB1 MEDIATES SYNAPTIC PLASTICITY IN THE NUCLEUS ACCUMBENS

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The Nucleus Accumbens (NAc) has a prominent role in the reward system and its activity is critical for the correct processing of relevant emotional information being involved in positive and negative reinforcement. Type-1 cannabinoid receptors (CB1), the main elements of the endocannabinoid system (ECS), participate in reward processing in part by its direct impact on synaptic plasticity in the NAc. Indeed, impaired endocannabinoid (eCB) signaling contributes to dysregulated synaptic plasticity, increased stress response, negative emotional states and cravings that propel addiction. Astrocytes play active roles in information processing by sensing synaptic activity and releasing neuroactive molecules – called gliotransmitters – that activate neuronal receptors. Through the release of gliotransmitters, astrocytes have been found to modulate neuronal activity and synaptic transmission in several brain areas and to impact animal behavior. CB1-mediated astrocyte-neuron communication has been shown in different brain regions, but the functional astrocyte-neuron interactions and its underlying mechanisms in the nucleus accumbens (NAc) are unknown. We used electrophysiology, pharmacology and cell-type specific transgenic mouse lines to investigate the role of astroglial CB1 in synaptic plasticity in the Nucleus accumbens core. We found that CB1 specifically expressed in astrocytes, but not in neurons, are necessary for spike-timining-dependent long-term depression (t-LTD) in the NAc, a form of plasticity critically involved in memory formation. We also found that astrocytes release purines downstream of the CB1 activation, which is a key step for t-LTD. Synaptic modifications in the NAc are important for appetitive/aversive-dependent learning. Here we show that astroglial CB1 mediate long-term synaptic depression in the NAc and reveals astrocytes as new potential targets for treatment of motivational disorders.

ASTRO-LIGHT: A NEW TOOL FOR MODULATION OF SPECIFIC ASTROCYTIC NETWORKS

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Unravelling the principles of information processing in complex cell circuits requires techniques capable of target and modulate specifically the activity of those elements involved. Although it has been demonstrated that astrocytes play an active role in neuronal transmission, the evolution of genetic tools to study and control these circuits has focused mainly on neuronal activity. Currently, there are available techniques to modulate astrocytic activity with precise temporal control (optogenetics), or in a sustained activation period (chemogenetics). Nevertheless, these tools act on the whole astrocytic population and it remains challenging to target gene expression or activity in specific populations of astrocytes, leaving the role of these cells in neural circuits or behaviour still unclear.

In this study, we present a new tool to translate the activity-mediated calcium signals of astrocytes into gene expression in a light-dependent manner, i.e. Astro-Light. Using a combination of electrophysiology, molecular, pharmacological and behavioural techniques, we have tested Astro-Light capacity to modulate the activity of specific astrocytic networks with implications in animal behaviour.

First, we engineered Astro-Light vectors under GFAP promoter and characterized Astro-Light expression after viral infection in the mouse Nucleus Accumbens (NAc). We apply Astro-Light to label astrocytes in the NAc that are activated during optogenetic stimulation of long-range excitatory inputs thought to regulate motivated behaviors. Finally, we tested the ability of Astro-Light to modulate animal behaviour.

Our results reveal Astro-Light as a functional and powerful tool for studying astrocyte-neuron interactions and enables dissection of astrocytic circuits underlying complex behaviors with high spatiotemporal precision.

BASAL AUTOPHAGY INHIBITION IN MICROGLIA DIMINISHES PHAGOCYTOSIS OF APOPTOTIC CELLS AND MICROGLIAL SURVIVAL

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Autophagy is the cellular process whereby cytoplasmic constituents such as long-lived proteins and damaged organelles are delivered to the lysosome for degradation. Autophagy is basally active in virtually all cell types, wherein it functions as a cellular quality control checkpoint to promote cellular fitness and survival. However, the role of basal autophagy in microglia, the brain resident macrophages, has started to emerge only recently. In this study, we have evaluated the effect of basal autophagy inhibition in microglial phagocytic function and survival. To monitor the autophagic activity of microglia, we first developed a 2-step model to separately assess autophagosome formation and degradation in microglia using conventional LC3 Western blot assays. Using this model, we confirmed that after treatment with the unc-like kinase1/2 (ULK1/2) inhibitor MRT68921, which inhibits the autophagy pre-initiation complex, reduced both autophagosome formation and degradation proportionally in primary microglia, leading to a reduced turnover ratio of autophagosomes and an inhibition of basal autophagy. Next, we assessed the effects of basal autophagy inhibition in microglial phagocytosis of apoptotic cells and survival using pharmacological in vitro and genetic in vivo approaches. In vitro, MRT68921 impaired microglial phagocytosis of apoptotic cells at low concentrations that did not induce microglial death. However, high concentrations of MRT68921 did induce significant microglial death, suggesting that basal autophagy disruption is critical for microglial survival. In vivo, constitutive deletion of autophagy-related genes such as ATG4B, also disrupted microglial phagocytosis and decreased microglial survival in the neurogenic niche of the hippocampus, where newborn neurons constantly undergo apoptosis and are rapidly engulfed by microglia. We are now extending our analysis to other microglia-specific autophagy-deficient mouse models such as ULK1 or BECN1. In conclusion, our results indicate that basal autophagy shapes microglial fitness regulating their function and survival.

BRAIN ESTROGEN SYNTHESIS REGULATES SYNAPTIC INHIBITION IN FEMALE HIPPOCAMPUS

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The aim of our work was to study the contribution of estrogen, the main feminine sex hormone, to the function of hippocampal inhibitory neurons (IN). IN play a critical role in controlling different forms of network activity and plasticity underlying cognition. Estrogens are synthesized in the brain by neurons that express the enzyme aromatase and regulate excitatory synaptic function and plasticity. However, the role of brain-derived estrogen role in controlling IN function is unknown.

We first studied the contribution of hippocampal CA1 IN to brain estrogen synthesis. We detected aromatase mRNA and protein in CA1 IN expressing the marker parvalbumin (PV), suggesting estrogen synthesis in this major IN subtype. We then studied the functional impact of brain estrogen synthesis on CA1 synaptic inhibition using patch-clamp recordings on brain slices from female ovariectomized mice. Our results revealed that blockade of brain aromatase increases CA1 synaptic inhibition by limiting the activation estrogen receptors and lead to maturation of perineuronal nets (PNNs), extracellular structures that control PV+ IN excitability. Enzymatic digestion of PNNs impaired aromatase regulation of synaptic inhibition, suggesting that plasticity of PNNs is part of the mechanism used by estrogen to regulate the function of PV+ IN.

Interestingly, aromatase regulation of PV+ IN was only present in female mice. Using a transgenic line in which sex determination is independent of sex chromosomes and manipulations of perinatal gonadal hormone levels, we found that female-specific regulation of PV+ IN has a gonadal origin and is independent of the genetic sex of the brain.

Our results reveal sex differences in the regulation of PV+ IN by brain synthesized estrogen. Since aromatase inhibitors are widely used in clinics, our results have implications for understanding the adverse effects that these treatments have on cognitive functions in humans.

CANNABINOID RECEPTOR TYPE 1 (CB1R) EXPRESSION IN THE BRAIN STRUCTURES OF GENETIC MODELS OF EPILEPSY

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The endocannabinoid system (ECS) is related to several physiological processes associated with the modulation of brain excitability, with impact on the expresión, susceptibility and control of epileptic seizures. The cannabinoid receptor type 1 (CB1R) is widely expressed in the brain, especially in the limbic structures of the forebrain. Changes in CB1R expression are associated with seizures in animal models and in humans. The Wistar Audiogenic Rat (WAR) strain and Genetically Audiogenic Seizure-Prone Hamster from Salamanca (GASH/Sal) are genetic models of epilepsy that present tonic-clonic and limbic seizures in response to intense sound stimulation. In this work we aim characterize the expression of CB1R by immunohistochemistry in brain structures important for the expression of limbic seizures. For this, we used an immunohistochemical protocol to evaluate the effects of acute and chronic audiogenic seizures on the expression of CB1R in different regions of the hippocampus and amygdala. WARs showed increased immunostaining for CB1R in the inner molecular layer of the hippocampus. Acute and chronic audiogenic seizures increased CB1R immunostaining in limbic structures of WARs. Furthermore, changes in CB1R expression in the amygdala, but not in the hippocampus, were associated with limbic recruitment and severity of limbic seizures in WAR. The expression of CB1R in GASH/Sal showed a wide distribution in many brain nuclei. These CB1R immunostaining patterns are practically identical between the GASH/Sal and the control animals, varying in the intensity of immunostaining in limbic regions, being slightly weaker in the GASH/Sal than in the control, mainly in brain regions associated with epileptogenic circuits. Our results suggest that endogenous alterations in CB1R immunostaining in genetic models of epilepsy could be associated with genetic susceptibility to audiogenic seizures. Also, we demonstrate neuroplastic changes of CB1R in amygdala and hippocampus is associated with acute and chronic seizures. Furthermore, the present study provides important information on CB1R and susceptibility to seizures in genetic animal models of seizures and supports the relationship between ECS and epilepsy.

CELL TO CELL COMMUNICATION MEDIATES THE NEURODEGENERATION CAUSED BY GLIOBLASTOMA

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Glioblastoma (GB) is the most aggressive and frequent primary brain tumor. Current treatments include radio-, chemotherapy and surgical resection of the solid core of the tumor. However, GB infiltrative cells cause that almost 100% of the patients undergo relapses and cause death in 16 months. GB cells produce cellular protrusions known as Tumor Microtubes (TMs) or cytonemes, which facilitate tumor expansion and cellular interaction among GB cells and with healthy surrounding neurons.

We use a Drosophila melanogaster GB model to study glia-neuron cellular interactions that contribute to the neurodegeneration induced by GB signals. TMs expand through the brain and connect GB cells, and with the healthy neurons through synapses.

As a consequence of GB-neuron interaction, WNT pathway and Insulin Receptor (InR) signaling attenuation play a central role in the neurodegeneration associated to GB. TMs accumulate specific Frizzled receptors that contribute to the depletion of WNT from surrounding neurons. This imbalance in WNT pathway causes JNK pathway activation and Matrix Metalloproteases (MMPs) secretion, MMPs degrade extracellular matrix and facilitates further TMs expansion. In consequence of WNT depletion, neurons undergo synapse loss and neurodegeneration that contribute significantly to the premature death caused by GB.

Besides, GB cells also produce ImpL2, an antagonist of the Insulin receptor known as IGFBP7 in humans. ImpL2 is secreted and impact on neighboring neurons, in consequence Insulin pathway is repressed, causes mitochondrial defects and synapse loss. Restoration of InR signaling in neurons counteracts neurodegenerative effects of GB.

CHARACTERIZATION OF PRIMARY AND IMMORTALIZED WHALE MÜLLER GLIAL CELLS

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Müller cells are the principal glia of the retina, expressing growth factors, neurotransmitter transporters and antioxidant agents with an important role in preventing excitotoxic damage to neurons. Although fish Müller cells can be transformed into neurons, this has never been described in mammals. In the present study, whale Müller cells were cultured and immortalized, with the aim of analysing the molecular characteristics as well as the division rate in vitro of primary and immortalized whale Müller cells.
The eye of Balaenoptera borealis was obtained, retina was isolated and Müller cells were cultured. Half of the cultures were immortalized with simian virus 40 T-antigen. Primary as well as immortalized Müller cultures were grown until primary cells reached senescence. Specific Müller molecules, dedifferentiated, neuronal precursors and neuronal markers were studied in both primary and immortalized cells. Ultrastructural morphology was also studied by scanning electron microscopy (SEM). In addition, the proliferation kinetics (time between divisions, percentage of dividing cells and division duration) was analyzed by time-lapse. Karyotype characterization was performed in immortalized whale Müller cells.
Whale Müller cells were immortalized after 10 passages and approximately 2 months of culturing. Müller markers were preserved, while expression of dedifferentiation markers was observed after the 5th passage. At high passages, neuronal precursor markers were weakly expressed. In addition, immortalized cells were stained extensively with neuronal markers. Immortalized Müller immunostaining and SEM revealed heterogeneous cell morphologies due to changes in the cytoskeleton. The proliferation kinetics demonstrated that primary whale Müller cells divides every 23 h, approximately, while after the immortalization process the time between divisions increased to 29 h.
In conclusion, we have generated a cell line from whale Müller cells that maintains primary Müller characteristics but presents a partially dedifferentiated state. In addition, we present a detailed analysis of the rate of cell division during the immortalization process.
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CHARACTERIZATION OF SYNAPTIC TRANSMISSION OCCURRING IN OLFACTORY GLOMERULI OF X. TROPICALIS TADPOLES IN VIVO

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Communication among neurons occurs in synapses in a highly regulated process that determines the way we see, smell or behave. In the olfactory system, the glomeruli of the olfactory bulb are the first site of odour processing in the brain. Here, many synapses are established between olfactory sensory neurons and olfactory bulb neurons. The western-clawed frog (Xenopus tropicalis) provides an excellent experimental platform to investigate the molecular mechanisms of synaptic transmission in vivo due to its experimental accessibility, transparency and possibilities of genetic manipulation. The aim of the present study is to characterize the bases of neurotransmission taking place in olfactory glomeruli. We investigated long-lasting depolarizations (LLDs) that were identified in local field potential recordings obtained from the glomerular layer of the olfactory bulb. Odorant responses were obtained after ipsilateral stimulation of the olfactory epithelium with amino acid solutions. Amino acids applied at 200 µM triggered responses in the majority of animals investigated: arginine 82% (n=9), histidine 76% (n=9), leucine 77% (n=14) and methionine 63% (n=39). This evidence shows a poor selectivity of the olfactory system of X. tropicalis tadpoles to detect odorants. To assess sensitivity, methionine was applied from 0.01 µM to 200 µM. The threshold for detecting responses was found at ~1 □M. The experimental approach was refined using the zHB9::GFP transgenic line, which allowed the recording of a single identified glomerulus. The amplitude of LLDs obtained after 20 µM and 200 µM application of methionine changed from 27 µV (n=5) to 49 µV (n=5), respectively. This result suggests that the amplitude of LLDs obtained in olfactory glomeruli is related to the concentration of the odorant applied. Future experiments are required to determine the precise contribution of presynaptic terminals of olfactory sensory neurons to LLDs.

CHEMOGENETIC STIMULATION OF MATURE OLIGODENDROCYTES DRIVES MYELIN-AXON METABOLIC COUPLING AND PREVENTS AXONAL DAMAGE

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Oligodendrocytes make myelin and support axons metabolically with lactate. Experience and neuronal activity can induce dynamic changes in myelination during development and in adult life, suggesting a new form of plasticity to adapt brain function to environmental stimuli. Myelin remodeling is driven mainly by newly-formed oligodendrocytes from precursors cells. However, the role of mature oligodendrocytes in plastic changes of myelin is practically unknown. We have generated transgenic mice, using the CreERT2-lox technology, overexpressing the DREADD receptor hM3Dq under the PLP promoter, specific of mature oligodendrocytes. Chronic stimulation of hM3Dq receptors induced an increase in myelination of axons in cerebral cortex and corpus callosum, and consequently, an increase in axonal conduction velocity of interhemispheric callosal connections. Importantly, acute stimulation of hM3Dq+ activates metabolism in oligodendrocytes. We detected an increase in glycolytic rate and in lactate production and release. Moreover, these higher metabolic coupling between oligodendrocytes and axons maintained axonal function under high frequency stimulation and prevented axonal damage secondary to oxygen glucose deprivation. We then tested the impact of mature oligodendrocytes stimulation to promote remyelination and protect axons in demyelinating disease models. Preliminary data show that chemogenetic oligodendrocyte stimulation ameliorates motor symptoms of mice with experimental autoimmune encephalomyelitis, a model of multiple sclerosis. Taken together, these findings indicate that this chemogenetic mouse line is a very useful tool to elucidate the contribution of mature oligodendrocytes to myelin remodeling in physiological and pathological conditions, and reveals a novel role of myelin-axon lactate shuttle in axonal protection.

COGNITIVE FUNCTIONS THAT RELY ON DORSAL HIPPOCAMPAL SYNAPTIC PLASTICITY PROCESSES INVOLVE A G-PROTEIN DEPENDENT MECHANISM THROUGH ADENOSINE A1 RECEPTOR-ACTIVATED GIRK CHANNELS

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A1 adenosine receptor-mediated GirK (G-protein-gated inwardly rectifying potassium) channels conductance is constitutively active in dorsal CA1 neurons contributing to the resting membrane potential. Its disruption has been linked to the etiology of many diseases that involve neural excitability alterations, such as Alzheimer's disease, suggesting a critical role of GirK channels for cognitive processes that depend on hippocampal neuronal activity.

Here, we aimed to explore the role of A1 adenosine receptor-mediated GirK basal activity in the regulation of synaptic plasticity processes supporting dorsal hippocampus-dependent cognitive capabilities.

To achieve this aim, we pharmacologically modulated basal GirK channel conductance in the dorsal hippocampus by using A1 adenosine receptor modulators or by direct selective manipulation of channel activity and we examined its involvement in controlling synaptic plasticity processes at different levels of complexity.

First, using mice dorsal hippocampal slice preparations, we examined pharmacological A1 receptor and GirK channel activity modulation effect on the induction and maintenance of long-term synaptic plasticity at CA3-CA1 synapse. Additionally, using an in vivo approach, we performed acute intracerebroventricular injections of GirK selective modulators to study their contribution to CA3-CA1 synaptic plasticity and subsequent learning and memory functions.

Our data indicates that a G-protein dependent mechanism through A1 adenosine receptor-activated GirK is required for long-term synaptic plasticity processes in dorsal hippocampus, as both A1 receptor and GirK channel activity modulation modified LTP/LTD induction threshold ex vivo and in vivo, even transforming HFS-induced LTP into LTD. Also, the disruption of such mechanism leads to hippocampal plasticity-dependent learning and memory deficits as shown during the behavioral tasks such as open field habituation.

Together, these results provide evidence that A1 adenosine receptor-mediated GirK basal activity governs hippocampal synaptic plasticity direction, which has a significant impact on hippocampal-dependent cognitive functions.

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COMPARATIVE EFFECT OF GLUTAMATERGIC RECEPTORS AGONISTS N-METHYL-D-ASPARTATE AND KAINATE ON MOUSE INNER RETINAL CELLS

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Purpose: Due to the heterogeneous sensitivity of the different retinal neurons to glutamate agonists, our objective is to compare the effect of two glutamate agonists, N-methyl D-aspartate (NMDA) and kainate (KA) on inner retinal cells. Recently, our group has developed a mouse model of inner retina degeneration induced by a combined intravitreal injection of those two glutamatergic agonists. Methods: C57BL6/J mice were intravitreally injected into the right eye with 1μL of PBS containing NMDA 10 mM or KA 3 mM (1μL PBS was inoculated into the contralateral eye). The effect on retinal function was evaluated post-injection by optomotor test and electroretinographic (ERG) recording. The structural retinal damage was assessed by immunohistochemical labelling of retinal cells on retinal sections. Results: Intraocular injection of NMDA/KA (10/3 mM) induces the abolition of optomotor response and a strong decrease of ERG b-wave at one-week post-treatment, but does not show any functional nor structural alterations in photoreceptors. Both excitotoxic agents, when injected independently, caused a complete loss of optomotor response just 3 days post-injection. The sole KA (3 mM) treatment produced the loss of b-wave ERG components, both in scotopic and photopic conditions. By contrast, NMDA-treated eyes preserved these ERG components, but a marked decrease in their amplitude was observed. Nonetheless, no significant changes on the “a” wave amplitude were found after the injection of both agents. Immunohistochemical labeling showed no effects of NMDA nor KA on the outer nuclear layer, but a moderate damage on the inner retinal layers in NMDA-injected eyes, and a deleterious effect in KA-injected eyes. Conclusion: Retinal damage induced by KA shows a stronger effect than NMDA. However, differences observed between NMDA and KA injection could be caused by the different sensitivity of the retinal neurons, maintaining the possibility of inducing a synergic interaction with lower concentration of KA.

COMPETITION OF TRANSCRIPTIONAL PROGRAMS FOR TRANSCRIPTIONAL CO-ACTIVATORS UPON NEURONAL ACTIVATION

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Activity-driven transcription is essential for the consolidation of memory processes and other long-lasting behavioral changes, contributing to the etiology of important neurological disorders such as epilepsy. CREB- and AP1-dependent gene expression have been identified as critical transcriptional factors for neuronal activity-driven transcription. Furthermore, histone acetylation has been correlated with neuronal transcription during memory acquisition and formation. Our laboratory has recently demonstrated the involvement of CBP and p300, two histone acetyltransferases and transcriptional co-activators that are known to interact with CREB and AP1, in the maintenance of neuronal identity. However, the functional cooperation between transcriptional factors and epigenetic complexes during neuronal activity is not yet defined. To investigate this cooperation, we integrated transcriptomic, epigenetic and chromatin structural data from the adult mouse hippocampus generated by our laboratory in the context of an experimental model of status epilepticus. Our multiomic analysis shows that neuronal activation causes a dramatic, but transient, genomic redistribution of CBP and p300, and CBP/p300-dependent H3K27 acetylation. Together our experiments and analyses unveil an intriguing competition between neuronal homeostasis and plasticity transcription, that can have important implications in neuropathology, and demonstrate a specific role for CBP/p300 in the transcriptional changes occurring during neuronal activation.

CONTRIBUTION OF ASTROCYTE EXTRACELLULAR VESICLES TO LOCAL TRANSLATION IN NEURONS

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Local protein synthesis is a conserved mechanism by which mRNAs are localized to the cell periphery and proteins are synthesized at target sites. Local translation is especially relevant in polarized cells like neurons so that neurites can rapidly react to changes in their environment. For instance, the exposure of isolated axons to β-amyloid oligomers (Aβo), central to Alzheimer's disease, induces local protein synthesis and mediates neurodegeneration contributing to the disease. However, axons are not isolated in the nervous system but surrounded by other compartments or non-neuronal cells. Our laboratory is interested in the contribution of glial cells to local translation in neurons. Others have reported that extracellular vesicles (EVs) secreted by astrocytes are involved in the regulation of different neuronal functions. Based on these data, we hypothesize that astrocyte-derived EVs are delivered to neurons to modulate local protein synthesis in physiological and Aβo- induced conditions.
To assess the relevance of astrocytes in neurons local proteome, we isolated somata and neurites from primary cortico-hippocampal neurons cultured in Boyden chambers in absence/presence of astrocytes and analysed the extracted proteins by LC-MS/MS. Results show that the presence of astrocytes in control conditions changes the neuritic proteome. Gene Ontology analyses show that proteins significantly regulated in presence of astrocytes vs their absence are mainly involved in RNA binding, processing and translation. In Aβo-induced conditions, astrocytes also change neuritic proteins compared to only- neuron cultures, with translation-involved proteins among them. To determine whether these proteins are locally synthesized in neurites, we have selected 176 and are analysing their corresponding transcripts in somata and neurites.
We have also assessed if EVs are directly involved in translation regulation. Isolated EVs from neuron-astrocyte cultures increase translation levels in neurites, suggesting that EVs are relevant for local protein synthesis in neurons. We are deeply studying EVs by LC-MS/MS to search for translation regulators.
Altogether, our data provide a new mechanism of local translation regulation in which astrocyte-derived EVs could play an important role.

CORTICAL ASTROCYTES EXHIBIT FUNCTIONAL HETEROGENEITY TO DISCRIMINATE SENSORY MODALITIES

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Layer-specific activity of cortical circuits critically depends on the interplay among distinct cell types to shape sensory stimulus selectivity and control gain. In this complex network, astrocytes are crucial as they can sense the synaptic activity within the layering circuits to modulate the strength and timing of both excitatory and inhibitory neurons. However, cortical laminar distribution of astrocytes does not correspond to the six excitatory neuronal layers, which leads to the question on whether astrocytes across cortical layers may exhibit functional heterogeneity or engage in different interactions with neighbouring neurons to modulate neuronal dynamics of sensory processing. Here, we used a combination of in vivo electrophysiology, behavior and genetic tools to explore the layering functional organization of astrocytes as well as their ability to modulate the layer-specific neuronal circuitries. By using brain slices injected with the AAV5-gfaABC1D-cyto-GCaMP6f, we found that astrocyte activity is inherently distinct across cortical layers, with L2/3 astrocytes being less excitable and L5/6 exhibiting higher number of spontaneous oscillations. To determine whether such astrocyte functional diversity play a role in the neuronal sensory processing and stimulus selectivity, we used in vivo electrophysiology to record neuronal evoked-potentials across all layers and sensory behavioral tests while modulating astrocyte activity. Up-regulation of astrocyte activity using GFAP-hM3D(Gq)-DREADD decreased evoked-potential magnitudes in response to high-stimulus intensity in L4/5/6 neurons, which was accompanied by an increase threshold of paw withdrawal following thermal stimulation. On contrary, astrocytes down-regulation using IP3R2-/- mice line decreased L2/3/4 evoked-potential in response to low intensity stimulation and consequent increased threshold in response to tactile stimuli. Therefore, our data indicates that astrocytes work as a buffer of neuronal activity by plausible controlling E:I balance in a stimulus-dependent manner. In addition, astrocyte functional heterogeneity may serve to control stimulus sensitivity in a layer-dependent manner with consequences in behavior output.

DIFFERENTIAL EFFECTS OF PARAQUAT IN HUMAN AND MOUSE ASTROCYTE'S MEMBRANES.

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During last decades it has been observed an increasing prevalence in ageing concomitant diseases; a longer life expectancy due to an improvement of life conditions is the main factor of this augmentation. As astrocytes are responsible for proper neuronal function, their importance is clear to the maintenance of brain health. Metabolism homeostasis alterations, such as oxidative stress, can trigger these conditions, producing cellular pathological changes as lipid peroxidation, oxidative modification of proteins and DNA damage.

One of the main mechanisms involved in cellular oxidation is Reactive Oxygen Species (ROS) formation, key deregulators of cellular stability. ROS can be produced by mitochondrial electron transport chain dysregulation by external pro-oxidant compounds action, such as Paraquat.

To study sex influence, species and paraquat exposure on the activity of mitochondrial respiratory chain in astrocytes of different species, we developed microarrays using cell membranes isolated from a human astrocytic cell line (1321N1) and primary cultures of male and female mouse astrocytes. Subsequently, the superoxide formation capacity of each sample was determined on cell membrane microarrays using complex I substrate (NADH) combined with specific inhibitors of mitochondrial complex I (rotenone), complex III (antimycin A) and complex IV (azide). Paraquat treated astrocytes showed a higher superoxide formation when compared with control group, not only in basal condition but also in presence of respiratory inhibitors. A significant difference between male and female was also observed in mouse astrocytes primary cultures, while no difference was detected between human and mouse in male astrocytes.

Furthermore, the difference seen between control and paraquat-treated human astrocytes was not only due to mitochondrial electron transport chain, but also other NADH oxidoreductases might be implicated in ROS generation. Thus, further investigation with specific protocols will be necessary to elucidate it. How astrocytes manage oxidative stress and ROS formation is central to their neuroprotective responses.

DISSOCIATION OF FUNCTIONAL AND STRUCTURAL PLASTICITY OF DENDRITIC SPINES DURING NMDAR AND MGLUR-DEPENDENT LONG-TERM SYNAPTIC DEPRESSION IN WILD-TYPE AND FRAGILE X MODEL MICE

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Many neurodevelopmental disorders are characterized by impaired functional synaptic plasticity and abnormal dendritic spine morphology, but little is known about how these are related. Previous work in the Fmr1-/y mouse model of fragile X (FX) suggests that increased constitutive dendritic protein synthesis yields exaggerated mGluR5-dependent long-term synaptic depression (LTD) in area CA1 of the hippocampus, but an effect on spine structural plasticity remains to be determined. In the current study, we used simultaneous electrophysiology and time-lapse two photon imaging to examine how spines change their structure during LTD induced by activation of mGluRs or NMDA receptors (NMDARs), and how this plasticity is altered in Fmr1-/y mice. We were surprised to find that mGluR activation causes LTD and AMPA receptor internalization, but no spine shrinkage in either wildtype or Fmr1-/y mice. In contrast, NMDAR activation caused spine shrinkage as well as LTD in both genotypes. Spine shrinkage was initiated by non-ionotropic (metabotropic) signaling through NMDARs, and in wild-type mice this structural plasticity required activation of mTORC1 and new protein synthesis. In striking contrast, NMDA-induced spine plasticity in Fmr1-/y mice was no longer dependent on acute activation of mTORC1 or de novo protein synthesis. These findings reveal that the structural consequences of mGluR and metabotropic NMDAR activation differ, and that a brake on spine structural plasticity, normally provided by mTORC1 regulation of protein synthesis, is absent in FX. Increased constitutive protein synthesis in FX appears to modify functional and structural plasticity induced through different glutamate receptors.

DOPAMINE RECEPTORS IN OLIGODENDROGLIA

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Several neurotransmitters have been shown to act on oligodendrocytes or their precursors but the function of this action is unknown. Neurotransmitter agonism or antagonism influences the development and differentiation of oligodendroglia and, as a result, the myelination process. Dopamine receptors have been reported to exist in oligodendrocyte precursor cells (OPCs) but evidences about their function are scarce. We have generated enriched oligodendroglia cultures from rat cortical neural progenitor cells, followed by O4+ cell sorting, as well as by direct conversion from adipose tissue stromal cells overexpressing Sox10 + Olig2 + Zfp536 (SOZ-induced oligodendroglia). RT-qPCR analysis showed that both D1-type (DRD1 and DRD5) and D2-type (mainly, DRD2) mRNA are expressed in brain-derived OPCs and that differentiation by growth factor (EGF, bFGF and PDGF-AA) withdrawal and T3 addition changed to a predominantly D1-type expression. SOZ-induced oligodendroglia showed a similar expression pattern of dopaminergic receptors. Functionality of these receptors was assayed by measurements of cAMP levels in response to dopaminergic agonists and antagonists. Immunofluorescence (O4 and NG2) analyses show that dopaminergic agonists of both types influence the proliferation and morphological maturation of oligodendroglia. These results support that dopamine inhibits oligodendrocyte differentiation and myelination. Excess of dopamine during the formation of neuronal tracts might have a negative effect on their myelination and thus impair their functionality. An impaired myelination of some corticofugal tracts due to a hyperdopaminergic environment at critical periods of brain development might be in the basis of neuropsychiatric disorders like schizophrenia.

EARLY SYNAPTIC IMPAIRMENT IN THE HIPPOCAMPUS OF A RAT MODEL OF PROGRESSIVE PARKINSONISM

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The loss of dopaminergic neurons and the aggregation of α-synuclein in intracytoplasmic Lewy bodies are key pathological features of Parkinson´s disease (PD). Although considered a predominantly motor disorder, PD patients often present non-motor signs, which could be related to alterations in limbic brain regions. The aim of our study was to evaluate synaptic plasticity and differential protein expression in the main limbic nucleus, the hippocampus, at different time-points in the neurodegenerative progress using an animal model of progressive parkinsonism. Rats were inoculated in the substantia nigra compacta with an adeno-associated viral vector coding for A53T mutated human α-synuclein (AAV-hαsyn) or empty viral vector as a control group (AAV-EVV), and evaluated at 1-, 2-, 4-, and 16-weeks post inoculation (p.i.). Synaptosomes were isolated from hippocampus and synaptic plasticity was evaluated by chemical stimulation of long-term potentiation (cLTP), double-staining of postsynaptic-GluA1 and presynaptic-Neurexin1β, and flow cytometry analysis, whereas protein expression was assessed by SWATH-MS proteomics. The effect of pramipexole and L-DOPA on cLTP were also tested. In the AAV-hαsyn group, a significant inhibition of cLTP was observed since 1w p.i. ($p<0.01$) coinciding with the presence of hαsyn in midbrain, confirmed by western blot. Incubation with pramipexole showed a recovery of cLTP in AAV-hαsyn group at all time points ($p<0.05$), whereas L-DOPA only recovered cLTP at 4 weeks p.i. ($p<0.05$). Of note, pramipexole partially inhibited hippocampal cLTP in AAV-EVV animals ($p<0.05$). The proteomic study identified a total of 7958 proteins, of which 131 were statistically differentially expressed in the AAV-hαsyn group (58 up-regulated, 73 down-regulated). Bioinformatic analysis revealed alterations related to synaptic structure and transport at early time-points, and related to membrane potential and plasticity at later time-points. Our results indicate that hαsyn impairs synaptic function and structure in the hippocampus of parkinsonian rats, which could be functionally recovered by dopaminergic treatment.

EFFECT OF SEI AND FIN WHALE MÜLLER GLIA IN THE SURVIVAL AND NEURITE GROWTH OF RGCS IN VITRO

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Müller cells are crucial in retinal homeostasis. In zebrafish and lower vertebrates, spontaneous retinal regeneration was observed. In the mammalian retina, there is no such evidence. However, recent studies have been demonstrated that Müller cells promote the survival and the neurite outgrowth of RGCs in vitro. Here, the effect of Müller cells from two of the largest mammals in the world, Fin and Sei whales, was analyzed on RGCs survival and neurite outgrowth.
The retinas of beached Balaenoptera physalus and Balaenoptera borealis whales, 24h post-mortem, were studied. Cultures of Müller cells were grown and, once whale Müller cells reached confluence, adult rat RGCs were seeded on Müller cells. The cells were cultured during six days. The number and length of the neurites of the RGCs were quantified. The conditioned media (CM) from whale Müller cells was analysed by mass spectrometry to identify and quantify proteins. Results were compared to the CM from pig Müller cells. NELL2, SEMA3F, CD56, NRCAM, OGN and PEDF were selected as candidate factors to promote neurite outgrowth and tested in pure rat RGCs cultures.
RGCs survival increased 60% when cultured with whale Müller cells. The length of the neurites (>200μm) increased 90% and the length of the longest neurites (>1000μm) increased more than 500%. Proteomic analysis from whale Müller cells CM showed that the function of the 10% of the most represented proteins detected were related to neurite outgrowth, compared to only 2% of the proteins in pig CM. The selected proteins induced an increase in the percentage of RGCs with long neurites (>200μm) and an increase of 200% in RGCs with very long neurites (>1000μm).
Whale Müller cells increase RGCs neurite growth, suggesting that whale Müller cells secrete a combination of neurotrophic factors promoting regeneration of RGCs. Besides, the selected proteins from the CM increase neurite outgrowth, confirming the capacity of the factors secreted by whale Müller cells to regenerate RGCs.

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EFFECTS OF BALANCED VS. DEFICIENT OMEGA-3 FATTY ACID DIETS ON ADULT HIPPOCAMPAL NEUROGENESIS AND GLIA

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Maternal dietary intake of the polyunsaturated fatty acids omega-3 (Ω3) and omega-6 (Ω6) impacts brain function, specifically hippocampal development. Current trends in dietary habits have dangerously reduced the intake of Ω3. The hippocampus is involved in memory and it is one of the most plastic regions of the brain. Indeed, newborn neurons continuously integrate into the hippocampal dentate gyrus (DG). We postulate that deficient-Ω3 diets affect the cellular composition of the DG after the postnatal periods, as its development continues through life. Thus, we fed young male and female mice (1.5 months old) with either Ω3 balanced or deficient isocaloric diets for ten weeks. As Ω3 and Ω6 are precursors of inflammatory regulators, we injected mice six weeks before sacrifice with bacterial lipopolysaccharide (LPS), a well-known inducer of inflammation and long-term negative modulator of neurogenesis. Our results revealed an effect of diet on adult neurogenesis (number of stem cells and new neurons) and microglia. Surprisingly, new neurons significantly decreased in female mice fed with the Ω3-deficient diet but not in male mice. However, LPS did not affect neurogenesis or microglia but induced a long-term reduction in astrocytes regardless of the diet. Further analysis is required to adequately evaluate the modulatory action of Ω3 dietary balance on the long-term effects of LPS mediated inflammation. In conclusion, dietary Ω3 deficiency alters the cellular composition of the young DG by affecting adult neurogenesis and microglia. Notably, adult neurogenesis is especially affected in female mice. Our study points to the relevance of balancing Ω3 dietary intake to preserve brain health.

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EFFECTS OF TRANSCRANIAL DIRECT-CURRENT STIMULATION (tDCS) ON THALAMOCORTICAL SENSORY PATHWAY IN AWAKE MICE

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The transcranial Direct-Current Stimulation (tDCS) is a non-invasive neuromodulatory technique that induces changes in the human cortex excitability dependent on the applied polarity. It has recently been proposed that tDCS may act by modifying the local- and long-range circuits involved in the excitation/inhibition (E/I) balance in the cerebral cortex. The aim of the present study was: 1) to describe the characteristics of field potentials recorded in primary somatosensory cortex (S1) in response to whisker and ventro-posterior medial thalamus (VPM) electrical stimulation, and 2) to compare the effects of tDCS on field potentials induced by these two different stimuli.

For that, we prepare C57 mice for chronic multilayer recording of LFPs, VPM electrical stimulation and tDCS in the alert head-restrained condition. tDCS was applied between a ring electrode over S1 and a reference electrode consisting of a rubber rectangle (6 cm2) attached to the back of the mouse. Evoked potentials in response to whisker or VPM stimulation were chronically recorded at two different depths (400 - 600 µm and 1000 – 1500 µm from the brain surface) before, during and after short pulses of tDCS (15 s) at different transcranial current intensities (±50, ±100, ±150 and ±200 µA).

The recorded VPM-induced potentials showed a reduction in the latency (~3 ms) of the first component with respect to whisker-induced sensory potentials. On the other hand, depth profile of VPM-induced evoked potentials showed the inversion of N1 component across layers similarly to reported for whisker-induced sensory potentials. Regarding to immediate effects of tDCS, anodal and cathodal pulses of tDCS successfully modulated the waveform of VPM-induced evoked potentials increasing and decreasing its amplitude during anodal and cathodal, respectively.

These results demonstrate that tDCS immediate effects on S1 recorded VPM-induced potentials reproduce those observed in sensory evoked potentials induced in response to peripheral whisker stimulation.

EFFECTS OF TRANSCRANIAL DIRECT-CURRENT STIMULATION (tDCS) ON THE FIELD POTENTIAL INDUCED BY PHOTOSTIMULATION OF GLUTAMATERGIC CELLS IN SOMATOSENSORY CORTEX

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Transcranial Direct-Current Stimulation (tDCS) is a non-invasive brain stimulation technique that can influence brain excitability. Although previous studies have demonstrated the modulatory effects of tDCS on cortical activity in different brain regions, the mechanisms underlying its physiological effects are not fully understood. It has been hypothesized that different neuronal populations diversely response to exogenous electric field according to their morphology and orientation. In this study, we use an optogenetic approach to know about the impact of the electric field on different neuronal groups.

For that, wild-type mice were injected with an adeno-associated virus containing the gene encoding ChR2 preceded by the CAMKII promoter to selectively photostimulate glutamatergic neurons in the primary somatosensory cortex (S1). Three weeks after virus infection, mice were prepared for chronic recording of LFPs in S1 during photostimulation and simultaneous tDCS in head-restrained condition. The evoked field potentials in response to blue light was characterized for different light duration and intensity by using optrodes. To describe the impact of tDCS on glutamatergic neuronal population, short pulses (15 s, including 5 s ramp up and 5 s ramp down) of transcranial currents were delivered over S1 at different intensities and polarities (±50, ±100, ±150 and ±200 μA). An electrical stimulus (0.2 ms, <0.2 mA) was applied to whisker pad 2 s before photostimulation as a control during tDCS protocol.

The induced field potentials in response to blue light were proved to be directly dependent on both light exposure and light intensity. During the application of anodal tDCS, we observed an increase in the amplitude field potentials induced by whisker and optogenetic stimulation whereas cathodal tDCS decreased it.

These results prove that optogenetics is a valid and effective technique to study the effects of tDCS on distinct neuronal populations, a fundamental knowledge to understand and optimize future clinical applications.

EXISTENCE OF FGFR1-5-HT1AR HETERORECEPTOR COMPLEXES IN HIPPOCAMPAL ASTROCYTES. PUTATIVE LINK TO 5-HT AND FGF2 MODULATION OF HIPPOCAMPAL GAMMA OSCILLATIONS

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The majority of the fibroblast growth factor receptor 1-serotonin 1 A receptor (FGFR1-5-HT1AR) heterocomplexes in the hippocampus appeared to be located mainly in the neuronal networks and a relevant target for antidepressant drugs. Through a neurochemical and electrophysiological analysis it was therefore tested in the current study if astrocytic FGFR1-5-HT1AR heterocomplexes also exist in hippocampus. They may modulate the structure and function of astroglia in the hippocampus leading to possible changes in the gamma oscillations. Localization of hippocampal FGFR1-5-HT1AR heterocomplexes in astrocytes was found using in situ proximity ligation assay combined with immunohistochemistry using glial fibrillary acidic protein (GFAP) immunoreactivity as a marker for astroglia. Acute i.c.v. treatment with 8-OH-DPAT alone or together with basic fibroblast growth factor (FGF2) significantly increased FGFR1-5-HT1AR heterocomplexes in the GFAP positive cells, especially in the polymorphic layer of the dentate gyrus (PoDG) but also in the CA3 area upon combined treatment. No other hippocampal regions were studied. Also, structural plasticity changes were observed in the astrocytes, especially in the PoDG region, upon these pharmacological treatments. They may also be of relevance for enhancing the astroglial volume transmission with increased modulation of the neuronal networks in the regions studied. The effects of combined FGF2 and 5-HT agonist treatments on gamma oscillations point to a significant antagonistic interaction in astroglial FGFR1-5-HT1AR heterocomplexes that may contribute to counteraction of the 5-HT1AR-mediated decrease of gamma oscillations.

GALECTIN-3 IMPAIRS GAMMA OSCILLATIONS AT HIPPOCAMPAL CA3 AREA EX VIVO: A SUITABLE TARGET TO COUNTERACT THE PROGRESSION OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a progressive multifaceted neurodegenerative disorder for which no disease-modifying treatment exists. Cognitive decline is a clinical hallmark of AD, and its severity correlates with the level of disruption of neuronal networks activity. Recently, neuroinflammation has been revealed central to the pathology progression with evidence suggesting that microglia-released galectin 3 (gal3) plays a pivotal role amplifying neuroinflammation in AD. However, possible involvement of gal3 in the disruption of cognitive-relevant neuronal network oscillations remains unknown. Here we investigated the functional implications of gal3 for neuronal network functioning by performing ex-vivo recordings of fast electrical rhythmic activity within gamma frequency range (gamma oscillations, 20-80Hz) in CA3 area of WT hippocampal mice brain slices. Gamma oscillations were induced by applying 100 nM KA either in an interface- or submerged-type recording chambers. Concomitantly to local field potential we performed patch-clamp recordings of relevant neuronal populations (fast-spiking neurons (FSN) and pyramidal cells (PCs)) with focus on FSN. We used two different general approaches (1) gamma induced after gal3 treatment and (2) gal3 application during ongoing gamma oscillations. We observed that gal3 application significantly decrease gamma oscillation power and rhythmicity which is mediated by the gal3-carbohydrate-recognition domain and prevented by the gal3 inhibitor TD139 in a dose-dependent manner. Such disruption resulted to be activity-dependent and was accompanied by the impairment of FSN- and PC-gamma phase-locking. Interestingly, TD139 also prevented Aβ42-induced degradation of gamma oscillations. Notably, we found that gamma oscillations are impaired at CA3 hippocampal area of 5xFAD mice model at 6 months old while gamma oscillations recorded from 5xFAD mice lacking gal3 (5xFAD-Gal3KO) remain similar to age-matched WT counterpart. Thus, we report for the first time that gal3 impairs cognitive-relevant neuronal network dynamics. Moreover, our findings suggest that removing/inhibiting gal3 could be beneficial to counteract the neuronal network instability typical of AD.

GSK-3β S9A OVEREXPRESSION LEADS MURINE HIPPOCAMPAL NEURAL PRECURSORS TO ACQUIRE AN ASTROGLIAL PHENOTYPE IN VIVO

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The addition of new neurons to the existing hippocampal circuitry persists in the adult dentate gyrus (DG). During this process, named adult hippocampal neurogenesis (AHN), adult hippocampal progenitor cells (AHPs) give rise to newborn dentate granule cells (DGCs). The acquisition of a neuronal lineage by AHPs is tightly regulated by numerous signaling molecules and transcription factors. In this regard, glycogen synthase kinase 3β (GSK-3β) is a master regulator of the maturation of AHPs in vitro. Here we analyzed the cell-autonomous effects of overexpressing a constitutively active form of GSK-3β (GSK-3β S9A) in AHPs in vivo. To this end, we stereotaxically injected a GSK-3β S9A-encoding retrovirus (GSK-3β-V5) into the DG of young adult C57BL6/J Ola Hsd female mice and studied the cell lineage acquisition, migratory and marker expression patterns, and the morphological maturation of the infected cells over time. Strikingly, GSK-3β S9A-transduced cells expressed glial fibrillary acidic protein (GFAP) and NG2, thereby acquiring an immature astroglial phenotype, which differed markedly from the neuronal phenotype observed in cells transduced with a control retrovirus that encoded GFP. Accordingly, the morphology and migration patterns of cells transduced by the two retroviruses are remarkably divergent. These observations support the role of GSK-3β as a cornerstone that regulates the balance between new astrocytes/neurons generated in the adult murine DG.

HYPERAMMONEMIA ALTERS THE FUNCTION OF AMPA, NMDA AND GABAA RECEPTORS AND EXTRACELLULAR cGMP REVERSES SOME OF THESE ALTERATIONS

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Hepatic encephalopathy (HE) is a neuropsychiatric syndrome caused by liver disease. Liver failure leads to hyperammonemia (HA) and inflammation, which act synergistically to induce neuroinflammation which alters neurotransmission leading cognitive and motor impairment. Extracellular cGMP levels are decreased in the hippocampus of hyperammonemic rats and increasing extracellular cGMP to normal levels normalizes neuroinflammation and cognitive and motor impairment, through the modulation of membrane expression of some glutamate and GABA receptor subunits. However, the effect of HA and extracellular cGMP on the function of these receptors are not known. The aim of this work was to analyze the function of AMPA, NMDA and GABAA receptors in the hippocampus of rats with chronic HA, and assess if extracellular cGMP modulates their function. Rats were sacrificed after four-five weeks of ammonium rich diet and we obtained transversal hippocampal slices. The MEA2100-system was used for postsynaptic Input-Output (I/O) curve recordings in the Schäffer Collaterals. One of the 120 available planar microelectrodes was selected in the distal part of CA1/CA3 border for tetanic stimulation. The recording electrode was in CA1 area. We perfused the slices with ACSF or with ACSF with different inhibitors (CNQX, AP5 and picrotoxin) to analyse the contribution of AMPA, NMDA and GABAA receptors to I/O curves separately. To assess the effect of extracellular cGMP, hippocampal slices were perfused with solutions containing or not extracellular cGMP. We analysed the effect of HA and extracellular cGMP on different parameter of the I/O curves at different times and stimulation intensities. The results show that HA reduces the function of AMPA receptors and results in a hyperfunctionality of NMDA and GABAA receptors. Extracellular cGMP reverses some of these alterations.

IMPACT OF AGING ON THE STRUCTURE AND NMDA RECEPTOR EXPRESSION OF SOMATOSTATIN EXPRESSING HIPPOCAMPAL INTERNEURONS

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Aging is a natural process related to the gradual loss of physiological, behavioral, and social functions. Understanding the neurobiology underlying age-related impairment is essential given the growing elderly population. Among these mechanisms, changes in the structure of neurons and particularly in their dendritic spines are thought to be crucial players in age-related cognitive decline. One of the most studied brain structures affected by aging is the hippocampus, known to be involved in different essential cognitive processes. While the aging-associated quantitative changes in dendritic spines of hippocampal pyramidal cells have already been studied, the relationship between aging and the structural dynamics of hippocampal interneurons remains relatively unknown. Spines are not a frequent feature in cortical inhibitory neurons, but these postsynaptic structures are abundant in a subpopulation of somatostatin expressing interneurons, particularly in oriens-lacunosum moleculare (O-LM) cells in the hippocampal CA1. Previous studies from our laboratory have shown that the spines of these interneurons are highly plastic and influenced by NMDA receptor manipulation. Thus, in the present study, we have investigated the impact of aging on this interneuronal subpopulation. The analyses were performed in 3-, 9-, and 16-month-old GIN mice, a strain in which somatostatin positive interneurons express GFP. We studied the changes of dendritic spines, en passant boutons, and NMDA receptors (GluN1 and GluN2B) using confocal microscopy and image analysis. We observed a significant decrease of the dendritic spine density in 9-month-old when compared with the 3-month-old animals. We also observed a decrease in the expression of the GluN2B subunit, but not of that of GluN1, during aging. These results will constitute the basis for more advanced studies of the structure and connectivity of interneurons during aging and their contribution to cognitive decline.

IMPACT OF BRAIN STATE ON TRANSCRANIAL DIRECT-CURRENT STIMULATION (TDCS) EFFECTS IN MICE

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Transcranial direct-current stimulation (tDCS) is a non-invasive brain stimulation technique capable of inducing polarity-specific changes in neuronal excitability. Whilst cathodal tDCS has been related to long-term depression in mice primary somatosensory cortex (S1), anodal tDCS have no long-term effects. The physiological changes induced in the brain during and after tDCS must be interpreted as the result of the interaction between the imposed electrical currents and fields and the on-going, endogenous cortical activities. The aim of this study was to determine the impact of different brain states (awake vs anesthetized) on the short- and long-term effects observed during and after tDCS on mice S1 cortex.

For that, we prepare C57 mice for chronic recording of LFPs in S1, ventroposterior-medial thalamus nuclei (VPM) electrical stimulation and tDCS in head-restrained animals. Evoked potentials (EPs) induced in S1 due to electrical stimulation of VPM (every 10±2s) were recorded on awake and under anesthesia condition (isoflurane). Short-term (15s pulses, ±50µA, ±100µA, ±150µA and ±200µA) and long-term (±200µA, 20min) effects of tDCS in both conditions were compared.

Differences within EPs were found between brain states (smaller amplitudes and longer latencies appeared under anesthesia). Short-term effects of tDCS on EPs were evident for both awake and anesthetized conditions inducing an increase and decrease in the amplitude during anodal and cathodal, respectively. On the other hand, clear long-term effects were observed in anesthetized animals whilst no significant long-term effects were observed on awake mice for anodal nor cathodal tDCS. Under isoflurane condition, a decreased amplitude in EPs was induced remaining up to 1 hour after cathodal tDCS offset. Contrarily, an increase in the amplitude of EPs was observed after anodal tDCS offset.

These results demonstrate an important role of the brain state on long-term plastic changes induced by tDCS in mice S1 region.

IMPAIRED STRIATAL PLASTICITY AND DENDRITIC SPINE REMODELING IN THE PREMOTOR STAGE OF AN ANIMAL MODEL OF PROGRESSIVE PARKINSONISM

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Parkinson's disease (PD) is characterized by a progressive loss of dopaminergic neurons in the substantia nigra compacta (SNc) and accumulation of α-synuclein (α-syn), which underlies complex functional and structural changes in striatal spiny projection neurons (SPNs). Although a reduction in SPNs dendritic spine density has been reported in either postmortem studies of advanced PD patients or rodent models of parkinsonism, it is unknown if these alterations occur since the onset of the nigrostriatal dopaminergic degeneration. Thus, our aim was to study the temporal sequence of functional and structural changes in striatal dendritic spines. For that purpose, animals were inoculated in the SNc with an adeno-associated viral vector coding for A53T mutated human α-syn (hα-syn) and evaluated at 72h, 1, 2 and 4 weeks post-inoculation (p.i.). Synaptic plasticity by chemical stimulation of LTP (cLTP) in isolated striatal synaptosomes and measurement of striatal dopamine by HPLC were assessed. The analysis of the density and morphology of SPNs dendritic spines was performed by microinjection and high-resolution confocal microscopy. The hα-syn group showed an inhibition of cLTP ($p<0.001$) and a decrease in dopamine content ($p<0.01$) since 72h p.i. even before the presence of hα-syn in the striatum and significant dopaminergic neurodegeneration. These functional alterations are associated with a dendritic spine remodeling, as observed by a significant loss of thin spines, along with an increase in the head volume of thin and mushroom spines. These structural changes occur before the development of parkinsonian motor signs and could represent a compensatory mechanism to enhance the function of existing spines, balancing the observed decrease in spine turnover. Thus, our results indicate dysfunctional neurotransmission by impaired striatal synaptic plasticity since very early time points, leading to dendritic spine remodeling, before the manifestation of motor impairment.

IMPLICATION OF SFRP1 IN ALTERED SYNAPTIC PLASTICITY ASSOCIATED WITH ALZHEIMER'S DISEASE

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We have demonstrated that SFRP1 is a novel, promising therapeutic target for Alzheimer Disease (AD). SFRP1 acts as a secreted endogenous regulator of ADAM10, a brain α-sheddase, which controls the activity of several substrates including APP and proteins regulating synaptic plasticity and neuroinflammatory crosstalk. Astrocyte-derived SFRP1 is upregulated in the brain of AD patients, localizing to amyloid plaques and interacting to Aβ peptides. Neutralization of Sfrp1 activity in AD-like mouse models decreases the formation of Aβ peptides, counteracts brain inflammation and maintains synaptic plasticity. Here we aimed at determining if Sfrp1 has a direct effect on synaptic plasticity.

We generated a transgenic mouse model overexpressing Sfrp1 in astrocytes (GFAP-tTA;TRE-Sfrp1) and analysed it by immunohistochemistry and RT-qPCR to determine the possible presence of neuroinflammatory and molecular alterations. Mice were further characterized for cognitive abilities with behavioural tests and analysed for possible dendritic and spine modification via viral-mediated visualization of their morphology.

We show that GFAP-tTA;TRE-Sfrp1 mice present an allele-dependent increase in Sfrp1 expression, which is associated with a decrease in dendritic and spine density already at two months of age. These defects are associated with an age-dependent appearance of cognitive decline and alterations in LTP. Our data support the idea that Sfrp1 has a direct impact on synaptic plasticity which is not secondary to its effect on APP processing, indicating that it might have a pleiotropic effect in AD.

IN SILICO SCREENING OF GMQ-LIKE COMPOUNDS REVEALS GUANABENZ AND SEPHIN1 AS NEW ALLOSTERIC MODULATORS OF ACID-SENSING ION CHANNEL 3

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Acid-sensing ion channels (ASICs) are voltage-independent cation channels that detect decreases in extracellular pH. Dysregulation of ASICs underpins a number of pathologies. Of particular interest is ASIC3, which is recognised as a key sensor of acid-induced pain and is important in the establishment of pain arising from inflammatory conditions, such as rheumatoid arthritis. Thus, the identification of new ASIC3 modulators and the mechanistic understanding of how these compounds modulate ASIC3 could be important for the development of new strategies to counteract the detrimental effects of dysregulated ASIC3 activity in inflammation. Here, we report the identification of novel ASIC3 modulators based on the ASIC3 agonist, 2-guanidine-4-methylquinazoline (GMQ). Through a GMQ-guided in silico screening of Food and Drug administration (FDA)-approved drugs, 5 compounds were selected and tested for their modulation of rat ASIC3 (rASIC3) using whole-cell patch-clamp electrophysiology. Of the chosen drugs, guanabenz (GBZ), an α2-adrenoceptor agonist, produced similar effects to GMQ on rASIC3, activating the channel at physiological pH (pH 7.4) and potentiating its response to mild acidic (pH 7) stimuli. Sephin1, a GBZ derivative that lacks α2-adrenoceptor activity, has been proposed to act as a selective inhibitor of a regulatory subunit of the stress-induced protein phosphatase 1 (PPP1R15A) with promising therapeutic potential for the treatment of multiple sclerosis. However, we found that like GBZ, sephin1 activates rASIC3 at pH 7.4 and potentiates its response to acidic stimulation (pH 7), i.e. sephin1 is a novel modulator of rASIC3. Furthermore, docking experiments showed that, like GMQ, GBZ and sephin1 likely interact with the nonproton ligand sensor domain of rASIC3. Overall, these data demonstrate the utility of computational analysis for identifying novel ASIC3 modulators, which can be validated with electrophysiological analysis and may lead to the development of better compounds for targeting ASIC3 in the treatment of inflammatory conditions.

IN VIVO ASTROCYTE ACTIVATION MODULATES SPONTANEOUS INHIBITORY ACTIVITY DURING SLOW WAVE OSCILLATIONS IN THE SOMATOSENSORY CORTEX

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During sleep and anesthesia, cortical spontaneous activity is dominated by slow wave oscillations (SWO, <1.5 Hz) consisting of alternating synchronized network activity (up-states) and generalized neuronal silence (down-states). Several evidence indicate distinct excitatory and inhibitory neuronal mechanisms involved in the SWA regulation in rodents. Nonetheless, in vitro and in vivo data indicate astrocytes as regulators of cortical up-states by controlling both the initiation and the frequency of synchronized oscillations. However, whether astrocytes are also able to regulate neuronal excitability during up-states or are involved in the mechanisms of down-states is still unknown. Here, we investigated the role of astrocytes in the spontaneous cortical oscillatory activity by using a combination of in vivo electrophysiology and pharmacogenetics. For that, a 32-channel multielectrode was lowered into the somatosensory cortex to record spontaneous neuronal activity, while astrocyte modulation was achieved by using a hM3Dq-Gq-DREADD under the astrocyte GFAP promoter and acute i.p. injection of its ligand clonazepine (CNO). Neuronal recordings obtained from the same animals before and 1-hour after CNO application showed that astrocyte activation exhibited a clear effect on the SWO neuronal firing. While firing rate during down-states was significantly enhanced, up-states presented a reduced spontaneous activity. To determine the neuronal cell-type modulated by astrocytes under our conditions, we used a spike-sorting clustering algorithm based in three measurements (width, Trough-to-peak and after-high-hyperpolarization). Our analysis showed that CNO increased the number of putative inhibitory neurons during down-states, without affecting the number of putative excitatory clusters. Such apparent enhancement of inhibitory activity shorten down-state duration and therefore increasing SWO power as well as induced a slower down-to-up transition and decreased up-state amplitude. In conclusion, our findings indicate that down-states in SWO are directly modulate by astrocytes. In addition, the appearance of new putative inhibitory clusters would suggest the modulation of GABAergic interneurons by astrocyte activity.

INTACT INDUCTION AND PRESYNAPTIC OCCLUSION OF SHORT AND LONG-TERM POTENTIATION IN SYNAPTOPHYSIN FAMILY KNOCKOUTS

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Synaptophysin family proteins are ubiquitous integral components of synaptic vesicle membranes. We recently found that baseline synaptic strength is enhanced in quadruple knockouts missing all four family members owing to elevated probability of release of the docked and primed readily releasable vesicles within presynaptic terminals. Deficits in both short- and long-term potentiation were detected previously in partial mutants missing one or two family members, and are more severe in the quadruple knockouts. The short-term deficits could result from the elevated baseline probability of release because: (1) short-term potentiation is also caused by elevated probability of release; and (2) a previous study identified a limit that would occlude further elevations if unaltered in the mutants. In contrast, long-term potentiation is widely thought to be caused by a different type of mechanism. Nevertheless, here we show that the deficit in long-term potentiation can be rescued by lowering extracellular calcium, which lowers the baseline amount of release back to wildtype levels. The result suggests that the most widely studied form of long-term potentiation is caused by elevated probability of neurotransmitter release from presynaptic terminals, and that the amount of potentiation can be limited by a native occlusion mechanism that was previously shown to heavily influence the timing of recovery from short-term depression.

LOSS OF TRESK BACKGROUND POTASSIUM CHANNEL ENHANCES ACUTE AND CHRONIC ITCH

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TRESK (K2P18.1) is a background K+ channel expressed in sensory neurons, where it modulates the resting membrane potential, action potential firing and neuronal excitability. A subset of these sensory neurons, which express specific TRPs and Mas-related G protein-coupled receptors (Mrgprs), are activated by pruritogens and mediate itch sensations. Because TRESK is involved in somatosensitivity and pain perception, we evaluated the contribution of this channel to pruritic sensitivity and its potential as a target for the treatment of chronic itch pathologies including renal or liver failure, Hodgkin's lymphoma and different types of dermatitis. By combining, RNA in situ hybridization, calcium imaging, electrophysiological and behavioral approaches, we found that TRESK is involved in the modulation of non-histaminergic itch. TRESK colocalizes in MrgprD+ and MrgprA3+ sensory neurons. Different populations of primary cultured sensory neurons from both wild-type and TRESK knockout mice were activated by chloroquine (CQ), β-alanine, BAM8-22 or histamine in calcium imaging experiments. At the behavioral level, subcutaneous injection of chloroquine in the cheek model produced an acute scratching response, which was significantly enhanced in mice lacking TRESK. Interestingly, TRESK ko mice also showed alterations in mice models of chronic itch. Induction of Allergic Contact Dermatitis or Dry Skin showed a significantly higher scratching response in mice lacking TRESK compared to their wild-type counterparts. In the mouse model of imiquimod-induced psoriatic itch, the absence of TRESK produced a significantly enhanced scratching behavior, which developed earlier and was more robust. In summary, our data indicate that TRESK is involved in regulating the excitability of a subset of sensory neurons that mediate histaminergic-independent itch. Given the prominent role of this neuronal subpopulation in chronic itch diseases, TRESK appears as a new potential candidate for therapeutic intervention.

M1 AND M2 MUSCARINIC RECEPTORS COORDINATELY REGULATE THE EXOCYTOTIC PROTEINS THROUGH PKC AND PKA AT THE ADULT NEUROMUSCULAR JUNCTION

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Synapses use plastic mechanisms to adjust the strength of the neurotransmitter release to any situation. At the neuromuscular junction (NMJ), muscarinic acetylcholine receptors (mAChR) participate in synaptic plasticity as presynaptic autoreceptors sensing and controlling the release of acetylcholine (ACh). M_1 and M_2 subtypes increase and decrease, respectively, neurotransmitter release. M_2 involves PKA and M_1 PKC. Several PKC and PKA targets contribute to the synaptic vesicle exocytosis and their coordination is fundamental to achieve the extraordinary speed, precision and plasticity of neurotransmission although the molecular signaling regulation is unknown. Therefore, the present study is aimed to know how M_1 and M_2 mAChRs regulate (1) their own and mutual expression, (2) PKA subunits dynamics and activity, (3) PDK1 activity, (4) nPKCε and cPKCβI, and (5) Munc18-1, MARCKS and SNAP-25 phosphorylation. We performed immunohistochemistry and confocal techniques to evidence the presynaptic location of the regulated molecules. Specific inhibitory reagents were used to block M_1 and M_2 mAChR, nPKCε, cPKCβI, PKA and PDK1 activity.

Main results obtained from Western blot, co-immunoprecipation and subcellular fractionation experiments showed that: (1) M_2 downregulates M_1. (2) M_2 inhibits PKA activity by downregulating Cβ subunit, upregulating RIIα/β and liberating RIβ and RIIα to the cytosol reducing the phosphorylation of SNAP-25 on Thr-138 and CREB. M_1 signaling opposes to M_2 by recruiting R subunits to the membrane. (3) M_1 and M_2 mAChR activate the master kinase PDK1, which promotes the priming of the presynaptic PKCβI and PKCε isoforms. (4)M_1 recruits both primed-PKCs to the membrane and promotes (5) Munc18-1, SNAP-25 and MARCKS phosphorylation. In contrast, M_2 downregulates PKCε through a PKA-dependent pathway, which inhibits Munc18-1 synthesis and PKC-phosphorylation.

The results demonstrate the coordinate and dependent action of the M_1 and M_2 mAChRs on ACh release SNARE-SM mechanism involving PKC and PKA to regulate neurotransmission and guide towards potential therapeutic targets.

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mGlu4 RECEPTORS RESCUE PARALLEL FIBER LTP AND MOTOR SKILLED REACHING DEFICITS IN A MOUSE MODEL OF FRAGILE X SYNDROME

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Fragile X patients and mice lacking the Fragile X Mental Retardation Protein (Fmr1 KO) suffer from multiple behavioral alterations including some motor deficits. We found that cerebellar parallel fiber to Purkinje cell Fmr1 KO synapses show an increase in synaptic vesicle (SV) docking and in spontaneous release that occludes further potentiation by β adrenergic receptors (β-ARs) and compromises parallel fiber Long Term Potentiation (LTP), a presynaptic form of synaptic plasticity. Diminishing the extracellular Ca2+ concentration, restored the readily releasable pool (RRP) size and rescued β-AR-mediated potentiation and parallel fiber LTP. Interestingly, VU 0155041, a selective positive allosteric modulator of mGlu4 receptors, also restored both the RRP size and parallel fiber LTP. Moreover, VU 0155041 injected into Fmr1 KO mice improved both parallel fiber LTP and a cerebellum based motor skilled reaching test. Thus, pharmacological activation of mGlu4 receptors may offer therapeutic relief in Fragile X Syndrome.

MICROGLIAL LOCAL TRANSLATION IN Aβ-INDUCED PATHOLOGY

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Protein synthesis is essential for the maintenance of cellular proteostasis. Neural cells (e.g neurons, astrocytes, oligodendrocytes) are highly polarized and therefore their proteins have to be asymmetrically distributed to fulfil their function. This distribution occurs through two different mechanisms: 1) the classical pathway where proteins are synthetized in the perinuclear region and delivered to their target site after maturation and/or 2) through local translation, where mRNAs are transported to the target site in a repressed state to be locally translated into proteins.
Local translation allows cells to react in spatial and temporal manner to numerous stimuli. Most data regarding local translation have been obtained in neurons. However, there is evidence that local translation plays a crucial role in other CNS cell types too. For instance, local translation of MBP in oligodendrocytes has been described in neurodegenerative conditions. More recently, the ability of peripheral astrocytic proteins to translate proteins has been described.
Microglia, although not being of neural origin, are the resident immune cells of the nervous system, and show a morphology as equally complex as neurons and neuroglia. In microglia, local translation has newly been described. Nonetheless, the involvement of local translation in these cell types in physiology and pathology is still to be elucidated. Taking into account that glial cells might be active participants in neurodegenerative diseases and based both on the literature and recent results of our group, our hypothesis is that local translation in microglial peripheral processes is involved in neurodegenerative diseases.
Previously, our group has obtained results supporting the idea that local translation in microglia is altered in the context of inflammation. It is well stablished that neurodegeneration and neuroinflammation are strictly related. Thus, we are currently analysing the effect of both parameters combined and their relation to changes in local translation of microglial peripheral processes.

MODELLING MICROSCALE DIFFUSION IN GEOMETRICALLY RESOLVED BRAIN EXTRACELLULAR SPACE IN LIVE TISSUE

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The extracellular space (ECS) is emerging as an important regulator of brain function involved in metabolite clearance and volume transmission signaling. It consists of highly convolved channels and reservoirs filled with interstitial fluid that facilitates diffusional spreading of molecules. However, state-of-the-art methods for investigating diffusional properties in brain neuropil do not reconcile sub-micron optical resolution with live tissue experiments. Consequently, it remains largely unknown how diffusion is shaped around individual cellular sub-structures. Knowing this is important for understanding how ECS structure can regulate signaling and metabolism, and how aberrant changes in the ECS properties may disrupt these processes.

Recently developed Super Resolution Shadow Imaging (SUSHI) reveals the nanoscale organization of the ECS in live tissue [1]. SUSHI images therefore provide new opportunities to model diffusion in the interstitial fluid channels around individual cellular structures. Accordingly, we aim to establish a numerical computational model of nanoscale ECS diffusion based on live tissue images, which is highly robust to image acquisition parameters and allows modelling differently sized molecules. In addition, we are using 2-Photon microscopy-based shadow imaging [2] to study how osmotic changes affect the ECS volume and consequently the concentration of neurotransmitters in live brain slices.

We will test the hypothesis that the local ECS geometry around a given synapse will channel away released transmitters in preferred directions, which may conceivably modulate extracellular crosstalk between neighboring synapses, and shape extrasynaptic transmission.

The proposed models are likely to facilitate groundbreaking new insights into the role of ECS structure in shaping diffusion and signaling on micro-scales, which remains a poorly understood phenomenon.

[1] Tønnesen J, et all. Super-Resolution Imaging of the Extracellular Space in Living Brain Tissue. Cell, 2018

[2] Kuo, S. P., et all. Spatial Organization and Dynamics of the Extracellular Space in the Mouse Retina. J. Neurosci. 40, 7785–7794 (2020).

MODIFICATION OF THE EXTRACELLULAR MATRIX IMPAIRS MICROGLIAL MOTILITY

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Beyond neurons and glia, the Central Nervous System (CNS) holds a plastic scaffold known as the extracellular matrix (ECM). Unlike the ECM from connective tissue, where collagen is the main unit, the neural interstitial matrix consists mostly on long chains of the glycan polymer hyaluronan. Protein components of the ECM bind to hyaluronan forming a self-assembled matrix that functions as structural framework and signalling hub. Microglia, the never-resting immune cell of the CNS, constantly surveys the brain parenchyma, interacting with neighbouring cells and the surrounding extracellular microenvironment. We have recently described in adult parkinsonian mice a bidirectional loop between hyaluronan and microglia, which affects both matrix structure and microglia reactive state, with consequences on neurodegeneration and tissue architecture (Soria et al., 2020, Nat Commun). Despite recent advances on matrix-glia interplay, it is unknown whether changes in the structural matrix affect microglial motility. Here we report on the interaction between microglia and hyaluronan, using an ex vivo approach to characterise microglia dynamics in response to matrix modification. Using 2-photon time-lapse imaging in acute slices of Cx3cr1+/eGFP mice, we show that microglial motility, ramification and territory surveyed are reduced upon hyaluronan fragmentation ex vivo, with no changes when other matrix components are degraded instead. We also report alterations in directed motility upon laser ablation after hyaluronidase treatment. These results suggest impairment of microglial motility upon matrix modification and shed light on the dual role of hyaluronan as scaffolding polymer and pro-inflammatory signal in the CNS.

MODULATION OF THE PRESYNAPTIC TRANSLATOME BY ASTROCYTIC EXTRACELLULAR VESICLES IN ALZHEIMER'S DISEASE

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Neurons are highly polarized cells with an asymmetric morphology, thus implying an asymmetric distribution of proteins. Protein synthesis is vital to guarantee the correct neuronal function. Under physiological conditions, proteins need to be appropriately sorted to the target cellular compartment where they elicit their function. Noteworthy, protein synthesis is not always carried out by the classical translation pathway, in which proteins are synthesized in the rough endoplasmic reticulum and after maturation, proteins are transported to the target compartment. Protein translation can also be executed by another way based on the delivery of the mRNA to the target site, where mRNAs will be locally translated into proteins. This process is known as local protein synthesis.

Neuronal local translation allows for a faster reaction of neural processes in response to environmental cues and contributes to the maintenance of axonal and dendritic homeostasis. In the Peripheral Nervous System, it has been described that extracellular vesicles (EVs) secreted by Schwann cells are capable of contributing to local protein synthesis and regenerate injured nerves. Nevertheless, it is so far unclear whether glial cells are involved in local protein synthesis.

Recent evidence show that in Alzheimer´s disease (AD) pathology local protein synthesis is involved in the transmission of β-amyloid pathology from the axons to the soma. In this way, the retrograde transport of proteins synthesized in the axon in response to amyloid peptide leads to pathological transcriptional changes that contribute to neurodegeneration in AD. Furthermore, previous results of our research group have found evidences supporting that EVs secreted in presence of astrocytes modifies the levels of translation in axons of the Central Nervous System in vitro, both under physiological and AD conditions. Based on these facts, the working hypothesis is that astrocytes contribute to presynaptic translatome through the transfer of EVs in physiological and AD conditions.

MOLECULAR MECHANISMS UNDERLYING NMDA RECEPTOR-BK CHANNEL COUPLING IN SPECIFIC BRAIN REGIONS

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Postsynaptic N-methyl-D-aspartate receptors (NMDARs) play a crucial role in excitatory synaptic transmission and plasticity, but their role must be framed into a more complex physiological picture, where they can interact with other ion channels shaping their function. A rather unexplored partnership is that of NMDAR with large-conductance calcium- and voltage-gated potassium (BK) channels, which until now had been exclusively studied in granular cells of the olfactory bulb and hippocampal pyramidal neurons. In these neuronal types, their expression seems restricted to the soma, regulating cellular excitability. We have recently shown that synaptic NMDAR–BK coupling occurs in a subpopulation of barrel cortex layer 5 pyramidal neurons, where it is promoted by a physical proximity maximizing NMDA receptor calcium access to the BK channel. This proximity allows a negative feedback mechanism to significantly and selectively filter plasticity, providing input-specific synaptic diversity to the thalamocortical circuit (Gomez et al, 2021, https://doi.org/10.1101/2020.12.30.424719). Using a panel of biophysical, cellular and imaging techniques in brain slices and heterologous expression systems, we now describe this mechanism in other brain areas and study the role of specific NMDAR and BK subunits in this coupling mechanism.

MONITORING OF ABERRANT NEUROGENESIS IN HIPPOCAMPUS DURING IN VITRO EPILEPTOGENESIS

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Epilepsy is among the most common and severe neurological disorders, yet epileptogenesis is poorly understood. The epileptic focus in temporal lobe epilepsy is frequently found in the hippocampus, where it causes sclerosis and aberrant neurogenesis in the dentate gyrus. Our goal here is to understand what alterations take place in the neurogenic niche during epileptogenesis.
Organotypic hippocampal cultures (OTCs) are a widely used in vitro model of temporal lobe epilepsy, and offers unique optical access to the hippocampal circuit over days and weeks in vitro. We have successfully established a model of OTCs and retro viral vector-based cell labeling of newborn neurons in order to assess aberrant neurogenesis in epileptic conditions induced by addition of the GABA-A receptor antagonist picrotoxin (PTX). We have verified that the epileptic environment reduces newborn neuron density, diminishes their morphological complexity and increases cell death. Through the addition of cerium nanoparticles (CeO2NPs), which harness strong anti-oxidative stress action, we have reversed cell death and recovered newborn neuron density. The reduction in morphological complexity of newborn neurons induced by PTX was however worsened by CeO2NPs. We have also observed that in epileptic conditions there is a reduction in GABA cell density, and specifically in GABAergic newborn neurons. Our hypothesis is that newborn neurons need GABA input to be GABAergic. By adding tetrodotoxin, which mimics the effect of increasing GABA input in the slices, we observed that the GABAergic newborn neuron percentage was recovered. This proves that blocking GABA-A receptors is not the reason why there are fewer GABAergic newborn neurons in epileptic conditions, but the activity that occurs after this blocking, which favors hyperexcitation. Therefore, we can conclude that GABAergic newborn neuron survival does not depend on GABA input, increasing our interest in trying to assess what occurs during the GABAergic period.

MORPHOLOGICAL CHARACTERIZATION OF THE WHALE RETINA

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The retina of the largest adult mammal in the world, the whale, was analysed morphologically by immunohistochemistry. The eye of these aquatic mammals have been poorly studied, thus, the aim of this study was to examine the different neurons and glial cells in the whale retina using a range of molecular markers.
The eyes of beached whales (n= 2, Balaenoptera physalus and Balaenoptera borealis) were obtained, and after dissection and fixation of the retinas, whole-mount preparations and cryostat sections were immunostained. The neurons and glial cells in these tissues were analysed using different antibodies to label RGCs, photoreceptors, bipolar cells, amacrine cells, microglia, astrocytes and Müller cells. Thioflavin S was also used to label misfolded proteins.
Most of the molecular markers used labelled their specific structures in the whale retinas as in terrestrial mammalian retinas. However, whale cones do not express cone markers (M/L and S opsin, and cone arrestin). It is important to highlight the large size of whale RGCs and there are a heterogeneity in NFs expression. It is also noteworthy that intrinsically photosensitive RGCs labelled with melanopsin form an extraordinary network in the whale retina, where these cells are more abundant in the centre, and different subtypes of melanopsin positive-cells were identified. Thioflavin S is weakly labelled of some RGCs in a punctuate pattern and it could easily represent an early sign of neurodegeneration. In addition, degenerative neuritic beading has been observed in RGCs when the retina was analysed after 48 hours postmortem.
In conclusion, there are some notable differences in the retina of the whales when compared with that of terrestrial mammals. Their rod-monochromatic vision due to an evolutionary loss of cone photoreception and the well-developed melanopsin-positive RGCs network could in part be responsible for their perception in the deep sea.
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NEUROMUSCULAR ACTIVITY REGULATES PKA CATALYTIC AND REGULATORY SUBUNITS AND ITS DOWNSTREAM SIGNALING PATHWAY FOR ACh RELEASE AT THE NMJ

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Protein kinase A (PKA) triggers intracellular pathways that modulate activity-dependent mechanisms such as neurotransmission and synaptic plasticity. At the neuromuscular junction (NMJ), PKA signaling enhances neurotransmitter release via the phosphorylation of the release machinery including SNAP-25 (synaptosomal associated protein of 25 kDa) which is a key protein for the exocytosis. Presynaptic impulse at the NMJ triggers the molecular mechanisms associated with the release of ACh and this process can be retrogradely regulated by the resulting muscle contraction. Therefore, both presynaptic and muscular regulation could influence PKA signaling pathway, although this regulation is unknown.
Accordingly, to study the effect of synaptic activity on PKA subunits and its activity, we stimulated the rat phrenic nerve (1 Hz, 30 min) resulting or not in contraction (abolished by μ-conotoxin GIIIB). Changes in protein levels and phosphorylation were detected by Western blotting and cytosol/membrane translocation by subcellular fractionation.
We show that muscle contraction decreases PKA-Cβ levels because the contraction-induced upregulation of AKAP150 is capable to recruit enough RIIβ regulatory subunits to permit Cβ catalytic subunits increase their activity enhancing pSNAP-25 T138 phosphorylation and synaptic vesicle exocytosis and, therefore, allowing the myocyte to receive enough ACh to complete a correct contraction. In addition, RIIα opposes to this regulation binding to Cβ to inhibit its activity and stops the ACh liberation mechanism contributing to balance the catalytical activity of Cβ on pSNAP-25 T138. This contraction-induced regulation opposes to the performed by the presynaptic stimulus that balancing the same mechanisms maintain stable endogenous pSNAP-25 T138 levels and therefore ACh exocytosis.
These results indicate that neuromuscular synaptic activity performed by the neuron and the myocyte regulates accurately the PKA catalytic and regulatory subunits in order to maintain an optimal ACh transmission at the NMJ.

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NEURON-DERIVED EXTRACELLULAR VESICLES ENHANCE SYNAPTIC PLASTICITY THROUGH RTP801

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Neurodegenerative diseases are characterized by an impairment in synaptic plasticity leading eventually to cognitive symptoms in patients. Extracellular vesicles (EVs), which are involved in intercellular communication, have been described to have an important role in synaptic plasticity, as they are carriers of bioactive miRNAs, proteins and lipids. These molecules are involved in synaptic processes and can influence firing rate in the recipient neurons. RTP801/REDD1 is a pro-apoptotic protein which levels are elevated in compromised neuronal populations from patients with neurodegenerative disorders.

The aim of this study is to examine how RTP801 modulates the synaptic effect of EVs at structural and functional levels.

EVs were isolated by ultracentrifugation from rat cortical neurons at DIV13 and used to treat other cultured cortical neurons for 24h. We next examined synaptic plasticity at several levels, assessing the number of consolidated synapses using PSD-95/VGLUT-1 contacts as readout, by immunofluorescence; measuring the levels of synaptic proteins in total neuronal lysates by western blot and investigating whether EVs could change individual neuron activity or collective events, including the topology of the neuronal network.

We next studied how the lack of RTP801 affects synaptic plasticity treating rat cortical cultures with EVs obtained from WT or RTP801 KO mouse cultured neurons.

We found that neuron-derived EVs enhance the consolidation of glutamatergic synapses in recipient neurons, effect that is lost with EVs isolated from RTP801 KO neurons. Moreover, our preliminary results suggest that RTP801 modulates synaptic plasticity by affecting the EVs content.

Further studies will be needed to frame RTP801 as a transcellular mediator of neurodegeneration and to put neuronal EVs in the spotlight to prevent synaptic plasticity impairment.

NMDA RECEPTOR CONTENT OF EXCITATORY SYNAPSES IN THE CA1 REGION OF THE HIPPOCAMPUS IS REDUCED IN P301S MICE

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N-methyl-D-aspartate receptors (NMDARs) are pivotal players in synaptic transmission and plasticity underlying learning and memory. Consequently, synaptic dysfunction of NMDARs has been implicated in the pathophysiology of Alzheimer´s disease (AD). The major structural correlate of the cognitive decline and related symptoms of Alzheimer disease (AD) are mainly attributable to synaptic failure. Given the predominant roles of synaptic NMDA receptors (NMDARs) in excitatory synaptic transmission in the brain, changes in their dynamic regulation have been involved in the pathophysiology of AD. Here we use the P301S tauopathy mouse model to examine possible alterations of GluN1, the obligatory subunit of NMDARs, in neurons that overexpress human tau (P301S mutated gene) in hippocampal neurons, using histoblots and high-resolution immunoelectron microscopic techniques. Histoblots showed that the total amount of NMDARs and their laminar expression pattern in different dendritic layers of the CA1 region of the hippocampus decreased significantly in ten-months-old P301S mice compared to age-matched wild type mice but was unaltered in three-months-old P301S mice. At the ultrastructural level, two synapse populations were examined using SDS-digested freeze-fracture replica labelling in the stratum radiatum in mice of 10 months of age: i) on spines of CA1 pyramidal cells; and ii) on dendritic shafts of CA1 interneurons. P301S mice exhibited a significant reduction of synaptic GluN1 compared with wild-type mice in both pyramidal cells (WT: 616,5 ± 25,2 immunoparticles/µm2; P301S: 392,5 ± 27 immunoparticles/µm2) and interneurons (WT: 541,6 ± 31,6 immunoparticles/µm2; P301S: 353,7 ± 23,1 immunoparticles/µm2) ($P<0,0001$). Our data demonstrate an age-dependent reduction of synaptic NMDARs in P301S mice. These findings support the notion that the progressive accumulation of phospho-tau is associated with synaptic alteration of NMDARs can take place in the absence of Aβ pathology.

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NMDA RECEPTORS CONTAINING GluN3A SUBUNITS INFLUENCE MYELINATION DURING DEVELOPMENT AND AFTER INJURY

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NMDA receptors containing GluN3A subunits (GluN3A-NMDARs) are key modulators of the experience-dependent refinement and consolidation of neuronal circuits during critical periods of postnatal development (Nat Rev Neurosci, 2016). Together with their known effects on synapse selection, unbiased RNAseq analyses suggested that GluN3A-NMDARs modulate myelination. Specifically, we find that: 1) mRNAs encoding a wide range of myelin-related genes are upregulated in GluN3A knockout (KO) mice relative to wild-type; 2) enhanced MBP levels and myelinated axons are observed in somatosensory, motor or visual cortices during the second postnatal week (postnatal days P11-P18), but not at later stages when GluN3A levels are normally down-regulated; 3) the effect can be observed in adult mice upon demyelinating lesions of the CNS.

At the mechanistic level, the myelination phenotype was not due to enhanced numbers of oligodendrocyte precursor cells (OPCs), but to a faster or increased differentiation of existing OPCs. Because GluN3A is expressed both by neurons and oligodendrocytes and the cross-talk between them is essential for proper circuit function, we are now applying: i) mouse genetics to delete GluN3A from oligodendrocytes using Sox10-Cre mice; and ii) neuronal activity stimulation by DREADDs in total and Sox10-Cre KO mice to discriminate between oligodendrocyte and neuronal contributions to the observed phenotypes. Our working hypothesis is that GluN3A coordinates the maturation and consolidation of neural circuits by controlling the on-off switch of regulatory pathways that modulate the selection of synaptic connections and ensuing myelination of associated axons.

NUCLEUS ACCUMBENS ASTROCYTES CONTROL THE COGNITIVE IMPAIRMENT DERIVED FROM CHRONIC EXPOSURE TO THC

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The nucleus Accumbens (NAc) is a key region of the reward system implicated in motivation, drug addiction and numerous neurological and psychiatric disorders. A remarkable feature of this nucleus is the integration of motor and limbic information from glutamatergic inputs. Due to the relevance of this communication, it is crucial the maintenance of glutamate homeostasis, which is altered by addictive drugs. Moreover, there is solid evidence that the modulation of synaptic transmission is mediated by activation of cannabinoid receptors type I (CB1Rs) in astrocytes, suggesting that astrocytic CB1Rs are involved in glutamate homeostasis and modulate long-distance communication between neuronal populations. However, the functional role of astrocytes in alterations derived from chronic drug exposure is not fully understood.

In this study, we have analyzed the role astrocytes play in alterations produced by tetrahydrocannabinol (THC), the psychoactive constituent of marijuana. For that purpose, we have removed specifically the protein p38αMAPK, which mediates exocytic release of glutamate, from NAc astrocytes (Navarrete et al., 2019). First, using fiber photometry in vivo we analyzed glutamate dynamics and astrocytic activity in NAc after 1mg/kg THC chronic administration in wildtype (wt) and p38αMAPK-/- (Astrop38α) mice. Then, we performed behavioral tests to assess whether THC had reinforcing properties or affected learning and memory. Furthermore, using a chemogenetic approach (DREADDs) we activated NAc astrocytes to analyze the behavioral implications. And finally, we performed electrophysiology experiments to analyze synaptic plasticity. We observed: 1)THC administration increases astrocytic calcium activity in wt and Astrop38α; 2)THC administration induces glutamate release in NAc in wt, which is not present in Astrop38α; 3)Astrocyte signaling mediated by CB1R induces NMDAR-LTD at NAc; 4)NAc astrocytes are involved in learning; and 5)Removal of p38αMAPK in NAc astrocytes restores the cognitive impairment derived from THC treatment.
Altogether, our results reveal astrocytes as critical elements for the maintenance of glutamate signaling, with a significant role in drug-consumption related alterations.

NUTRIENT-MEDIATED REGULATION OF GluA1 SURFACE LEVELS

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It is widely known that brain needs a lot of energy to carry out all its functions. Neurons use most of this energy to maintain glutamatergic synapsis in which AMPA receptors play an important role. Our group has recently demonstrated that the basal transport of AMPAR subunit GluA1 to the plasma membrane is downregulated upon glucose depletion. Moreover, it has been described that specific nutrients also modulate synaptic strength, as well as some diets have an impact on learning and memory processes. The aim of this study is to elucidate the molecular mechanisms by which different nutrients can regulate synaptic function, analyzing its effects on AMPAR trafficking. In order to address this objective, primary cortical mouse neurons were treated with different fatty acids or ketone bodies at 14-15 days of culture. Then, GluA1 surface levels were analysed by immunocytochemistry. Our results indicated that, on the one hand, palmitic acid, a saturated fatty acid mostly found in palm oil, decreased the amount of GluA1 surface levels in neurons. However, oleic acid, an unsaturated fatty acid which comprises the majority of olive oil, did not show a negative impact on GluA1 synaptic level. Nevertheless, a polyunsaturated fatty acid found in fish like salmon, the ω-3 docosahexaenoic acid, increased the amount of GluA1 subunit at plasma membrane. On the other hand, the β-hydroxybutyrate, a ketone body used as a source of energy in the brain during ketogenic diet (based on low carbohydrate and high fat intake), raised GluA1 surface levels. In summary, we demonstrate that saturated fatty acids reduce GluA1 surface levels, while polyunsaturated fatty acids and ketone bodies seem to have beneficial effects in neurons. These results give insight into why certain diets are able to delay cognitive impairment in neurodegenerative diseases.

ON THE G PROTEIN-COUPLED HETERORECEPTOR COMPLEXES NEUROMODULATION OF THE CLAUSTRUM

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G protein-coupled receptors (GPCRs) modulate the synaptic glutamate and GABA transmission of the claustrum. The work focused on the transmitter-receptor relationships in the claustral catecholamine system and receptor-receptor interactions between, dopamine D2 receptor (D2R), kserotonin 5HT2A receptor (5HT2AR), neurotensin NTS1 receptor (NTS1R), kappa opioid receptors (KOR) and SomatostatinR2 (SSTR2) in claustrum. Methods used involved immunohistochemistry and in situ proximity ligation assay (PLA) using confocal microscopy. Double immunolabeling studies on dopamine (DA) D1 receptor (D1R) and tyrosine hydroxylase (TH) immunoreactivities (IR) demonstrated that D1R IR existed in almost all claustral and dorsal endopiriform nucleus (DEn) nerve cell bodies, known as glutamate projection neurons, and D4R IR in large numbers of nerve cell bodies of the claustrum and DEn. However, only a low to moderate density of TH IR nerve terminals was observed in the DEn versus de few scattered TH IR terminals found in the claustrum. These results indicated that DA D1R and D4R transmission in the rat operated via long distance DA volume transmission in the rat claustrum and DEn to modulate claustral-sensory cortical glutamate transmission. Large numbers of these glutamate projection neurons also expressed, 5-HT2AR, NTS1R, KOR and SSTR2 which formed 5HT2AR-D2R, D2R-NTS1R, and KOR-SSTR2 heteroreceptor complexes using PLA. Such receptor-receptor interactions can finetune the activity of the glutamate claustral-sensory cortex projections from inhibition to enhancement of their sensory cortex signaling. This can give the sensory cortical regions significant help in deciding on the salience to be given to various incoming sensory stimuli.

ORGANIZATION OF A Sox2-POSITIVE GLIAL CELL POPULATION IN THE OPTIC NERVE ASSOCIATED WITH GROWING FIBERS IN THE FISH VISUAL SYSTEM

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The visual system of teleost fish shows growth and regeneration capacities during the entire animal's life. Therefore, the pre-encephalic visual system [retina, optic nerve head (ONH), and optic nerve (ON)] of adult fish serve as a model for studying neurogenesis and regeneration of the vertebrate central nervous system (CNS). Previous work has shown differences of Sox2 expression in areas of pre-encephalic visual system, which indicates that this transcription factor could have several functions in the CNS. Our study focused on a detailed characterization of a Sox2 positive cell population located in the first portion of the optic nerve. We have used adult specimens of the cichlid fish Astatotilapia burtoni as an animal model, which shows substantial growth adding retinal tissue and optic nerve fibers throughout life. Clearing samples of pre-encephalic visual system (the whole eye with an optic nerve piece), and immuno staining, we identified a population of glial cells positive for Sox2. These cells are arranged around the axons from newborn neurons identified by staining for doublecortin and location in the optic nerve. Our results suggest that this glial population is associated with the pathway navigation of the new axons from the retina. Besides known functions in stem cell pluripotency, Sox2 could be involved in neurochemical signaling and/or a pool of potential proliferative cells, in possible combination with other regulatory factors, in the fish visual system. Understanding the variety of cell types and subtypes in the visual system of fish and their plasticity could be the key to comprehend the growing and regenerating processes in the adult vertebrate CNS.

p11 (S100A10) KNOCKDOWN IMPACTS SYNAPTIC STRENGTH AND USE-DEPENDENT SHORT-TERM PLASTICITY AT EXCITATORY SYNAPSES IN RAT HYPOGLOSSAL MOTONEURONS

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Chaperone p11 (S100A10) is an adaptor protein that regulates trafficking and plasma membrane expression of several ion channels and receptors. Thus, p11 sets up motoneuron (MN) intrinsic excitability (Nat Commun. 2019;10(1):3784). Although there is evidence supporting a presynaptic role of p11 in synaptic dynamics, little evidence exists so far on its involvement in neurotransmission and synaptic plasticity. In brainstem slices from rat pups (P7-P9), a high frequency stimulation (HFS, @60-Hz, 10-s) protocol was applied to the ventrolateral reticular formation and excitatory postsynaptic currents (EPSC) from hypoglossal MNs (HMNs) were analyzed during and after HFS. A small-interfering RNA against p11 (siRNAp11; 5 □g/5 □l) was injected into the fourth ventricle at P5, taken administration of a non-interfering siRNA (cRNA) as control. siRNAp11 reduced (-56.6 ± 5.2%) mRNAp11 expression in brainstem of P7-P9 rats. P11 knockdown was accompanied by a reduction in the amplitude of recorded EPSCs (cRNA: 0.467 ± 0.056 nA; siRNAp11: 0.338 ± 0.048 nA). Interestingly, siRNAp11 altered both synchronic and asynchronic neurotransmitter release during HFS. Whilst the kinetic of reduction in charge transfer for the synchronic component of the synaptic response across the train experienced only a subtle delay after p11 knockdown, the asynchronic component was strongly attenuated by this treatment (cRNA: 1.58 ± 0.372 nC; siRNAp11: 0.66 ± 0.119 nC). Since the asynchronic component increases during train by accumulation of presynaptic [Ca2+]i, siRNAp11-induced reduction in this component also supports a pre-synaptic site of action of endogenous p11. Kinetic of EPSC recovery after HFS was also altered in siRNAp11 relative to cRNA-treated pups. A more detailed analytical processing is needed to clarify particular synaptic events regulated by this protein in use-dependent synaptic plasticity.
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Keywords: Motoneurons, S100A10, short-term synaptic plasticity.

PARALLEL PROCESSING OF QUICKLY AND SLOWLY MOBILIZED RESERVE VESICLES IN HIPPOCAMPAL SYNAPSES

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Neurotransmitter in presynaptic terminals is stored within synaptic vesicles, and is released into the synaptic cleft via exocytosis. However, a synaptic terminals contains hundreds of vesicles in the interior, whereas fewer than ten can dock at once to the active zone area of the plasma membrane where exocytosis occurs. The vesicles in the interior are thought to be held within so called reserve pools, but the concept of a pool remains poorly defined. Here we use optical imaging dyes and a combination of low and high frequency stimulation to confirm that individual hippocampal presynaptic terminals in cell culture contain multiple reserves that can be distinguished functionally by how quickly the contents can be mobilized for exocytosis. Quickly and slowly mobilized reserves were mobilized in parallel, and did not mix, even during heavy stimulation. The results are not consistent with long-standing models where reserve pools are connected in series, but instead support an alternative that emerged from a series of previous electrophysiology studies, where: 1) active zones contain multiple independent docking/release sites; (2) the release sites vary in probability of catalyzing exocytosis following individual action potentials; and (3), each docked vesicle is connected to a separate reserve.

POTENTIAL NEUROPROTECTIVE ROLE OF LYSOPHOSPHATIDIC ACID RECEPTOR 1 OVEREXPRESSION BY HIPPOCAMPAL NEURONS IN A MODEL OF TEMPORAL LOBE EPILEPSY

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Lysophosphatidic acid receptor 1 (LPA1) is a G-protein coupled receptor involved in cell proliferation, survival differentiation and other biological processes. In the adult rodent brain, LPA1 specifically labels hippocampal neural stem cells (NSCs) which generate newborn neurons throughout postnatal and adult life in most mammals.

Interestingly, LPA1 also labels Reactive-NSCs (React-NSCs). The reactive glia-like counterparts of NSCs induced by seizures and that abandon neurogenesis to transform into reactive astrocytes and contribute to gliosis.

Further, using a transgenic mouse line in which the enhanced green fluorescent protein is expresses under the regulatory elements of LPA1 (LPA1-GFP) we have stablished that React-NSCs lose LPA1 expression several weeks after seizures, as they differentiate into reactive astrocytes. In parallel, neurons of the granule cell layer start to express LPA1 gradually in the epileptic brain and maintain its expression in the long term.

Using confocal microscopy imaging of control and epileptic LPA1-GFP mice we are currently evaluating whether LPA1 expression promotes the survival of neurons in granule cell layer. In addition, we are using hippocampal NSC-derived neuronal cultures to activate or inhibit LPA1 inducing cell death to better asses it potential role in neuroprotection.

PRE- AND POSTSYNAPTIC ORGANIZATION OF C-TYPE SYNAPSES ON MOTOR NEURONS ARE REGULATED BY THE DIFFERENT ISOFORMS OF NEUREGULIN 1

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C-type synapses on motor neurons (MNs) originate from local cholinergic interneurons and display a particular postsynaptic ER-related organelle called subsynaptic cystern (SSC). Neuregulin-1 (NRG1) was detected close to C-boutons in association with SSCs. As the NRG1 antibody used in these studies cannot distinguish different isoforms of NRG1, we took advantage of transgenic mouse lines to examine NRG1 isoform-specific functions in C-boutons.

We have performed electron and confocal microscope analyses of the MN C-boutons of transgenic mice that overexpress NRG1 type I (NRG1-typeI) or NRG1 type III (NRG1-typeIII).

Samples from NRG1-typeIII mice showed NRG1 area that, in contrast with the wt mice, overpass the afferent presynaptic limits on the surface of MN cell bodies. We found that these C-boutons were specially enriched in sigma-receptor 1, Kv2.1 and M2 muscarinic receptor, with a similar pattern to the expanded NRG1-typeIII. However, the number of cholinergic terminals contacting MN somata was not altered. The examination of NRG1-typeIII mice by electron microscopy showed an accumulation of abnormally expanded and reduplicated SSC-like structure. Regarding NRG1-typeI, the immunostaining for NRG1 also produced a stronger signal at the MN surface of NRG1-typeI mice compared with WT. However, in contrast to NRG1-type III mice, the vesicular acetylcholine transporter immunostaining revealed an increase in the number and size of presynaptic terminals innervating the MN surface. The ultrastructural examination confirmed the presence of enlarged presynaptic terminals on the MN soma surface, matching partially with postsynaptic SSC. However, the amplified formation of SSC-like was not observed in NRG1-typeI mice.

Altogether, these findings suggest that: 1) NRG1-typeIII acts as a specific organizer of postsynaptic SSC-like membrane compartments without a major impact on the C-bouton presynaptic counterpart; 2) NRG1-typeI promotes presynaptic C-bouton synaptogenesis with no influence on biogenesis or molecular architecture of coaligned SSC.

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RAS SIGNALING DURING METABOTROPIC GLUTAMATE RECEPTOR DEPENDENT LONG TERM DEPRESSION (mGluR-LTD)

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RASopathies are the most common type of neurodevelopmental disorders, affecting approximately 1 in 1000 individuals. Ras proteins are small GTPases that can act as a molecular switch with its downstream effectors, such as PI3K/Akt or MAPK/Erk pathways. Both, Ras and its effectors are essential regulators of cell proliferation, differentiation and survival. In addition, they have been related with synaptic plasticity events, key processes for learning and memory. It is known that Ras, PI3K/Akt and MAPK/Erk pathways are required for long term potentiation; however, the role of Ras in long-term depression induced by metabotropic glutamate receptors (mGluR-LTD) is still controversial but clinically relevant, especially for Costello Syndrome or SynGAP-deficient patients. To address this question we generated a collection of Ras mutants and produced Sindbis virus to infect rat hippocampal organotypic cultures used for electrophysiological recordings and biochemical experiments. To monitor protein synthesis, we used SUnSET assay, a non-radioactive method based on the use of puromycin. We also optimized a FRET system in our organotypic cultures to monitor Ras activity using live imaging techniques. Firstly, using this FRET system, we observed that Ras activity increases upon chemical mGluR-LTD induction (DHPG 100μM stimulation) in dendritic spines. Subsequently, to elucidate if Ras is required for mGluR-LTD we occluded Ras activity using a dominant negative form which prevented the synaptic depression, measured electrophysiologically. Blocking Ras activity with this mutant also prevented the increase in pErk levels and in protein synthesis upon chemical mGluR-LTD induction compared to control infected slices. To sum up, Ras activity is required for mGluR-LTD, mediating the increase in Erk signaling and in protein synthesis.

REACTIVE NEURAL STEM CELLS AND ABERRANT NEUROGENESIS IN A NEURON-SPECIFIC MODEL OF DRAVET SYNDROME

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Hippocampal neurogenesis (HN) is a form of neuroplasticity which implicates the generation of new neurons from neural stem cells (NSCs) in the dentate gyrus (DG). Although HN persists throughout adulthood, it reaches maximum values during early postnatal periods, when the population of NSCs is at its largest.

NSC activity and HN are particularly regulated by neuronal activity and severe alterations have been found in the hippocampal neurogenic niche in mouse models of epilepsy. Induction of reactive-like and gliogenic NSCs (React-NSCs) besides aberrant neurogenesis, defined by altered newborn neuron morphology, migration and functional properties, are induced by epileptic seizures.

We are thus interested in Dravet Syndrome (DS), a severe form of infant epilepsy characterized by the early onset (3-6 months of age) of seizures. DS is caused by mutations in the Scn1a gene encoding the α1 subunit of sodium channel Nav1.1, and provokes febrile seizures, hyperexcitability, neurological comorbidities and premature death. Therefore, we hypothesize that early seizures could have a greater impact and longer-lasting on the neurogenic niche in DS due to their early onset.

Through confocal microscopy imaging we are analysing the neurogenic niche of a novel inducible knock-in mouse model of DS (Syn-Cre/Scn1aWT/A1783V) at postnatal day 25 (soon after the onset of seizures) which consist in the neuron-targeted expression of a missense mutation (A1783V) in the Scn1a gene. We have observed the induction of React-NSCs, characterized by more and thickened branches plus overproliferation. We have also observed a strong induction of aberrant neurogenesis. Newborn immature neurons, identified by the expression of doublecortin are present in much higher numbers; migrate abnormally towards the hilus and the molecular layer; and have basal dendrites and V-shaped proximal apical dendrites. We are currently investigating other possible alterations such as cell death/survival, differentiation imbalance and changes in astroglia and microglia.

REDUCTION IN THE DENSITY OF GROUP I MGLU5 RECEPTORS ALONG THE NEURONAL SURFACE OF HIPPOCAMPAL CELLS IN A MOUSE MODEL OF ALZHEIMER´S DISEASE

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Metabotropic glutamate receptor subtype 5 (mGlu5) is implicated in the pathophysiology of Alzheimer´s disease (AD). However, its alteration at the subcellular level in neurons is still unexplored. Here, we provide a quantitative description on the expression and localisation patterns of mGlu5 in the APP/PS1 model of AD at 12 months of age, combining immunoblots, histoblots and high-resolution immunoelectron microscopic approaches. Immunoblots revealed that the total amount of mGlu5 protein in the hippocampus, in addition to downstream molecules (i.e., Gq/11 and PLCβ1), was similar in both APP/PS1 and age-matched wild type mice. Histoblots revealed that mGlu5 expression in the brain and its laminar expression in the hippocampus was also unaltered. However, the ultrastructural techniques SDS-FRL and pre-embedding immunogold, demonstrated that the subcellular localisation of mGlu5 was significantly reduced along the neuronal surface of hippocampal principal cells, including CA1 pyramidal cells and DG granule cells, in APP/PS1 mice at 12 months of age. The decrease in the surface localisation of mGlu5 was accompanied by an increase in its frequency at cytoplasmic sites in the two neuronal populations. Together, these data demonstrate for the first time a redistribution of mGlu5 from the plasma membrane to intracellular sites in different principal cells of the hippocampus in APP/PS1 mice, suggesting an alteration of the excitability and synaptic transmission that could contribute to the cognitive dysfunctions in this AD animal model.

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RELATIONSHIP OF FAIM-L AND OVARIAN TUMOR (OTU) DEUBIQUITINASES IN SYNAPTIC REMODELATION

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A correct regulation of the nuclear factor-kappa B (NF-κB) transcription factor is critical for synaptic processes, neurotransmission and neuroprotection. Recently, deubiquitinase A20, a well known negative regulator of NF-κB in immune system, has been involved in synaptic functions. In primary hippocampal neurons, A20 negatively regulates dendritic spine density in an NF-κB activation-dependent manner; however the molecular mechanism requires further clarification.
A20 belongs to a family of deubiquitinating cysteine proteases named ovarian tumor (OTU) family which contains other A20-like proteins such as OTUD7A (Cezanne2) and OTUD7B (Cezanne). Primary cortical neurons from Cezanne2-null mice show decreased total spine density and activity compared to wild-type neurons, showing an opposite function of what described for A20. However, little is known about the molecular pathways and partners associated to Cezanne2 in neurons.
Our group has previously shown that the neuronal form of Fas Apoptotic Inhibitory Molecule (FAIM-L) participates in non-apoptotic functions of caspases such as axonal degeneration and synaptic plasticity. By stabilizing XIAP, FAIM-L regulates AMPAR internalization after NMDA-induced LTD and regulates axonal pruning after NGF deprivation.
Since FAIM-L, A20 and Cezanne2 participate in synaptic remodelation and plasticity, we first studied whether they are able to interact. We observed that A20 interacts specifically with neuronal isoform, FAIM-L, and not with ubiquitously isoform, FAIM-S, under overexpression conditions. In addition, preliminary results reveal that FAIM-L could regulate the stability of A20 either directly or indirectly. Furthermore, we are studying whether the other A20-like proteins, in particular Cezanne2 could also specifically interact with the neuronal form of FAIM.
Given all this, we hypothesize that FAIM-L interaction with A20 or Cezanne2 could modulate their role in synapses. Therefore, examining the implications of these interactions in physiological and pathological conditions, such as neuroinflammation and neurodegeneration, will provide crucial information for better understanding the molecular mechanisms of dementia.

RNA LOCALISATION AND LOCAL TRANSLATION IN MICROGLIAL PERIPHERAL PROCESSES

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Local RNA translation allows the cells to respond fast and efficiently to environmental stimuli. Local translation is especially important in highly polarized cells, such as neurons, because it provides axons and dendrites a means for an accurate response to fast environmental changes. Although most of the work on local protein synthesis in brain cells has been performed in neurons, we now know this phenomenon is not restricted to these cell types. For instance, local translation has been described in peripheral astrocytic processes. In astrocytes local protein synthesis is essential for astrocytes to be involved in synapsis (Sakers et al., 2017). Furthermore, in oligodendrocytes it has been seen that MBP is translated locally in neurodegenerative conditions and during differentiation (Quintela-Lopez et al., 2019). Only recently localized translation in microglia has been established (Vasek et al., 2021), however its role in the pathophysiology of the brain has not been addressed. We propose that local translation in microglia plays fundamental roles in brain function and dysfunction.
We have exposed microglia to different stimuli, like LPS, ATP, Aβ and MCSF, and analysed how they affect local translation in microglial peripheral processes. LPS is the only stimulus inducing changes in local protein synthesis, as well as inducing changes in global RNA localization at the interface between microglial lamellae and filopodia (the leading edge). Additionally, Actb transcripts are increased in microglial lamellae and filopodia in response to LPS with LPS.
So far, our results indicate that local protein synthesis might be required for the inflammatory response in microglia cells. We are currently analysing localized translation of Actb and other transcripts involved in cell polarity and cytoskeletal rearrangements using puromycilation combined with proximity ligation assay (Puro-PLA).

ROLE OF GABAA AND AMPA RECEPTORS IN THE GENERATION AND PROPAGATION OF EPILEPTIFORM ACTIVITY IN THE CINGULATE CORTEX OF A MOUSE MODEL OF LISSENCEPHALY.

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The mutant mouse Lis1/sLis1 is a model of mild human lissencephaly that is very useful to study the role of the gene LIS1 in the pathophysiological mechanisms related to this disease. A prominent clinical feature of lissencephaly is the presence of intense epileptic seizures, and we have studies the pharmacology of the generation of epileptiform electrophysiological activity in the anterior cingulate cortex (ACC) of the Lis1/sLis1 mouse. The experiments were done using coronal brain slices and extracellular recording of epileptiform discharges (ED) in layer 2/3 of the ACC; the ED were evoked by electrical stimuli applied to layer 1 and in the presence of bicuculline (a blocker of GABAA receptors).

The sensitivity to bicuculline (tested at concentrations of 0.1–20 microM) of then generation of ED was similar in WT and Lis1/sLis1 ACC. The bicuculline D50 was 3.26±1.16 microM, n=11 and 2.95±1.22 microM, n=9 (WT and Lis1/sLis1 respectively); the D50 was measured from the dose-response relationship between the concentration of bicuculline and the size of the ED. To explore the role of AMPA receptors in the generation and propagation of ED we tested the effects of AMPA receptors antagonists. CNQX (1microM; a blocker of AMPA/kainate receptors) produced an increase of the latency of ED that was significantly larger in WT than in Lis1/sLis1 slices (WT: 15.35±2.67ms, n=11; Lis1/sLis1: 9.40±4.02ms, n=8; p=0.029 RankSumTest). GYKI53655 (2microM; a selective AMPA receptor blocker) produced a dose-dependent increase of the latency that was larger in WT than in Lis1/sLis1 (WT: 39.03±13.27%, n=4; Lis1/sLis1: 3.27±3.88%, n=6; p=0.038, RankSumTest), and a similar decrease of the size of ED (WT: 32.12±20.8%, n=4; Lis1/sLis1: 31.76±15.4±3.88%, n=6; n.s.)

These data suggest that in Lis1/sLis1 ACC the sensitivity of the generation of ED was normal, but there were abnormalities in AMPA receptors.

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ROLE OF LYSOPHOSPHATIDIC ACID RECEPTOR LPA1 IN USE-DEPENDENT SHORT-TERM DEPRESSION AND RECOVERY AT EXCITATORY SYNAPSES IN RAT HYPOGLOSSAL MOTONEURONS

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The bioactive phospholipid LPA impacts excitatory synaptic strength in hypoglossal motoneurons (HMNs) by a LPA1-mediated presynaptic mechanism influencing neurotransmitter release (PLoS Biol. 2015 13(5):e1002153). Here, we hypothesized that use-dependent short-term plasticity at excitatory synapses engages, at least in part, LPA-LPA1 signaling. In brainstem slices from rat pups (P5-P9), a high frequency stimulation (HFS, @20-Hz, 60-s) protocol was applied to the ventrolateral reticular formation and excitatory postsynaptic currents (EPSC) from HMNs were analyzed during and after (@0.2-Hz) HFS. Exogenous application of LPA (1 □M), but not vehicle, to the bath solution altered synchronic and asynchronic neurotransmitter release during HFS. The asynchronic component is known to increase during the train by accumulation of presynaptic [Ca2+]i. Furthermore, LPA delayed EPSC recovery after HFS. Addition to the bath solution of the LPA1 inhibitor AM095 (10 □M) or preceding microinjection of a small-interfering RNA against lpa1 (siRNAlpa1) into the fourth ventricle at P4, both affected synchronic and asynchronic release in an opposite direction that LPA. A non-interfering siRNA was taken as control in siRNAlpa1 experiments. AM095 and siRNAlpa1 both accelerated EPSC reestablishment after HFS. Finally, addition of a non-permeable Ca2+-chelator EGTA (11 mM) into the internal solution of the recording pipette or permeable AM-EGTA (300 □M) to the bath solution, differentially effected AM095-induced alterations on EPSCs during and after HFS. Altogether, these outcomes indicate that LPA-LPA1 signaling mediates short-term depression and recovery kinetics during and after HFS, respectively. The mechanism of action of endogenously synthesized LPA seems to depend on both pre- and post-synaptic [Ca2+]. A more detailed analytical processing is needed to clarify particular synaptic events regulated by this signaling pathway in use-dependent synaptic plasticity.

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Keywords: Motoneurons, Lysophosphatidic acid, short-term synaptic plasticity.

ROLE OF PI3K CATALYTIC ISOFORMS IN NEURONAL METABOLISM

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Among all organs, the brain is unique, not only because it consumes 20% of total body glucose, but also due to its unique metabolic profile, which appears compartmentalized between astrocytes and neurons. Thus, under basal conditions, astrocytes are responsible for most glucose uptake, and its processing into lactate, which is then shared with neurons through the astrocyte-neuron lactate shuttle (ANLS). However, there is increasing evidence that these metabolic fluxes are modulated in response to neuronal activity.

Phosphatidyl inositol 3-kinases (PI3Ks) are lipid kinases responsible for the conversion of phosphatidylinositol (4.5)-bisphosphate (PIP2) into phosphatidylinositol (3,4,5)-triphosphate (PIP3). PI3Ks are important mediators of synaptic plasticity processes. In addition, the PI3K/mTOR pathway is a central hub for the regulation of energy balance and cellular metabolism. However, little is known about the function of PI3Ks in neuronal metabolism.

We have addressed this problem using two lines of loxP mice, for the main catalytic subunits of PI3K: p110α or p110β. Neuronal specific knock-outs (KO) are generated in the hippocampus of adult animals by injecting an adeno-associated virus that expresses the Cre recombinase under the neuron-specific αCamKII promoter. Mice injected with saline were used as control.

Using proteomic approaches, we have observed differential effects of p110α and p110β KO in glycolytic enzymes, as well as in proteins related to mitochondrial function. Alterations in mitochondrial function are also suggested by changes in mitochondrial morphology, as evaluated by electron microscopy.

The functional relevance of these changes and the implication of each PI3K catalytic subunit in neuronal metabolism and synaptic plasticity will be discussed.

ROLE OF PI3-KINASE REGULATORY SUBUNIT (P85) IN THE STRUCTURAL PLASTICITY OF DENDRITIC SPINES

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Class I Phosphatidylinositol-3-kinases (PI3Ks) constitute a complex family of enzymes formed by a P110 catalytic (p110α, p110β, p110γ, p110δ) and a P85 regulatory (p85α, p55α, p50α, p55γ, p85β) subunit, with important roles in virtually all physiological processes in the body including, synaptic plasticity and cognitive function. Synaptic plasticity has a functional component (changes in synaptic strength) and a structural component (changes in synapse size, associated to cytoskeleton modifications in dendritic spines). The differential interactions and contributions of the PI3K isoforms for these functional and structural aspects of synaptic plasticity are still unknown. To address these issues, we have carried out loss-of-function (RNA interference) of specific p85 isoforms in rat hippocampal slices. After specific knockdown of p85α or p85β, most PI3K complexes are detected as heterodimers of p110 interacting with the alternative p85 isoform. In this manner, we can assess the function of p110/p85α versus p110/p85β forms of PI3K. This manipulation is associated with changes in PI3K signalling, as reported by phosphorylation of the protein kinase Akt and the ribosomal protein S6. In addition, using life imaging experiments in organotypic hippocampal slices, we detected changes in structural plasticity after long-term potentiation (LTP) induction, particularly with respect to the recruitment of the cytoskeleton proteins actin and cofilin into the dendritic spines. These differences had also functional consequences at the level of synaptic potentiation, as evaluated using electrophysiological recordings. The molecular aspects of this differential contribution of p85α and p85β to synaptic plasticity will also be discussed.

RUNNING AND SWIMMING DEPENDENT FAST-TO-SLOW BDNF/TrkB SIGNALLING OPTIMISATION AT THE NMJ

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Exercise is the most common physiological stimulus and has the capacity to modify tissues functionality. It improves motor control and cognitive abilities and reinforces neuroprotective mechanisms in central and peripheral nervous system. Peripheral nerves interact with skeletal muscles at the neuromuscular junction to guarantee an appropriated functionality of each other and of the neuromuscular synapse. Thus, modifications of this bidirectional communication through physical activity preserve this synapse as it increases quantal content and resistance to fatigue, acetylcholine receptors expansion and myocytes fast-to-slow functional transition.
Here, we provide the intermediate step between physical activity and functional and morphological changes by analysing the molecular adaptations of the full BDNF/TrkB downstream signalling in the skeletal muscle pathway, directly involved in acetylcholine release and synapse maintenance. After 45 days of training at different intensities, the BDNF/TrkB molecular phenotype of trained muscles from male B6SJLF1/J mice evidence a fast-to-slow transition without affecting motor neuron size. We provide further knowledge to understand how exercise induces muscle molecular adaptations towards a slower phenotype, resistant to prolonged trains of stimulation or activity that can be useful as therapeutic tools.
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SIGNALING MEDIATED BY THE CREB-REGULATED TRANSCRIPTION COACTIVATOR-1 (CRTC1) REGULATES NMDA-DEPENDENT SYNAPTIC PLASTICITY

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Activity-dependent remodeling of synapses (i.e, synaptic plasticity) is considered the cellular basis of several brain physiological processes, including learning and memory. Synapse-to-nucleus signaling plays an important role in glutamatergic synaptic plasticity by linking the activation of N-methyl-D-aspartate receptors (NMDARs; GluN) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs; GluA) to gene transcription. The synaptonuclear factor CREB-regulated transcription coactivator-1 (CRTC1) connects glutamate receptor activation to CREB-dependent genetic programs at the nucleus, contributing to neuronal development, survival and plasticity. By contrast, CRTC1 deregulation is associated with dendritic pathology and cognitive dysfunction in neurodegenerative disorders. The CRTC1-dependent molecular mechanisms regulating NMDA-mediated synaptic plasticity in brain physiology and pathology remain poorly understood. In this study, we employed biochemical, cellular and electrophysiological techniques and gain and loss of function gene expression approaches to elucidate the role of CRTC1 in glutamate receptor modulation in the hippocampus. We found that CRTC1 overexpression or silencing have opposite effects on GluN1 expression, protein kinase C/A (PKC/PKA)-mediated phosphorylation and synaptic localization without affecting total and phosphorylated GluA levels. CRTC1 mediates NMDAR transmission and synaptic potentiation in the hippocampus by regulating (PKC)-induced phosphorylation and synaptic recruitment of GluN1. These results suggest that besides contributing to expression of neuroplasticity-related genes into the nucleus, CRTC1 may play a local role by regulating NMDAR localization at synapses.

THE MICROGLIAL P2Y6 RECEPTOR MEDIATES NEURONAL LOSS AND MEMORY DEFICITS IN NEURODEGENERATION

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Summary

Microglia are implicated in neurodegeneration, potentially by microglia phagocytosing neurons, but it is unclear how to block the detrimental effects of microglia while preserving their beneficial roles. The microglial P2Y6 receptor (P2Y6R) – activated by extracellular uridine-5'-diphosphate (UDP) released by stressed neurons – is required for microglial phagocytosis of neurons. We show here that injection of amyloid beta (Aβ) into mouse brain induces microglial phagocytosis of neurons, followed by neuronal and memory loss, and this is all prevented by knockout of P2Y6R. In a chronic tau model of neurodegeneration (P301S tau mice), P2Y6R knockout prevented tau-induced neuronal and memory loss. In vitro, P2Y6R knockout blocked microglial phagocytosis of live but not dead targets, and reduced tau-, Aβ- and UDP-induced neuronal loss in glial-neuronal cultures. Thus, the P2Y6 receptor appears to mediate Aβ- and tau-induced neuronal and memory loss via microglial phagocytosis of neurons, suggesting that blocking this receptor may be beneficial in neurodegenerative diseases.

Keywords: Microglia, phagocytosis, neurodegeneration, P2Y6 receptor, memory deficits, Alzheimer's disease, cell death.

THE PRIMARY CILIUM AS AN ORGANELLE FOR ASTROCYTE-NEURON COMMUNICATION.

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Primary cilia are microtubule-based organelles present in the plasma membrane of most cell types, including mature astrocytes and neurons. The primary cilium has emerged as a major signalling hub in the cell; however, little is known about the role of this organelle in the mature brain. Data from our lab show that neuronal cilia is required for soluble amyloid beta oligomer signalling and modulation of autophagy, and that these events are modulated by physiological aging.

Here, we hypothesize that similarly to neuronal cilia, astrocytic primary cilium senses and transduces extracellular signals and that it reacts to changes in neuronal cilium. We also hypothesize that aging might alter cilia-related events in old astrocytes. To test our hypothesis, we have studied how the loss of primary cilia in neurons induces changes in astrocytes, astrocytic primary cilia, and cilia-related autophagy. For that, we have studied by IHC astrocyte reactivity and morphology in young and old IFT88::SLICK-H mice, a mouse model where cilia is lost in mature Thy1+ neurons. In these mice, we have also characterized astrocyte cilia presence and their morphology, as well as changes in the major autophagy markers. Moreover, we have deleted primary cilia in human astrocytes as well as in human neurons and established an in vitro model of astrocyte-neuron co-culture, with the aim to study the dynamics of astrocyte and neuronal cilia changes between these two cell types. Overall, we aim to understand the role of mature astrocytic primary cilia in the brain, as well as its interplay with neurons and their possible changes during aging.

THE ROLE OF CASPASE 8 IN THE DOPAMINERGIC SYSTEM

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Our group was a pioneer in identifying new non-apoptotic roles of caspases-8 and -3/7 in the CNS demonstrating a caspase-dependent mechanism governing microglia activation. Our propose is not only to continue studies about non-apoptotic roles of caspases in the control of brain inflammation, but to extend them to neuronal phenotypes. The aim of the present study was to understand the role of caspase-8 in the dopaminergic system. For this reason we have generated a novel animal model with a specific gene deletion of caspase-8 within catecholaminergic cells.
We performed behavioral, molecular and cellular analysis to study the nigro-striatal system in these animals. Therefore, we have measured dopamine levels in striatum by HPLC, expression levels of main dopaminergic components in substantia nigra by RT-PCR and motor status by behavioral tests. Our results show differences in the behavioral tests performed in mice lacking caspase-8. Moreover, we found no differences on mRNA expression levels of main dopaminergic proteins but higher levels of dopamine accompanied by an increase in the number of dopaminergic neurons by means of stereological studies. All these results could be the cause of the changes observed in the behavioral tests. Therefore, our results suggest that this animal model could open the field to future studies on the role of caspase-8 on the dopaminergic system, being applicable as a model for different brain disease with the dopaminergic system involved.

THE Y172 ANTIBODY AGAINST PHOSPHO-C-JUN (SER63) SELECTIVELY DETECTS AN UNIDENTIFIED PROTEIN PRESENT IN MOTONEURONS AND SCHWANN CELLS, TWO SIDEKICKS OF THE NEUROMUSCULAR SYSTEM

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To drive motor behavior, the excitation state of motoneurons (MNs) is controlled by different inputs. The C-bouton is one of the main excitatory synapses on MNs. Alterations in C-boutons appear to play an important role in MN pathology, particularly in atrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). During an immunocytochemical study on the role of c-Jun in MNs with a monoclonal (clone Y172) antibody against phospho-c-Jun (serine [Ser]63), we observed an unexpected labeling closely associated to C-boutons.

We further analyzed the Y172 immunostaining in the spinal cord MNs of CD1 mice and mouse models of SMA (Smn2B/-) and ALS (SOD1G93A). Additionally, we extended the study to the sciatic nerve.

In adult spinal cord, MNs displayed strong Y172 immunostaining in cytoplasmic structures associated with C-boutons, but not with other synapse types on MN somata and proximal dendrites. By ultrastructural analysis, cytoplasmic Y172 immunostaining was selectively located at the subsurface cistern of C-boutons. The analysis of Y172 immunoreactivity in injured MNs after peripheral nerve transection, and in SOD1G93A and Smn2B/- mice, revealed a significant depletion of cytoplasmic immunostaining. RNA interference experiments to knockdown c-Jun in vitro resulted in no reduction in the density of cytoplasmic Y172-positive profiles. Studies in the sciatic nerve revealed that the Y172-immunoreactivity was also present in the cytoplasm of Schwann cells ensheathing MN axons.

Overall, we show a novel unidentified molecular component of the C-bouton organization, which expression is lost in damaged MNs even before the occurrence of cholinergic deafferentation. Moreover, the presence of Y172 in Schwann cells suggests that this protein may play an important role in MN maintenance. Our results lay the foundation for further studies aimed at identifying the Y172-related protein and determining its role in the context of the development, maintenance, plasticity and pathology the neuromuscular system.

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TOWARDS PHARMACOLOGICAL MODULATION OF MICROGLIAL PHAGOCYTOSIS

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Microglia, the immune cells of the central nervous system, display a variety of functions to maintain homeostasis in the brain. Microglia are professional phagocytes and remove apoptotic cells to prevent the spillover of toxic components. Although phagocytosis is a key process to maintain brain homeostasis and is very efficient in physiological conditions, little is known on how to modulate microglial phagocytosis when it is impaired or exacerbated, such as in epilepsy or schizophrenia, respectively. Therefore, our goal is to find pharmacological modulators of microglial phagocytosis. Using a high throughput screening strategy in primary cultures of microglia, we tested 600 compounds from the Prestwick library, already approved by the Federal Drug Administration (FDA) and the European Medicines Agency (EMA) to be used in humans, in an in vitro model of phagocytosis. We found a subset of drugs that could be classified as promoters of phagocytosis (pro-phagocytosis drugs) or inhibitors (anti-phagocytosis). To validate the phagocytosis modulators in a more complex system, we used organotypic cultures and confirmed that some compounds promoted phagocytosis, while others blocked it. Currently, we are validating the compounds in vivo in two different models: anti-phagocytosis drugs are tested against physiological phagocytosis of apoptotic newborn cells in the adult hippocampal neurogenic niche; pro-phagocytosis drugs are tested against the pathological phagocytosis impairment induced in a model of epilepsy by intrahippocampal administration of kainic acid. Considering the lack of strategies to modulate phagocytosis, our compounds may represent a new therapeutical strategy to restore brain parenchyma homeostasis in pathologies where phagocytosis is impaired or exacerbated.

TRANSCRANIAL DIRECT CURRENT STIMULATION EFFECTS ACROSS MOTOR CORTEX LAYERS ON AWAKE MICE

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Transcranial direct-current stimulation (tDCS) is a non-invasive brain stimulation technique which involves the application of weak electric currents on the scalp. Although previous studies have demonstrated the value of tDCS for modulating sensory, motor, and cognitive functions, there are still lacks in our understanding of the underlying physiological mechanisms. To evaluate the impact of tDCS on cortical excitability, human experiments usually assess motor evoked potentials by motor cortex stimulation, for this reason, we aimed to stablish a mouse model of M1-tDCS in order to be able to explore physiological mechanisms of tDCS in more detail.
For this purpose, we performed electrophysiological recordings in M1 of alert mice during and after administration of M1-tDCS. Mice were prepared for chronic recordings of neuronal activity in layer 2-3, layer 5 and layer 6, evoked by stimulation of ventral lateral nucleus of the thalamus (VAL). M1-tDCS was performed at different current intensities (50, 100, 200 and 300 µA) for 5 s to test the acute effects on neuronal excitability, and for 15 min to analyze neuroplastic effects of the intervention.
tDCS acutely increased and decreased the amplitude of the VAL-evoked local field potentials (LFP) in a polarity- and intensity-dependent manner. We observed an increase of excitability for anodal stimulation, and a decrease for cathodal stimulation. This modulatory effect was observed across distinct cortical layers, but was more prominent for superficial layers. For 15 minutes of anodal or cathodal tDCS, we observed a similar polarity- and intensity-dependent modulation of the VAL-evoked LFP amplitude during stimulation, but when tDCS was switched off, only the protocols with higher intensities were able to induce a robust plastic effect, showing an increase of LFP amplitudes after anodal and a decrease after cathodal tDCS.
The current study demonstrates the feasibility of a mouse model of M1-tDCS that resembles the modulatory effects of the intervention observed in human experiments, highlighting the importance of properly adjusting the tDCS parameters to obtain results translationable to humans.

TRANSGENIC EXPRESSION OF MUTANT VERSIONS OF CSPalpha/DNAJC5 CAUSES LIPOFUSCINOSIS IN MICE

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Mutations in the DNAJC5 gene, that encodes the synaptic vesicle protein Cysteine String Protein alpha (CSPα) cause adult-onset autosomal dominant neuronal ceroid lipofuscinosis (CLN4) (Noskova et al, 2011), a devastating neurodegenerative disease affecting young adults. In humans, two-point mutations (Leu115Arg and Leu166Del) independently cause CLN4 by unknown mechanisms. In order to investigate the disease mechanisms in vivo, and since the disorder is autosomal-dominant, we have used a pronuclear injection approach to generate mouse lines overexpressing CSPα variants under control of the neuronal specific Thy1 promoter. We have generated three independent lines that express GFP-fusion proteins of three different versions of CSPα/DNAJC5: WT, Leu115Arg and Leu166Del. The mice are viable and do not show any obvious morbidity or mortality increase. Transgenes are well expressed all over the hippocampus, however, the WT version in particular is strongly expressed at mossy fibers in contrast to the mutant versions. The existence of autofluorescent punctate structures that are labelled with antibodies against ATP synthase subunit C in Leu115Arg, but also in Leu166Del transgenics, suggest an increase of lipofuscinosis in the mutants. Immunohistochemical analysis with markers of granulovacuolar degeneration bodies, excluded the involvement of this neurodegenerative lysosomal pathology. Next, we used transmission electron microscopy (TEM) to find at pyramidal neurons of the CA3 region structures similar to granular osmiophilic deposits (GRODs) previously described in NCL4 patients. The GROD-like structures were present only in the mutants but not in the negative controls or the transgenics overexpressing the WT version. Furthermore, we could not detect GROD-like structures or other signs of increased lipofuscinosis in conventional or conditional knock-out mice lacking CSPα/DNAJC5. We concluded that CSPα/DNAJC5 mutant versions might cause lipofuscinosis by preferentially hijacking key proteins that could include, but not only, endogenous CSPα/DNAJC5. We expect our novel mouse models will help to identify those proteins.

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TRANS-SYNAPTIC EFFECTS AFTER INDUCING LONG-TERM POTENTIATION IN THE HIPPOCAMPAL CIRCUIT

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Long-term potentiation (LTP) evoked by high frequency stimulation (HFS) is a very well-known experimental procedure that shares certain mechanisms with learning and memory processes. LTP is a typical example of synaptic plasticity, which appears after applying an HFS train to the afferent pathway of a CNS synapse. Basically, it consists in an increase of the synaptic response to a control stimulus following the presentation of the HFS train. This technique is studied mostly in the hippocampus due to its high susceptibility to LTP induction and its laminar nature that allows the study of synapses with different ultrastructural dispositions. Although most of preceding studies have been performed in vitro, we have developed a new experimental approach to carry out these experiments in behaving animals. The main goal of this study was to confirm that there are synaptic changes in strength not only in the synapse where LTP is induced, but also in those post-synaptic to it. We studied field excitatory post-synaptic potentials (fEPSP) evoked in five hippocampal synapses, located in both ipsi- and contralateral hemispheres (PP-CA1i, PP-CA3i, PP-CA1c, CA3-CA1i, and CA3-CA1c). HFS was presented to the perforant pathway (PP). Animals were prepared for chronic recordings in the mentioned synapses following procedures described elsewhere (Gruart et al., J. Neurosci., 2006). We have characterized input/output curves, paired pulse facilitation (PPF) and LTP of these synapses. We also performed depth-profile recordings, which showed differences in the synapses' latency. Data from input/output curves and PPF proved that the five studied synapses have similar basic properties, which makes their later comparison easier for subsequent analysis. Importantly, regarding HFS of the PP, we observed the presence of significant LTP both at the CA3-CA1c and PP-CA1c synapses, lasting at least two hours in the first day of recording. In conclusion, these results indicate that LTP can be evoked at synapses located far away from the stimulated afferent pathways.

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TREK CHANNELS AND THEIR PHYSIOLOGICAL ROLE IN THE INTRACARDIAC NEURONS: FOCUSING ON TEMPERATURE AND INTRACELLULAR ACIDIFICATION

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The intrinsic activity of the intracardiac nervous system is determined by cardiac pacemarkers and is strongly modulated by the sympathetic and parasympathetic branches of the autonomic nervous system. Although general mechanisms controlling cardiac activity have been extensively studied, little is known about the electrical properties and function of parasympathetic neurons in the intracardiac ganglion (ICG).

TREK channels, a subfamily of two-pore domain potassium (K2P) channels, are proteins with a crucial role in maintaining resting membrane potential and controlling excitability, but their function in the ICG remains unclear. In this work we investigated the effect of changes in physiological parameters such as increased temperature and intracellular acidification and how these variations affect to cultured mouse ICG neurons behaviour. First, the presence of TREK channels in the ICG was assessed by RT-qPCR and western-blot analysis. Using electrophysiological patch-clamp technique (perforated-patch), passive and active properties were examined at 24 and 37 ºC and before and after cytosolic acidification. In current-clamp experiments, the excitability of ICG neurons was clearly reduced when the temperature was increased from 24 to 37 °C, the neurons resulted hyperpolarized, the action potential firing rate decreased, and some action potential characteristics were also affected. The same phenomenon occurred when the cytosolic pH was diminished. Consistently, in voltage-clamp both physiological temperature and intracellular acidification induced outward currents and an increase in conductance through K+ channels. These currents showed similar characteristics to those driven through TREK-type channels.

Altogether, these results highlight the contribution of TREK channels in establishing neuronal excitability properties at physiological temperature and their role as neuroprotective channels in both temperature and intracellular acidification responses.

TRESK BACKGROUND K+ CHANNEL REGULATES SENSORY NEURON EXCITABILITY AND CONTRIBUTES TO MECHANICAL AND COLD PAIN

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TRESK (K2P18.1) is a background K+ channel highly expressed in spinal cord, dorsal root and trigeminal ganglia sensory neurons, where it has been involved in modulating sensory neuron excitability and firing. Changes in channel expression and function have been reported to enhance nociceptor excitability after injury or inflammation. To determine the role of TRESK in sensory transduction, we first compared the excitability and membrane properties of small/medium-sized sensory neurons in whole cell patch clamp recordings of cultured DRG neurons from wild type and TRESK knockout mice, which presented a reduced action potential threshold, increased membrane resistance and enhanced repetitive firing upon depolarization. Recordings of skin nociceptive fibers showed strong activation in response to cold in the absence of TRESK channel. In agreement, behavioral experiments in TRESK ko mice revealed a decreased mechanical threshold to von Frey hairs and an enhanced cold sensitivity. No significant changes were found for thermal sensitivity to warm or hot temperatures. Nocifensive behavior after capsaicin injection was unaltered while the response to AITC was slightly diminished. Interestingly, TRESK ko mice presented a reduced response to hypertonic and hypotonic stimuli even after sensitization with PGE2. During inflammation, ko mice showed a decreased phase I response in the formalin test, while phase II was unaltered. In the CFA-induced inflammatory model, both mechanical and thermal sensitivity were enhanced compared to wt animals. Mechanical and thermal hyperalgesia were also enhanced in the sciatic nerve cuffing model of neuropathic pain. Finally, the oxaliplatin-induced cold sensitization was absent in ko mice, probably due to the already enhanced cold sensitivity. In summary, our results indicate that TRESK has a significant contribution regulating the excitability of certain populations of sensory neurons mainly involved in mechanical and cold pain sensing. Moreover, a down-regulation of its expression as occurs after nerve injury might contribute to the generation of the hyperalgesia and allodynia observed during chronic pain.

TRESK BACKGROUND POTASSIUM CHANNEL MODULATES THERMAL SENSITIVITY IN MICE

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TRESK is a background potassium channel activated by intracellular calcium through the phosphatase calcineurin. The channel is highly expressed in distinct populations of primary sensory neurons involved in nociception, where it modulates their excitability. We previously described that TRESK ablation induces an increase in mechanical and cold sensitivity, and its downregulation after nerve injury has been linked to chronic pain. Interestingly, calcineurin inhibition by the drug tacrolimus (FK-506) can induce cold allodynia and hyperalgesia in human patients as a side effect. Here, we explore the behavioral effects of tacrolimus and the role of TRESK in the modulation of heat and cold sensitivity. First, we proved that the expression of major background potassium and TRP channels expressed in primary sensory neurons is not modified by TRESK ablation in the TRESK knockout mice model used in our studies. We also found that, in addition to its high expression in sensory ganglia, TRESK is also expressed at lower levels in spinal cord, brain, cerebellum and hippocampus. Behavioral studies show that, when exposed to a cold ramp, TRESK knockout mice present nocifensive behaviors in response to higher temperatures than wild-type animals. Interestingly, only wild-type male mice treated with tacrolimus present an enhanced sensitivity to cold. To better understand the cellular basis of this differences, responses to cold stimuli of different populations of primary sensory neurons are under study. TRESK ablation also induces an increase in heat sensitivity in male mice, but not in females. Besides, heat sensitivity of female mice is increased after tacrolimus treatment in a TRESK-independent manner. In summary, TRESK modulates cold sensitivity and its indirect inhibition by tacrolimus in wild-type mice resembles the cold allodynia observed in knockout animals. Given the channel's role in thermal sensitivity modulation and its mainly peripheral expression, TRESK activation is a potential therapeutic approach for the treatment of tacrolimus-induced allodynia and hypersensitivity to painful stimuli.

Topic 3

Systems Neuroscience

Posters

A COMPARATIVE STUDY OF THE SOMATOSENSORY CORTEX AND THE HIPPOCAMPUS IN ADULT MICE. FROM THE SYNAPTOME TO THE CONNECTOME

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Unveiling the brain's map of connections has become one of our times' great current scientific challenges. For this purpose, we have developed a tracer tool within the software package Espina. In the present study, we have traced the skeletons of all the axons and dendrites. We have connected each skeleton with the 3D reconstruction of the synapses related to them.

Three male C57 mice were sacrificed at postnatal week 8. The brain was then extracted from the skull and processed for electron microscopy. Three-dimensional brain (neuropil) tissue sample from layers 1 (L1) and 3 (L3) of the somatosensory cortex (hindlimb representation) and another one from stratum radiatum (SR) of hippocampal CA1 were obtained using focused ion beam milling and scanning electron microscopy (FIB/SEM). Synaptic junctions were visualized, identified (excitatory/inhibitory), and segmented in 3D with Espina software. The axons and dendrites involved in establishing the synapses were then followed and traced through the stack of images.

In spiny dendrites, the linear density of synapses was higher in the SR (more than 3 synapses/µm) than in L1 and L3 (less than 2 synapses/µm). Most synapses were established on dendritic spines, although around 4% of spines did not establish synapses. In dendrites that lack spines, the mean density of synapses was also higher in the SR than in L1 and L3. The excitatory axons established more synapses per micron in the hippocampus (0.63 synapses/µm) than axons from the somatosensory cortex (0.46 and 0.41 synapses/µm in L1 and L3, respectively). However, the inhibitory axons had a similar linear density of synapses in all the regions studied (between 0.38 and 0.43 synapses/µm).

Each region manifests structural parameters that make them different from other regions. Dendrites and excitatory axons from the hippocampus have a higher density of spines and synapses than those from the somatosensory cortex. However, the inhibitory axons showed similar synaptic densities in all the samples. The tracer tool developed allows in-depth characterization of the connectome from brain samples.

A CRITICAL PERIOD FOR THE ITCH SPINAL CORD NEURAL CIRCUIT?

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Itch is a widespread symptom associated with a diverse array of diseases. Despite its prevalence in the world population, our understanding of the development, maturation and mechanisms associated with itch neural circuits is lacking behind that of other somatosensory modalities. We have previously characterized the importance of spinal Ptf1a-derived inhibitory neurons in controlling the entry of innocuous mechanosensory information into the spinal cord dorsal horns. Loss of Ptf1a-derived adult neurons leads to the development of an intense chronic itch phenotype and increased hairy skin sensitivity. Here, we study the consequences of ablating Ptf1a-derived neurons both in early embryonic development, well before somatosensory input becomes active, and in newborns, when somatosensory circuits are being refined with the arrival of extrinsic stimuli. Our results show that postnatal loss of Ptf1a-derived neurons cannot be compensated, even as early as postnatal day 0, resulting in the development of chronic itch. However, elimination of these neurons during embryonic development leads to apparently normal itch sensation. The potential mechanisms explaining these observations point to the possible existence of a critical period for the establishment of functional itch circuits in the spinal cord. Moreover, these results suggest that loss of specific populations of inhibitory neurons in the dorsal horns activates endogenous compensatory mechanisms that yields a functionally normal mature spinal itch circuit.

A NEUROANATOMICAL PATHWAY FOR THE INTEGRATION OF PHEROMONAL AND SPATIAL INFORMATION

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Vomeronasal information plays a key role in rodents' individual recognition. This particular type of information could be integrated into the spatial context encoded in the hippocampus, thus constituting the neural substrate of the individual's integration into the cognitive map. Hence, pheromonal coding could represent the "who" component of episodic memory. In this work we describe an amygdalo-entorhino-hippocampal circuit from which vomeronasal stimuli may integrate into hippocampal processing. Through tract-tracing methods we demonstrate a glutamatergic reciprocal connection between the accessory olfactory bulbs and the posteromedial cortical amygdala (considered as the primary vomeronasal cortex), as a first potential nucleus for pheromonal information relay. To elucidate the connections from the vomeronasal amygdala to the dorsal hippocampus, the retrograde tracer FluoroGold was injected in the dorsal CA1 and the anterograde tracer TBDA (dextranamines labelled with biotin and rhodamine) in the primary vomeronasal cortex. Since no direct connection was found, we looked for areas where convergent retrogradely labelled cells and anterogradely labelled fibres were present. We found a restricted population of reelin-positive neurons retrogradely labelled in the dorsolateral entorhinal cortex layer II, where anterogradely labelled fibres with glutamatergic synaptic boutons were also present. To confirm this amygdalo-entorhinal pathway, retrograde tracer was injected in the dorsolateral entorhinal cortex. The results show that all the anteroposterior extent of the vomeronasal cortex projects to the dorsolateral entorhinal cortex, which in turn relays the pheromonal information to the dorsal CA1. Thus, we suggest that this circuit is the neural substrate of territorial behaviour in rodents, as well as the circuit allowing the integration of social and spatial information, that is, the "who" and "where" components of the episodic memory.

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AGE-DEPENDENT NEURAL CODING IN THE BASAL FOREBRAIN DURING A PAVLOVIAN TASK

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Acetylcholine mediates multitude of cognitive functions including arousal, attention, sensory processing, reinforcement expectation, reward and addiction. The basal forebrain cholinergic input to the cortical mantle plays a central role in these modulatory processes. Age-related changes in the morphology of cholinergic neurons has been observed across species, characterized by dendritic, synaptic, and axonal degeneration. However, the link between cholinergic activity during learning and the normal or pathological age-related neurodegeneration is still missing.

To clarify the role of the basal forebrain neurons in learning during the normal aging process, we performed neuronal recordings in the nucleus of the horizontal limb of the diagonal band, in head-fixed ChAT-cre mice during an auditory cued-outcome task. Animals from 3 to 18+ months of age were injected with an adeno-associated virus carrying channelrhodopsin-2, transfecting cholinergic neurons, allowing us to optically tag them.

Animals showed decreased anticipatory licking correlated with aging, being old animals less likely to lick during reward-predicting trials, compared to young ones. Surprisingly, tagged cholinergic neurons in the young animals seemed to respond to the reward but not punish-related cue, whereas in the old animals no response to tone was observed. Additionally, both punishment and reward activated part of the recorded neurons in all the animal groups, while only from 12+ months these contingencies had an inhibitory effect on the cell population.

We additionally performed fiber photometry experiments with a fluorescent sensor in order to determine the cholinergic influx over basolateral amygdala. Results showed a similar pattern to that observed in the basal forebrain cholinergic neurons.

These changes in coding of outcome contingencies of basal forebrain neurons can reflect the ongoing degenerative process or even be the cause, at least partially, of the learning deficits observed during natural aging.

AN ANALISYS OF TIMING CORRELATION REVEALS THAT MOTOR CORTEX NEURONS ARE RELATED, BUT NOT THE ORIGIN, OF CLASSICALLY CONDITIONING EYELID AND VIBRISSAE RESPONSES IN MICE

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Classical eyeblink conditioning is one of the experimental models more widely used for the study of the neuronal mechanisms underlying the acquisition of new motor and cognitive skills in behaving animals. Currently, certain studies are pointing out the motor cortex as the putative structure responsible of this type of learning, although other studies give this main role to the cerebellum. Other brain areas might be involved too. In order to determine the specific contribution of the motor cortex to the generation of learned movements, we studied the temporal correlation between unitary activities of identified eyelid and vibrissae motor cortex neurons, and the electromyographic activity of the orbicularis oculi and vibrissae muscles and magnetically recorded eyelid movements, during classical conditioning of eyelid and vibrissae responses, using both delay and trace conditioning paradigms, in behaving mice. Mice were prepared for classical eyeblink and vibrissae conditioning and for recording of the unitary activity of motor cortex neurons. Chronic electrodes for stimulation and recording were implanted in the eyelid and vibrissae muscles, and a craniotomy was carried out in the cerebellar skull. Eyelid movements were recorded with the help of a magnet fixed to it. Neurons were identified by their antidromic activation from the ipsilateral red nucleus or the contralateral facial nucleus. We also studied the involvement of motor cortex neurons in reflexively evoked eyelid responses and the kinematics and oscillatory properties of eyelid movements evoked by motor cortex micro-stimulations. Results show the involvement of the motor cortex in the performance of conditioned responses elicited during the classical conditioning task. However, a timing correlation analysis showed that both electromyographic activities (from orbicularis oculi and vibrissae muscles) preceded the firing of motor cortex neurons, which must therefore be related more with the reinforcement and/or proper performance of the conditioned responses than with their acquisition and storage.

AREA-SPECIFIC PATTERNS OF CONVERGENT THALAMIC INPUTS TO THE MOUSE MOTOR CORTEX

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A large region of the mouse dorsal and medial frontal isocortex is commonly labeled 'motor'. As such, this region is often compared with premotor and motor areas of the primate brain. However, the published histological and electrophysiological data in rodents provide ambiguous and often conflicting evidence for delineation of individual areas. Since multiple thalamic nuclei innervate the motor cortex, fine quantitative differences between mouse motor cortex regions in the thalamocortical input composition and/or intracortical distribution could provide an independent and consistent reference for area delineation, as well as for cross-species comparisons.

To map and quantify thalamus neurons innervating different motor cortex regions in the adult C57BL/6 mouse, we microinject in cortex retrograde tracers (Fast Blue and choleratoxin B conjugated with fluorochromes, CTB-Alexa) and use cytochrome oxidase activity to help in cortical layer and nuclei delineation. We calculate the percentage of labeled cells in each nucleus within all labeled cells in each case and compare then between cases. In subsequent experiments, the tangential/laminar convergence/divergence of these multiple pathways will be analyzed using micropopulation and single-cell anterograde labeling methods.

Our preliminary data show that, in all points explored, the most numerous sources of thalamic innervation comes from VM (ventromedial nuclei) and the complex VAL (ventral anterior-lateral nuclei), to a lesser extend from the posterior, intralaminar nuclei and the midline nuclei. Our data also reveal a remarkable heterogeneity in the set of nuclei whose projections converge at different points in the cortex, in addition to a complex topography within each nucleus. All of this supports the idea that the analysis of thalamic connections can provide valuable data for the consistent delineation of functional domains in the motor cortex of the mouse.

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BBI: A BRAIN-BACTERIA INTERFACE TO REVEAL AND COMPUTE REAL-TIME CHANGES IN NEURONAL ACTIVITY INDUCED BY BACTERIAL PRESENCE

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The interaction of bacteria with various somatic cell types is an exciting emerging field. Despite the known effects of microbiota on the gut-brain axis, very little is known about the direct interactions that bacteria could have with neurons, both in terms of molecular mechanisms and information transfer. Here, we show how bacteria and neurons can be cultured together, and demonstrate a novel integrated platform that facilitates the analysis of neuronal-bacteria communication. Using optical (calcium signaling) real-time readouts, we show that neurons react to the nearby presence of bacteria, and that this response depends on the bacterial species and density. Thus, neurons sense the presence, type, and amount of bacteria, and can readily be interrogated in the living state to extract information about aspects of their microbial microenvironment. In addition, by fitting the bacterial-induced neuronal experimental data to a differential equation model, we ascertain Gompertzian parameters that can be used to predict neuronal response to bacteria presence as a function of time. Our proof-of-principle data in this highly tractable platform reveal crosstalk mediated by electrical and chemical signals and illustrate a novel example of cross-kingdom communication between highly diverse cell types. The ability to eavesdrop on information passing between these two very different levels of biological organization will facilitate insight into evolutionary cell biology and could impact the understanding of brain-bacteria communication for diagnosis of neuronal states in health and disease.

CELL-TYPE SPECIFIC WIRING BETWEEN VENTROPOSTERIOR THALAMIC NUCLEUS NEURONS AND SOMATOSENSORY CORTICES

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The ventral posterior nucleus (VP) of the thalamus is the relay station for somatic sensory inputs to the cortex. Thalamocortical axons carrying mechanoceptive information from mystacial vibrissae follicles to the primary somatosensory area (S1) in rodents are a key model in sensory systems neuroscience. In contrast other thalamocortical pathways originated in rodent VP have received much less attention, to the point that even their cellular wiring remains poorly defined. Here, using micropopulation and single cell-axon labeling, systematically charted and analyzed structural differences between the various output circuits established between VP cells and the cerebral cortex.

Our data show that, while, as expected, axons coming from populations in rostrodorsal VPM target area S1 upper layer 4 (L4) in focal, point-to-point fashion, the cells in a large ventrocaudal domain of VPM target instead the second somatosensory area (S2) L4, also in point-to-point fashion. Besides, cells from a ventrolateral VPM domain target S1, arborize in layers 2-3 and deep L4, avoiding “barrel” cores. Cells in the lateral ventroposterior nucleus (VPL) target S1 and S2 simultaneously, often via collaterals of the same axons. Reconstruction of the complete axonal tree of transfected single cells (Neurolucida) reveals quantitative differences in the distribution of axonal arborizations in different areas and layers. Our results indicate the existence of several parallel thalamocortical pathways between VP and somatosensory cortices, depending each of a specific projection neuron subtype.

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CHRONIC FULL BAND RECORDINGS WITH GRAPHENE MICROTRANSISTOR NEURAL INTERFACES FOR THE DISCRIMINATION OF BRAIN STATES

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Brain states (such as sleep, anesthesia or wakefulness) are characterized by specific patterns of cortical activity dynamics. Some of these patterns are observable in the infraslow frequencies of the spectrum (<0.1Hz) but are often missed due to the intrinsic limitations of the recording devices. We previously demonstrated that flexible arrays of graphene solution-gated field-effect transistors (gSGFETs) can record full-spectrum signal, including the infraslow component (DC, direct current-coupled), acutely in multiple sites of the rat brain. Here, we performed chronic implants of 16-channel gSGFET arrays on rat cerebral cortex and recorded full-band neuronal activity in order to test the long-term stability and biocompatibility of implanted devices, and to study the DC band during the transition between different brain states across different levels of anesthesia. We found that chronic epicortical gSGFET implants can record full-band signals with high stability, fidelity and spatiotemporal resolution for up to 6 months. Further, different brain states generated by different levels of anesthesia can be identified by the high pass filtered (AC, alternating current-coupled) spectrogram, which was complemented by the DC band for the quantification of the depth of anesthesia. We conclude that recording the infraslow activity by gSGFET interfaces provides an additional value for the identification of anesthesia levels and their associated brain states, and further supports the preclinical and clinical use of graphene neural interfaces for long-term multi-site minimally-invasive recordings of cortical activity.

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CHRONIC SENSORY DEPRIVATION ALTERS CORTICAL RHYTHMS IN THE SOMATOSENSORY CORTEX

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The somatosensory cortex is arranged in six layers characterized by different cellular populations and input/output connections. The laminar organization represent a key evolutionary feature that serve to transfer the spontaneous cortical activity vertically across layers from the same column and horizontally among different cortical areas. In this context, a spinal cord injury (SCI) produces a massive sensory deprivation of the somatosensory cortex that strongly affects the cortical activity in the long-term. However, whether spontaneous neuronal activity is equally affected across deprived cortical layers in time is less understood. Therefore, the main aim of this work is to identify physiological features of spontaneous neuronal activity localized in each cortical layer at different time points from acute to chronic stage of sensory deprivation (SCI). Experiments were performed in adult rats under control conditions and after SCI or sham lesion. To measure neuronal activity, we used electrophysiological recordings of local field potentials simultaneously obtained from all cortical layers by using a vertical array of 32 electrodes (50 m spaced). Data were obtained weekly after SCI to elucidate the temporal windows of cortical physiological changes. The spontaneous activity from each cortical layer were analysed by using a Fast Fourier Transform analysis to identify the power of frequency bands of interest: slow-wave (<1Hz), Delta (1-4Hz), Beta (12-25Hz) and Gamma (25-80Hz). Results show that physiological changes take place only in the SCI group during the 3rd and 4th weeks after SCI, while control group remain unchanged. Specifically, Delta activity was reduced in layers IV and V, while Beta and Gamma activity were increased in the infragranular layers V and VI. We conclude that a chronic sensory deprivation after SCI affects the cortical rhythms differently across layers and in a time-dependent manner. Changes in spontaneous activity could indicate alterations of neuronal network excitability, which could be part of the cortical reorganization phenomenon.

CORTICAL PYRAMIDAL CELLS EXPRESS THYROID HORMONE TRANSPORTERS MCT8 AND OATP1C1 IN HUMAN AND MONKEY BRAIN.

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Monocarboxylate transporter 8 (MCT8) and organic anion-transporting polypeptide 1C1 (OATP1C1) are thyroid hormone (TH) transmembrane transporters that play a crucial role in the availability of systemic THs for neural cells allowing their appropriate development and function. MCT8 mutations are the underlying reason of Allan-Herndon-Dudley syndrome which expresses a dramatic motor disfunction. This study aims to analyze the presence of these two proteins at the cellular level in the monkey and human cerebral cortex.

We analyzed the distribution of these two transporters in the cerebral cortex of 30-50 µm floating frozen cerebral cortex sections taken from three cynomolgus monkeys and four adult humans by Nissl staining, immunohistochemistry, double labeling immunofluorescent and immunohistochemistry combined with NADPH-diaphorase histochemistry.

OATP1C1 is expressed in pyramidal neurons in layers II, III, V, and VI in both monkey and human brain, as evidenced by colocalization with RC3/Neurogranin. Non-colocalization with NADPH-diaphorase implies that OATP1C1 is not expressed in multipolar, bitufted and stellate NOS-expressing GABAergic interneurons in the cortex. MCT8 distribution is similar to that of OATP1C1 in monkey and human brain, although the intensity of its signal is lower. Interestingly, Cajal-Retzius like cells expressing OATP1C1 are observed in layer I. OATP1C1 and MCT8 immunostained cells with very small soma and large processes compatible with astrocyte morphology are found in the subcortical white matter in close relation to vessels. MCT8 is also expressed in the endothelial cells of the different size vessels and capillaries throughout the monkey and human cortex, while OATP1C1 is occasionally observed in the endothelium of large and medium-sized vessels.

Our results demonstrate the abundance of MCT8 and OATP1C1 TH transporters in the long and short projection pyramidal cortical neurons and in the astrocyte-vessel complexes in adult human and non-human primates, which suggest their critical position in the efferent cortical motor system.

DISTINCT HEMISPHERICAL RESPONSIVENESS TO TRANSCRANIAL STATIC MAGNETIC FIELD STIMULATION?

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Transcranial static magnetic field stimulation (tSMS) is a non-invasive brain stimulation technique able to reduce corticospinal excitability when applied over the primary motor cortex (M1). Recently a few studies have been questioning this inhibitory effect of tSMS. Methodological differences between studies such as stimulating the right or left M1 might explain opposed findings.
Thus, we investigated whether tSMS over M1 does modulate corticospinal excitability, exploring whether tSMS modulates the right or left hemisphere differently. We recruited 40 right-handed healthy subjects (females: 22; mean age: 30.6±7.7 years). Separated into two same-sized groups, age and gender-matched, each subject underwent one tSMS session for 30 minutes applied either over right or left M1. We recorded 30 motor evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS) from the first dorsal interosseous (FDI) muscle before and after (at 0, 15 and 30 minutes) tSMS intervention. The experiment was carried out with neuronavigation and threshold tracking methods to determine the individual resting motor threshold (RMT) and the stimulus output intensity to evoke a 1mV peak-to-peak MEP amplitude. Both measures were repeated after tSMS application.
The results show that 30 minutes tSMS produced a significant decrease in MEP amplitude at all time points (two-way repeated measures ANOVA, Dunnett, 0min, P=0.023; 15min, P=0.010; 30min, P=0.008), with no interaction between hemispheres (TIMExHEM: F3,114=1.16, P=0.328). However, when we analysed the hemispheres individually, we obtained a significant reduction of MEP amplitude for the right hemisphere (-21.5±28.9%; one-way repeated measures ANOVA, F3,57=4.36, P=0.008) with no significant differences for the left (-8.6±25.3%; one-way repeated measures ANOVA, F3,57=1.24, P=0.304).
Here we confirm the previously reported evidence that tSMS can modulate corticospinal excitability in an inhibitory sense for at least 30 minutes. Collectively, this effect is observed on both hemispheres. Nonetheless the results suggest a distinct responsiveness of hemispheres to tSMS, which could explain opposed findings between studies.

DOPAMINERGIC BLOCKADES DECREASE PHYSICAL EXERCISE MAINTENANCE RESPONSE

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The motor responses during physical activity are the result of a hierarchically arranged central nervous system circuits. Motor programs planned in the prefrontal cortex, are refined in different cortical and subcortical circuits. Finally, the motor output arrives to the spinal cord, the first layer in the hierarchy of muscle control. One key aspect of the circuits involved in motor control is that they are modulable. In previous studies we determined that the physical capacity, measured as the maximal response during an incremental test, is dependent of D1 striatal and D2 extra-striatal actions in Sprague-Dawley rats. Here we want to determine the role of dopaminergic antagonists in physical activity maintenance responses.

For that aim, we first defined a model of physical activity maintenance that consisted in performing three incremental tests after a habituation protocol to forced wheel exercise, each one separated with three days with an active rest session to maintain the performance throughout the tests. In the second test, we administered a D1-like receptors antagonist (SCH23390) in a dose of 0.1 mg/kg intraperitoneally or saline (Sodium Chloride 0.9%).

Sprague Dawley rats administered with D1 SCH23390 (0,1mg/kg) antagonist decreased the performance in the second test compared to the first and third ones (Test 1: 25.39±4.58 min.; Test 2: 5 min. (SCH23390, $p<0.05$) and 23.06±6.42 min. (Saline); Test 3: 25.42±3.4 min.). Rats injected with saline were able to maintain the performance throughout the incremental tests. Rats injected with SCH23390 significantly decreased their performance in the second test but recovered performance in the third test. This suggests that a habituation protocol and active rests between tests allow the maintenance of the performance and that the dopaminergic system is involved in the maintenance response. Finally, our model significantly reduces the number of experimental animals.

ELECTRIC FIELDS MODULATION OF EPILEPTIFORM DISCHARGES IN THE CEREBRAL CORTEX IN VITRO

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Transcranial direct current stimulation (tDCS) is a technique extensively used in the clinical realm to modulate cortical activity. tDCS has been suggested, among other applications, to induce suppression of epileptiform activity. However, the understanding of the mechanisms underlying tDCS neuromodulation over cortical dynamics is still limited. The goal here was to explore how DC fields modulate both pre-epileptic and epileptiform cortical activity in vitro, and their effects over the different components of cortical emergent activity.

We applied exogenous constant electric fields (EF) (between -5 and +5 V/m) to in vitro ferret cortical slices expressing slow oscillatory activity under control conditions and following the administration of bicuculline methiodide (BMI), a GABAA receptors antagonist. The blockade of GABAA inhibition resulted in the emergence of pre-epileptic discharges and, eventually, full-blown epileptiform discharges.

The frequency of epileptiform bursts was linearly modulated along the range of EFs used. The modulation was mainly exerted over the silent periods, that elongated for larger negative amplitude of the EFs, eventually reaching a complete suppression of epileptiform discharges. The duration of the bursts was hardly modulated by the EFs used, but their firing rate was. Interestingly, both positive and negative fields decreased firing rates, albeit probably by different mechanisms which will be discussed. As reported, the linear modulation of the frequency of bursts differs from the exponential modulation reported for the frequency of spontaneous (non-epileptic) Up states (D'Andola et al, bioRxiv, 246819, 2018), illustrating how the lack of inhibition decreases the range of flexibility of network responses.

Our results confirm that DC stimulation at the investigated intensities can modulate not only physiological but also disinhibited and more synchronous epileptiform activity, even reaching the silencing of full-blown epileptiform discharges. The present model might be useful to better understand the neuromodulation mechanisms of tDCS in the treatment of epilepsy patients.

EXERCISE-ASSOCIATED miRNA PROFILE IN miR-29a/b1 DEFICIENT MOUSE BRAIN

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The microRNA-29 (miR-29) family is decreased in brain tissue in 30% of late-onset Alzheimer's disease (LOAD) patients, increasing the levels of their target mRNAs, such as BACE1 (implicated in the formation of the β-amyloid peptide). This family also shows a decrease in their circulating levels in both plasma and cerebrospinal fluid from LOAD subjects. However, whether this affects disease progression has not been studied yet. LOAD is associated with sedentarism, among other risk factors. Interestingly, results from our laboratory in murine models of both resistance and endurance exercises showed that plasma circulating levels of miR-29 family (c-miR-29), along with other c-miRs, and adult hippocampal neurogenesis (also affected in LOAD) are increased after long-term exercise intervention. To better understand how exercise-associated miRs can counteract the loss of miR-29 family, we took advantage of the previously described miR29a/b1 cluster knockout mouse model (miR-29 KO), which presents severe ataxia. Thus, in this study, we first explored maximal endurance and resistance performance in 16-week-old symptomatic miR-29 KO and their corresponding wildtype (WT; n=6/genotype). Then, we determined the expression of the found exercise-associated miRs in four brain areas: cortex, hippocampus, striatum, and cerebellum; along with mRNA levels of some proteins associated with AD and neurogenesis pathways, such as Bace1, Ranbp9, Dcx, Bdnf, Syt1, and Atg5. Our results showed that physical performance is impaired in miR-29 KO mice. Moreover, some brain exercise-associated miRs, along with their mRNA targets, are altered in specific brain areas regarding WT mice. Thus, the absence of miR29a/b1 cluster affects not only to physical fitness, but also modifies brain miR profile. Future studies will provide further information on whether exercise-modulated miRs can counteract the loss of this cluster in miR-29 KO mice after training. Translating these results to patients, knowing which exercise-associated miRs are beneficial for LOAD will allow to design therapies focused on modifying disease progression.

EXPRESSION OF c-Fos IN THE VOMERONASAL AMYGDALA AND LATERAL ENTORHINAL CORTEX OF FEMALE MICE INDUCED BY MALE PHEROMONAL SIGNALS

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In mice, individual recognition is based on the major urinary proteins present in urine, detected by the vomeronasal system. These signals are integrated into the posteromedial cortical amygdala (PMCo). The PMCo projects to the dorsal lateral entorhinal cortex (dLEnt), which also receives olfactory inputs from the main olfactory bulb and piriform cortex. Thus, vomeronasal information from the PMCo may converge in the dLEnt with olfactory information, from where it would be relayed to the dorsal hippocampal CA1 (dCA1), reaching the hippocampal memory system as a complex chemosensory input. To assess the effect of urine stimuli on the c-Fos activity of the PMCo-dLEnt-dCA1 circuit, we tested the preference of CD1 female mice to investigate male urine (n=5) or a control odorant (citralva; n=5) located in a particular corner of the cage during a 90 minutes test. Exploratory behavior was quantified with DeepLabCut, a software based on deep learning methods, and subsequent data were processed with self-written Python code. This analysis showed that male urine induced a significantly higher exploration than citralva. The immunofluorescent detection of c-Fos revealed a significantly higher expression in the PMCo and dLEnt in mice exploring urine. Since neurons in dLEnt projecting to dCA1 are reelin-positive, we performed a double immunofluorescence detection of c-Fos and reelin. The results showed a significantly higher number of c-Fos/reelin-positive neurons in the experimental group and a positive correlation with exploration time. In contrast, the number of c-Fos-positive cells in dCA1 did not differ between mice exploring urine or the neutral odorant. The results suggest that vomeronasal information encoding individual identity reaches the hippocampal memory system through the dLEnt. The lack of differential c-Fos expression in CA1 may indicate that citralva is also inducing memory formation, but following a different path.

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FUNCTIONAL ANALYSIS OF CHOLINERGIC NEUROMODULATION OF CHANDELIER CELLS FROM SINGLE-CELL TO CIRCUIT

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Synaptic inhibition is responsible for orchestrating spontaneous and evoked activity in the neocortex. Chandelier cells (ChCs) are a subclass of GABAergic cortical interneurons that innervates the axon initial segment (AIS) of pyramidal neurons, controlling the cell firing output. Mostly localized at the boundary of layers 1 and 2 (L1-L2) in prefrontal cortex, they present an asymmetric distribution of dendrites, which are mostly oriented towards L1, which suggests that ChCs may receive input from other cortical areas and deep nuclei, such as basal forebrain, which projects the strongest cholinergic innervation to L1, implying a plausible role for ChCs as circuit switches. Our interest lies in studying the role of ChCs in the control of cortical network activity, with a special focus in the presumable cholinergic modulation of these cells. To identify the population of cortical ChCs, we used a mouse model expressing td-Tomato under control of precise Cre- and Flp- dependent promoters (Tasic et al., Nature 2018). Using immunohistochemical and electrophysiological techniques, we have described the existence of cholinergic neuromodulation through specific nicotinic receptors and the intrinsic electrophysiological properties of ChCs. To clarify the role of ChCs in the regulation of the prefrontal cortical circuitry, we performed in vivo 2-photon imaging experiments in awake animals using GECIs, showing that ChCs are active during arousal, and we used DREADDs to modulate their activity to uncover its inhibitory role in the control of the excitatory network. Our results demonstrate that prefrontal ChCs are a subpopulation of fast-spiking parvalbumin interneurons modulated by cholinergic inputs activated during arousal states in awake mice, with a prominent role in the control of the pyramidal neurons.

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FUNCTIONAL DIVERSITY OF MOTONEURONS INNERVATING EXTRAOCULAR MUSCLE FIBERS

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Extraocular muscles contain singly innervated fibers (SIF), that receive one en plaque motoneuronal synapse and twitch upon electrical stimulation, and the atypical and less abundant multiply innervated fibers (MIF), which receives multiple en grappe motoneuronal contacts along its entire length and are non-twitch. Previous morphological studies have reported a distinct anatomical distribution and afferent pattern of SIF and MIF motoneurons, suggesting that SIF motoneurons would participate in the whole repertoire of eye movements, while MIF motoneurons would contribute only to slow eye movements.

We aimed to discern the function of electrophysiologically-identified abducens and medial rectus SIF or MIF motoneurons by extracellular single-unit recordings in awake cats during fixations, saccades, disjunctive and vestibularly-induced eye movements. Additionally, retrograde tracing of MIF motoneurons and the combination with ChAT immunolabeling allowed us to carry out a morphological study comparing between both types of motoneurons.

We have demonstrated that abducens and medial rectus SIF and MIF motoneurons participated in all different types of eye movement. However, MIF motoneurons exhibited lower firing rate, were recruited earlier and presented lower eye position and velocity sensitivities than SIF motoneurons. Anatomically, MIF and SIF motoneurons distributed intermingled within the abducens nucleus, and both in the dorsal and ventrolateral part of the medial rectus subdivision of the oculomotor complex, with MIF motoneurons being smaller and having a lesser somatic synaptic coverage.

In conclusion: 1) MIF and SIF motoneurons both discharge during all types of eye movements, although with different thresholds and sensitivities; 2) the smaller size of MIF motoneurons could explain their longer antidromic activation latencies and lower recruitment thresholds; 3) the larger synaptic coverage of SIF motoneurons could explain their higher firing rates and eye sensitivities; and 4) MIF motoneurons appear randomly distributed in the abducens and oculomotor nuclei.

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HUMAN α-SYNUCLEIN OVEREXPRESSION IN MOUSE SEROTONIN NEURONS ELICITS A DEPRESSIVE PHENOTYPE: FOCUS ON BRAIN CONNECTIVITY AND SYNAPTIC DENSITY

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Anxiety and depression are the most prevalent neuropsychiatric disorders in Parkinson's disease (PD) population (≃50%), often preceding the appearance of motor symptoms. Neuropathological and neurochemical changes in the serotonin (5-HT) system occur during PD prodromal phase and contribute to a variety of non-motor symptoms. However, anxiety/depression brain circuits in PD are not known in detail. In this study, we hypothesized that synaptic impairments occur in the early stages of the disease onset before the neuronal cell loss. Dysregulating the synaptic junction would block neurotransmitter release, thus triggering a retrograde neurodegenerative process ending with neuronal cell loss by proceeding through the axons. We aimed to study whether synaptic density and functional connections are affected by α-synuclein accumulation in efferent brain regions from 5-HT raphe nuclei in the early stages of a depression/PD-like mouse model. We overexpressed human wild-type or A53T mutant α-synuclein (WTα-Syn and A53Tα-Syn, respectively) in 5-HT neurons using AAV vectors and two different synaptic markers, microtubule-associated protein 2 (MAP-2) and synaptic vesicle glycoprotein 2A (SV2A) were examined 4 and 8 weeks later. Mice overexpressing WTα-Syn showed progressive reductions of MAP-2 levels in different 5-HT-innervated cortices (e.g. prelimbic, infralimbic, cingulate and motor cortices), caudate putamen and different sub-fields of hippocampus. In parallel, WTα-Syn mice also showed an accumulation of SV2A protein in cingulate and motor cortices, and caudate-putamen. In addition, changes of MAP-2 and SV2A synaptic markers were also found in different 5-HT projection brain areas of A53Tα-Syn mice, although these changes were of greater magnitude. Brain functional activity was examined in these mice using rsfMRI and early gene zif628 expression. Altogether, these data suggest that changes in the density of MAP-2 and SV2A synaptic markers linked to α-synuclein-induced axonal pathology lead to reduced 5-HT neurotransmission evoking a depressive phenotype, and these effects precede neurodegeneration.

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IDENTIFICATION OF A FAST HIPPOCAMPAL RECOGNITION SYSTEM IN HUMANS USING INTRACEREBRAL EVOKED POTENTIALS

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The role of the hippocampal formation in memory recognition has been well studied in animals, with different pathways and structures associated to specific memory processes. However, due to the limited accessible information, the hippocampus is commonly analyzed as a unique responsive area in humans. Combining intracerebral electroencephalogram recordings in epileptic patients and blind source separation (BSS) methods, we identified several components emerging from the hippocampus, likely reflecting the activity of different substructures as CA1 or the dentate gyrus. In a memory task involving the recognition of old and new images, we found one hippocampal component with fast responses to the stimuli that could not be directly identified from raw recordings. This component was locally generated in the hippocampus and was different from the responses in other structures, including the entorhinal cortex. These results suggest that the hippocampus may have a fast memory recognition system that can be retrieved thanks to the use of BSS methods. This challenges previous studies pointing the entorhinal cortex as the first area involved in memory recognition, with similar delays to the novel hippocampal early response. We hypothesize that both regions are elements of the same recognition system and they would be virtually coactivated via the hippocampal-entorhinal loop, with time differences in the order of the synaptic delays between both structures.

INCREASED EXCITABILITY OF PARVALBUMIN-POSITIVE INTERNEURONS IN PREMOTOR CORTICAL AREA IN A MOUSE MODEL OF OBSESSIVE-COMPULSIVE DISORDER

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Obsessive Compulsive Disorder (OCD) is a severe, chronic, and ubiquitous neuropsychiatric disorder that affects 2-3% of worldwide population. A cortico-striatal dysfunction is considered a major factor in OCD pathogenesis. However, integration of other brain structures is necessary to generate a satisfying explanatory model of OCD pathophysiology and symptom generation. It has been reported in Sapap3 knockout (KO) mouse, a well-validated model of compulsive-like behavior, that striatal region receives increased levels of synaptic inputs coming from the secondary motor area (M2). M2 is thought to be homologous to the Supplementary Motor Area in humans, an area showing hyperactivity in OCD patients. SAPAP3 is a scaffolding protein of the glutamatergic synapses, predominantly expressed in the striatum and cortical areas, whose absence has been associated with obsessive compulsive-spectrum disorders. The Sapap3 KO mouse shows a decrease in parvalbumin-positive (PV+) interneurons in the striatum. The relationship between the absence of SAPAP3 and the reduction in PV+ cells is still unknown, but it has been proposed that a cortical disinhibition in OCD patients due to GABAergic deficits could be linked to its pathogenesis. Therefore, we study, using a combination of in vitro and in vivo 2-photon experiments, the cortical GABAergic circuitry involving PV+ interneurons in the premotor area (M2). We have developed a Sapap3 null mouse line that express td-Tomato under control of the PV promoter. Preliminary results, using electrophysiological recording in brain slice preparation, show increased input resistance, decreased rheobase and increased firing frequency gain. All together indicate that PV+ interneurons are hyperexcitable. In vivo calcium imaging experiments in awake animals are in progress to confirm the hyperexcitability of PV+ neurons, and how the excitatory network is affected.

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INHIBITION IN A MIDBRAIN CIRCUIT CONTROLLING INSTINCTIVE ESCAPE DECISIONS

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To avoid predation, animals must choose from an array of defensive actions that are instinctive, yet adaptable to the environment. In mice, excitatory (VGluT2+) neurons in the dorsal periaqueductal gray (dPAG) compute the decision to escape from imminent threats by integrating synaptic input from the medial superior colliculus. Here, we show that inhibitory (VGAT+) dPAG neurons are an integral part of the escape initiation circuit, imposing a local inhibitory tone that determines the threshold for escape initiation.

We first characterised the intrinsic firing properties of dPAG neurons using loose-seal cell-attached recordings in acute midbrain slices of transgenic mice. We found that, even in the absence of synaptic inputs, VGAT+ neurons in the PAG fire action potentials spontaneously, whereas VGluT2+ neurons do not. Next, we combined whole-cell patch-clamp recordings with optogenetic and chemogenetic manipulations and found that VGAT+ neurons provide synaptic input onto neighbouring excitatory cells and exert local phasic inhibition on the dPAG network. Optogenetic inactivation of VGAT+ dPAG neurons in freely behaving mice increased the probability of initiating escape from innately aversive stimuli. Conversely, optogenetic activation of the same neurons during threat presentation inhibited escape initiation. To further understand the biophysical basis of local inhibition in the dPAG and its modulation, we performed single-cell RNA-sequencing on VGAT+ and VGluT2+ neurons individually isolated from acute midbrain slices. Differential expression analysis of these data identified candidate ion channel subunit and neuromodulator genes for setting and regulating key biophysical properties of PAG neurons.

This work shows that both the activity of excitatory dPAG neurons and the initiation of instinctive escape are controlled by a local GABAergic network, and provides a framework for studying how molecularly defined biophysical properties might underpin behavioural control by the PAG.

INPUT-OUTPUT RELATIONSHIPS OF THE POSTERIOR INTRALAMINAR THALAMIC NUCLEI IN THE MOUSE

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The posterior intralaminar thalamic nuclei (PIN = Parafascicular nucleus + Ethmoid-Limitans nucleus) are a massive source of subcortical excitatory inputs to the striatum. Moreover, PIN neuron axons simultaneously innervate the cerebral cortex as well; however, the functional logic of this divergent projection is currently unclear. Likewise, sources of cortical and subcortical input to PIN neurons remain poorly defined.

Here, using wild-type adult C57BL/6 male mice as experimental subjects, we set out to a) map PIN afferents; b) sort out the relationship between PIN subregions and the cortical + striatal territories targeted by their projections; and c) elucidate the existence of differences in axon varicosity morphology/size in PIN axons in specific cortical layers or areas and/or striatal (matrix/striosome) domains. We made selective microinjections of biotin dextran amine (BDA) or a mixture of BDA and Cholera Toxin B Subunit in different PIN subdomains to visualize their efferent and afferent projections. We applied immunolabeling against μ-opioid receptor or glycine transporter 2, or histochemical stainings as thionin, cytochrome oxidase or acetylcholinesterase to delineate relevant brain territories.

Our data show that the mouse PIN receive massive inputs from, among other structures, motor cortex layer 5 cells and the multimodal intermediate/deep layers of the superior colliculus. Cortical layer 6 projections are robust as well. In turn, PIN projections to striatum and cortex are topographically organized, and the same PIN neurons innervate somatotopically-congruent regions in both structures. Axonal varicosity sizes vary depending on the target structure and the origin of the axons within the various PIN subregions. These observations suggest that the divergent PIN output to the striatum and the cerebral cortex is segregated into parallel, somatopically-related subcircuits.

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INTERFERENCE-BASED FORGETTING IN A GOAL-DIRECTED SPATIAL NAVIGATION TASK FOR RODENTS

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To avoid catastrophic accumulation of information during learning the brain needs to forget to retain only what is subjectivity relevant to each individual. Interference-based forgetting occurs when new information acquired before or after a learning event attenuates memory strength. In our daily life we are continuously exposed to new associations, each with its own value and volatility, which limits the interpretation of results obtained in lab conditions using only few associations. For this reason, we developed a novel task that allows us to observe the effect of memory interference in more ethological conditions.

We utilized our high-throughput behavioral task where animals learn the location of the reward among 8 possible positions, that randomly changes across days. We test the memory recall of the location of the reward position 2 hrs after the training session and we observe how memories from previous sessions (i.e. days) interfere with this recently learned memory. To manipulate the interference between previously learned memories, we infused human N-Methyl-D-aspartic (NMDA) receptor and Leucine-rich glioma-inactivated 1 (LGI1) antibodies into animals' brain ventricles to mimic a partially anterograde amnesic state.

We confirmed that continuous learning of new associations decreases the strength of recent memories due to the interference with previous ones. We showed for the first time the effect of interference from all previous memories up to 3 days in the past. We also found that antibodies mediated amnesia is capable of reducing interference significantly ($p<0.01$) enhancing recent memories at the cost of considerably reducing (~40%) the strength of old ones. Our findings support the theory of retroactive interference as the mechanism to eliminate memories from associations with high volatility.

INVESTIGATING THE ROLE OF AUDITORY CORTEX ON DECISIONS ABOUT SOUND LATERALIZATION

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Decisions are an essential part of life and almost all decisions involve some comparison between different options. Previous work from our group relates the mechanism for Weber's Law - accuracy of the discrimination between the intensity of two sensory stimuli depends only on their ratio - with a new mathematical regularity, the Time Intensity Equivalence in Discrimination (TIED) - changes in the absolute intensity of two stimuli being discriminated under a fixed intensity-ratio are completely equivalent to a change in the effective unit of time with which the discrimination duration is measured. The TIED is incredibly restrictive and has allowed us to list a set of three computations required for the Weber's Law to be present: sensory relay, evidence accumulation and representation of the decision bound. We aim to identify the different brain regions responsible for these computations through testing of different candidate areas. We have predicted how perturbations to each of these computations would affect the behaviour through changes in accuracy and reaction time.
The focus of this project is on testing the involvement of the ACx as the sensory relay. Lesion studies based on the injection of an excitotoxic agent which led to a permanent lesion of ACx show how behavioural outputs do not seem to be affected by the ablation of ACx.
Permanent lesions allow for compensatory mechanisms to arise and do not allow a trial by trial manipulation of the decision, so we also show the results of optogenetic silencing of the ACx. We show that by using the stGtACR2 opsin we manage to achieve effective and robust silencing of the ACx while shining blue light onto the cortex during acute recordings. After this we developed a novel way to chronically implant LEDs in the skull of the animal in order to allow for optogenetic silencing of the whole ACx in behaving, freely moving rats. We also present preliminary data on how this perturbations affect the animals' decisions.

LACK OF AVERSIVE BEHAVIOR IN FEMALE MICE EXPOSED TO PREDATOR ODORS

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The avoidance of danger and the detection of possible threats to the survival are behaviours present in all animal organisms. In fact, detecting and avoiding danger has probably been a strong evolutionary pressure for the development of sensory systems allowing this survival behaviour.

In animals, detecting the presence of a predator nearby and avoiding the territories with abundant predator signals is an important advantage for the survival of prey. In many mammals this detection is based on chemical cues. The chemical cues of other species that signal the presence of a predator are called kairomones. In Mus musculus, previous studies have reported possible kairomones for a variety of natural predators as foxes, cats or rats.

In this work we report a series of experiments with the objective of investigating the aversive or avoidance behaviour of mice against some chemical cues obtained from cats (cotton impregnated with saliva; cotton impregnated with secretions of the perianal gland of a cat; and cat bedding) and trimethylpirazine 13, a molecule found in wolf urine that has been reported to elicit avoidance behaviour in mice. The predator-derived stimuli were presented in preference tests against the same type of substrate containing the stimuli (cotton, bedding or filter paper), either clean of with saline.

Our results show no statistical difference in the time spent investigating the neutral and the predator odours used, and we observed no sign of avoidance or freezing behaviour. We conclude that the presence of these chemical cues is not sufficient to elicit avoidance or defensive behaviours. We hypothesize that, for the occurrence of this species-typical behaviours, it might be necessary the co-occurrence of other factors, related with the context, the internal state of the animal and the chemical characteristics of the stimuli.

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LARYNGEAL EFFECTS OF STIMULATION OF THE CUNEIFORM NUCLEUS IN SPONTANEOUSLY BREATHING ANAESTHETIZED RATS

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Background: The cuneiform nucleus (CnF) is a mesencephalic area that has been involved in sympathetic activity due its connectivity with several nuclei involved in cardiorespiratory control, e.g. dorsolateral periaqueductal gray matter (dlPAG), the parabrachial/Kölliker-Fuse complex (PBc/KF), the solitary tract nucleus (NTS) and the rostral ventrolateral medulla (RVLM). In previous studies we have demonstrated a functional interaction between hypothalamic and mesencephalic structures (DMH-PeF, dlPAG) with several pontine regions (PBc, A5) (Díaz-Casares et al., 2009, López-González et al., 2020). We have also shown that rostral and ventral pontine structures are involved in the changes of laryngeal caliber (Lara et al., 2002). The aim of this study was to characterise the relationship between mesencephalic-pontine neuronal circuits to understand their role in laryngeal control and its effect on vocalization.

Methods: Experimental studies were carried out with non-inbred male rats (n=7), [SPF, Sprague-Dawley (300-350 g)]. Animals were anesthetized with sodium pentobarbitone (60 mg/kg i.p., initial dose, supplemented 2 mg/ kg, i.v., as necessary). A double tracheal cannulation (upwards for the "glottis isolated in situ" technique, and downwards in the direction of the carina) was done. Subglottic pressure was recorded with a precision differential pressure transducer (ADInstrument model FE141, ± 0,03 psi) by passing a stream of humidified medical air upwards through the larynx at a constant rate of 30-70 ml/min with a thermal mass digital air flow meter controller (Bronkhorst Hi-Tec F-201CV-AGD-22-V). Electrical stimulation of the CnF using concentric bipolar electrodes (1 ms pulses, 20-40 µA, 100 Hz for 5 s) was performed. Respiratory flow, pleural pressure, blood pressure, heart rate and ECG activity were also recorded.

Results: CnF stimulation evoked a decrease of laryngeal resistance (subglottal pressure) ($p<0,01$) accompanied with an inspiratory facilitatory response consisted of an increase in respiratory rate ($p<0,01$), together with a pressor ($p<0,001$) and a tachycardic response ($p<0,01$).

Conclusions: The results of our study contribute with new data on the role of the CnF in the mechanisms controlling subglottic pressure and laryngeal activity.

M1- MUSCARINIC CONTROL OF SLOW OSCILLATIONS AND EPILEPTIFORM DISCHARGES BY LIGHT

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Different brain states are associated with specific patterns of cortical emergent activity. A highly synchronized activity pattern, slow oscillations, not only is associated to periods of deep sleep and anesthesia, but also to pathological conditions like non-responsive wakefulness syndrome and coma, and locally, to perilesional areas such as stroke. Neuromodulation techniques attempt to control neural activity, and their development is relevant for basic neuroscience and eventually, for the repair of disrupted functions in neurological disorders with altered activity patterns. A promising neuromodulation tool is photopharmacology, which is the control by light of drug activation, following chemical drug manipulation to be photoswitchable. Here, we investigated the effects of one of such novel drugs, BQCAAI (BAI), a photoswitchable type 1 muscarinic agonist obteined by the combination of benzyl quinolone carboxylic acid (allosteric part) with the muscarinic agonist Iperoxo (orthosteric part), to determine its effects over synchronized network activity - slow oscillations- in the cerebral cortex in vitro and in vivo. Our results show that slow wave activity was transformed into a faster oscillatory pattern in both preparations of the cerebral cortex following the illumination of the brain tissue containing BAI (1 micromolar BAI was used for in vitro and 5 micromolar BAI was used for in vivo experiments), demonstrating the effectivity of BAI. Higher BAI concentrations (> 10 micromolar) resulted in an epileptiform effect at in vitro. Interestingly, such epileptiform effect was not evoked by M2 muscarinic agonists (Barbero-Castillo et al, 2021, Advanced Science). Our results demonstrate that epileptiform effects of classical epilepsy models (pilocarpine) are indeed, M1-mediated, and sensitive to blockade by pirenzepine. These results shed light on the contribution of M1 acetylcholine receptor to cortical dynamics and validate the use of photoswitchable drugs for the spatiotemporal modulation of brain networks by light without requiring any genetic manipulation.

MORPHOLOGICALLY DISTINCT AFFERENCES TO THE LATERAL SUPERIOR OLIVE

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To locate sound sources, animals use interaural level differences (ILD), as sounds reach the nearest ear with higher intensity. ILDs are encoded in the lateral superior olive (LSO), a nucleus innervated by four neuronal types of auditory brainstem nuclei: spherical bushy cells (SBCs) and planar multipolar neurons of the ipsilateral ventral cochlear nucleus, principal neurons of the ipsilateral medial nucleus of the trapezoid body (MNTB), and small multipolar neurons of the contralateral ventral nucleus of the trapezoid body (cVNTB) (Gómez-Álvarez and Saldaña, 2016, J Comp Neurol 524:2230-2250). Neurons in the LSO are excited by sounds reaching the ipsilateral ear, and inhibited by sounds reaching the contralateral ear, yet how this integration occurs remains unclear.

We designed four tract-tracing experiments to label selectively each one of the projections to the rat LSO. We injected the tracer biotinylated dextran amine (BDA) into the anteroventral and dorsal cochlear nucleus (AVCoN and DCoN), superior paraolivary nucleus (SPON) and VNTB and studied the axons labeled in the LSO.

All LSO afferents form flattened plexuses that follow the laminar organization of the LSO. These projections differ in morphological aspects, including the caliber and branching pattern of the axons, and the size and abundance of terminal and en passant boutons.

Injecting BDA into the DCoN, SPON, and VNTB labels selectively the axons of planar multipolar neurons, MNTB principal neurons, and cVNTB neurons, respectively. Conversely, injecting BDA into AVCoN to label the axons of SBCs leads to confusing results, since AVCoN is innervated by MNTB neurons that also innervate LSO. The axons of SBCs and MNTB principal neurons, which represent 80% of the neurons that innervate the LSO, have fewer synaptic boutons than those of planar multipolar neurons and VNTB neurons. Therefore, the functional relevance of the latter two projections should not be underestimated.

NEURAL MECHANISMS OF SERIAL DEPENDENCE ACROSS VISUAL HEMIFIELDS AND BILATERAL PREFRONTAL CORTEX

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It has been shown that previously perceived working memory (WM) items have an effect on current WM reports. This effect is called serial dependence and has been shown to rely on the interaction of active neural representations and long-lasting activity-silent mechanisms in prefrontal cortex (PFC) [Barbosa, Stein et al., 2020]. Furthermore, it has been shown that WM representations are more frequent for contralateral than for ipsilateral memorized locations in PFC [Funahashi et al., 1989] and that active representations transfer between hemispheres when midline-crossing saccades occur in the delay [Brincat et al., 2021]. This indicates the consistent specialization of each hemisphere for the corresponding visual hemifield in WM. However, serial dependence challenges this view as it is unclear how it can emerge when consecutive stimuli appear in different hemifields, which engage independent neural substrates. Here, we investigate the transfer of serial dependence between visual hemifields and the associated prefrontal correlates across hemispheres, in order to shed light on the mechanisms of integration of lateralized WM storage. We collected simultaneous multi-unit recordings in bilateral PFC of 3 monkeys performing an oculomotor visuospatial delayed response task. We analyzed behavioral responses, and population coding in neural data in relation to serial dependence. Decoding from neural traces was used to predict behavioral biases of the monkeys for separate hemifields and hemispheres. We found that serial dependence of stimuli presented across hemifields was diminished in comparison to trials within the same hemifield. Furthermore, the decoded neural traces between hemispheres reflected these differences. We conclude that small biases towards previous memories in WM are partly supported by lateralized mechanisms. This shows an incomplete continuity of serial dependence in WM, which is in line with the activity-silent theory for serial dependence.

NEURAL PROBES FOR MULTIMODAL INTERROGATION OF BRAIN LAMINATION

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Understanding the laminar organization of brain areas is crucial to appreciate function and dysfunction. A diversity of cell types and input pathways distribute unevenly through neocortex and brain areas. Regional differences of cell-type specific innervation by local GABAergic interneurons shapes brain parcellations. Importantly, recent studies suggest that in some regions, such as the hippocampus, a more granular lamination can be defined across the CA1 deep and superficial sublayers. Strikingly, this fine-grained microstructure is revealing critical to evaluate histopathological entities associated to a range of neurological diseases.

Here, we describe a toolbox of neural probes suitable for evaluating the laminar organization of the brain at the neurophysiological, optogenetic and spectroscopic levels. Using ultra-dense Neuropixel probes, we obtain laminar recordings and well-isolated spiking activity of hundreds of neurons across several brain regions. Non-canonical 3D-oriented penetrations allow for simultaneous assessment of neocortical, hippocampal and brainstem nuclei of awake head-fixed mice. Using spectral analysis and unsupervised spike sorting techniques, we isolate the different contribution from neocortical and hippocampal activity, which when histologically validated permits resolving neurophysiological laminar profiles at high resolution. Next, we use high-density silicon probes with integrated micro-light emitting diodes (LEDs) combined with cell-type specific optogenetic for dissecting sublayer composition in the hippocampal area CA1. We show successful optotagging of a wealth of different neuronal types, including pyramidal cells and a diversity of GABAergic interneurons. Finally, we map depth-resolved Raman signals using tapered optical fibers to obtain spectroscopic laminar profiles of the chemical composition of brain parenchyma. We identify specific Raman signatures of fatty acid and triglycerides, as well as lipid and proteins differentially expressed across brain depth, which can be tracked histologically.

We discuss how this sophisticated toolbox of neural probes may allow for an unprecedented multimodal evaluation of brain structure and function in health and disease

NEURONAL ACTIVITY REFLECTING SENSORY AND BEHAVIOURAL VARIABLES IN THE MOUSE SOMATOSENSORY AND POSTERIOR PARIETAL CORTEX

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Real-world signals such as communication sequences unfold over time with a characteristic temporal structure. Recognizing temporally ordered patterns is key to survival. To explore how cortical neuronal activity underpins this capacity, we recently developed a task in which mice distinguished between tactile 'word' sequences constructed from distinct vibrations delivered to the whiskers, assembled in different orders. We combined task performance with two-photon imaging and optogenetics.

Animals licked to report the presence of the target sequence. Mice could respond to the earliest possible cues allowing discrimination, effectively solving the task as a 'detection of change' problem, but performed better when responding later, after more evidence could be collected.

Sequence selectivity can emerge generically in neurons through widespread forms of synaptic plasticity, and our expectation was that learning the task would induce cortical neurons to refine their sensory tuning simply by becoming more selective to the target sequence. Instead, two-photon imaging showed that, upon learning, neurons in both the primary somatosensory cortex (S1) and posterior parietal cortex (PPC) responded to multiple task variables, including not just sensory input but also the animal's action decision (goal-directed licking) and the trial's outcome (presence or absence of the predicted reward). However, optogenetic inactivation showed that while S1 was necessary for sequence discrimination, PPC was not. This indicates a dissociation between the response properties or "codes" that neurons in a cortical area exhibited in the task, and the causal involvement of that area in the task.

Our results show that (1) conditioning on a goal-directed sensory discrimination task results in neurons within sensory and association cortex whose activity reflects the learnt links between target stimulus and licking; (2) cortical neuronal activity reflecting task variables can simply broadcast those variables without playing a causal role in task performance.

NMDAr BLOCKING BY MK801 PRODUCES SPECIFIC OSCILLATORY CHANGES IN THE HIPPOCAMPUS AND THE PREFRONTAL CORTEX IMPAIRING WORKING MEMORY AND PLACE CELL FIRING

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Information processing in the brain depends on the dialog between different regions orchestrated by oscillatory activity, especially theta (4-12 Hz) and gamma rhythms (30-100Hz). Clinical and basic research has confirmed a strong relationship between cognitive impairment and distortion of these rhythms in Schizophrenia. It is hypothesized that these oscillopathies originate from alterations of the excitatory-inhibitory (E-I) balance. Therefore, unveiling the relationship between brain rhythms and cognition is crucial for understanding the cognitive manifestations of Schizophrenia. In this work, we aimed to investigate how changes in the E-I balance might reproduce rhythm and behavioral alterations. We used a mouse pharmacological model of schizophrenia, administering MK801 to adult wild-type mice, an NMDAr blocker known to alter interneuron and pyramidal activity. Mice were implanted with microdrives mounting tetrodes aimed to the dorsal hippocampus and medial prefrontal cortex (mPFC). Then brain activity was recorded while mice performed spontaneous exploration in an open field, and while testing working memory in the y-maze, after the administration of MK801 or vehicle. Our results proved that NMDAr blocking exerts differential effects in the oscillatory activity of the hippocampus and the mPFC. While in CA1 and subiculum MK801 produced an increase in gamma oscillations and a distortion of theta/gamma coupling, mPFC activity was characterized by an augmentation of theta, gamma and emergence of high frequency oscillations (HFO, 155-185 Hz). Interestingly HFO in the mPFC was strongly modulated by local theta activity. In addition, we observed an increase in CA1-mPFC coherence in the delta and alpha band, changes in locomotor behavior, defective place cell function and impaired working memory in the y-maze. We observed that CA1 theta/gamma modulation was enhanced during right alternation in the y-maze, but none of the oscillatory changes produced by MK801 could predict performance in the y-maze.

NORADRENALINE INNERVATION AND ALPHA ADRENOCEPTORS IN THE HUMAN AND MACAQUE HIGHER-ORDER THALAMIC NUCLEI.

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Noradrenaline (NA) modulates processes like sensorimotor gating and prepulse inhibition through higher order (HO) thalamic nuclei. Besides, the macaque HO nuclei are within the most densely innervated by NA and within those displaying the highest Alpha adrenoceptor concentrations. The purpose of this study is to compare the patterns of NA innervation and adrenoceptors in the macaque and human HO thalamic nuclei.

Human brain sections containing the thalamus were immunostained against the NA transporter to reveal the NA axons. Quantitative autoradiography was performed to reveal the adrenoceptors: the ligands [3H]-Prazosin (Alpha-1 receptors), [3H]-RX-821002 (whole Alpha-2 adrenoceptor population), and [3H]-UK-14,304 (high-affinity state Alpha-2 adrenoceptor) were used. The distributions of axons and receptors were compared to similar data from macaques, previously published.

The human mediodorsal nucleus (MD) showed moderate NA innervation, with the highest densities in the medial and ventral regions of the nucleus. Within the human pulvinar complex, the nucleus with the highest NA innervation was the oral pulvinar (Pul O); the medial pulvinar (Pul M) displayed moderate densities of NA axons, and the lateral and inferior pulvinar (Pul L, Pul I) presented low NA axon densities. The highest densities of Alpha-1 receptors were present in the dorsal and medial regions of MD, and in the medial regions of Pul M. The highest Alpha-2 receptor concentrations were present in Pul I. Pul O showed high Alpha-2 receptor revealed by [3H]-RX-821002 but rather low revealed by [3H]-UK-14,304 pointing to a low proportion of high-affinity Aplha-2 receptors in this nucleus.

The distributions of NA axons and Alpha adrenoceptors in the human HO nuclei are highly comparable to those in macaques. The main differences were in the Alpha-1 receptor concentrations, which were higher in macaques than in humans, and in the high-affinity Alpha-2 receptor concentrations in the PulO, which were also higher in macaques.

PHENOTYPE CHARACTERIZATION OF A MICE MODEL OF VISUAL BLINDNESS

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The presente work shows the phenotype characterization of a murine genetic model of absolute blindness. The animal model is based on the combination of a mutation in the Pde6brd10 gene, which results in a photoreceptor degeneration, together with a mutation in the Opn4 -/- gene, responsible of the melanopsin expresion in intrinsically photosensitive retinal ganglion cells.
The characterization of the visual functions of a double mutant Opn4 -/- x Pde6brd10 (OxRd) murine model has been carried out, applying a battery of behavioral tests, as well as in vivo electrophysiological recordings, which allowed us to know the degree of functionality of the retina and visual pathways. A structural characterization of the retina was also carried out using immunohistochemical labeling on retinal sections. The results were compared with wt mice and murine animal models that present both mutations separately.
The OxRd animals showed a total suppression of all visual abilities. The different behavioral tests showed that characteristic physiological visual reflexes and visual behavior of these animals, such as the rejection of illuminated spaces or the pupillary reflex, were totally inhibited. A complete decrease in visual acuity was observed by the optomotor test, as well as the absolute disappearance of the various components of the waves of the full-field and pattern electroretinogram (ffERG, pERG), indicating the functional loss of the different cellular components of the retina. Likewise, no visual evoked potential (VEP) could be recorded in these animals. Immunohistochemical labeling support these data, showing a marked degeneration of the outer retinal layers, due to the of the Pde6brd10 mutation, as well as the absence of melanopsin labeling.
The combination of the mutations in the Opn4 -/- and Pde6brd10 genes has allowed us to generate an animal model that does not show any photosensitive element in its retina. This animal is unable to recognize light stimuli, which makes it a potential tool for the study of new therapeutic agents such as optosensitive agents.

PREFRONTAL-HIPPOCAMPAL CIRCUIT ALTERATIONS AND RESCUE IN A MOUSE MODEL OF SCHIZOPHRENIA

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Disruption of neural synchrony and spatio-temporal communication in brain circuits involving the prefrontal cortex (PFC) and the hippocampus (HPC) has been suggested to be a hallmark characteristic of neuropsychiatric disorders such as schizophrenia (SCZ). SCZ patients show positive symptoms that can be effectively managed with antipsychotic drugs (ADPs). However, negative symptoms and cognitive deficits are inadequately treated. Therefore, a better understanding of the prefrontal-hippocampal neural basis of these symptoms is essential for the development of new treatments. We investigated the alterations of prefrontal-hippocampal circuits in the acute (PCP) and subchronic (sPCP) phencyclidine mouse model of SCZ, that assess the positive and cognitive symptoms of SCZ, respectively, and examined the recovery by typical and atypical APDs. First, we recorded neural activity in the PFC and HPC of C57BL/6J mice treated acutely with PCP alone or PCP followed by one APD (risperidone, clozapine or haloperidol). Acute PCP produced hypersynchronization and disrupted communication of prefrontal-hippocampal pathways that were efficiently recovered by ADPs. In another set of mice, we recorded the activity before and after administration of sPCP during an open field exploration, three auditory tasks and the novel object recognition test that assesses working memory and long-term memory abilities. We also investigated behavioral and neurophysiological rescue by a 14-day risperidone treatment. sPCP-treated mice showed abnormal gamma oscillations (30-100 Hz) and theta-gamma cross-frequency coupling during rest. Notably, auditory perception, working memory and long-term memory were profoundly impaired in sPCP-treated mice and were accompanied by disrupted prefrontal-hippocampal functional connectivity. Both, memory deficits and functional connectivity were rescued by risperidone. Our findings suggest that abnormal prefrontal-hippocampal neurodynamics may contribute to the neural mechanisms of SCZ and some of these alterations can be rescued by ADPs.

PRE-TRAINING RNNS ON ECOLOGICALLY RELEVANT TASKS EXPLAINS SUB-OPTIMAL BEHAVIORAL RESET

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When faced with a new task, animals' cognitive capabilities are determined both by individual experience and by structural priors evolved to leverage the statistics of natural environments. Rats can quickly learn to capitalize on the trial sequence correlations of two-alternative forced choice (2AFC) tasks after correct trials, but consistently deviate from optimal behavior after error trials, when they waive the accumulated evidence. To understand this outcome-dependent gating, we first show that Recurrent Neural Networks (RNNs) trained in the same 2AFC task outperform animals as they can readily learn to use previous trials' information both after correct and error trials. We hypothesize that, while RNNs can optimize their behavior in the 2AFC task without a priori restrictions, rats' strategy is constrained by a structural prior adapted to a natural environment in which rewarded and non-rewarded actions provide largely asymmetric information. When pre-training RNNs in a more ecological task with more than two possible choices, networks develop a strategy by which they gate off the across-trial evidence after errors, mimicking rats' behavior. Our results suggest that the observed suboptimal behavior reflects the influence of a structural prior that, adaptive in a natural multi-choice environment, constrains performance in a 2AFC laboratory task.

PROJECTIONS OF THE RAT MEDIAL SUPERIOR OLIVE

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Sounds do not usually reach both ears at the same time. This difference in arrival time (interaural time difference [ITD]) is an extremely useful cue to localize the sources of low-frequency sounds in the horizontal plane. ITDs are encoded in the medial superior olive (MSO), one of the nuclei of the superior olivary complex.

Although the rat is a favorite experimental model in auditory neuroscience, the projections of the rat MSO remain to be studied in detail with anterograde tracers. This may be due not only to the high-frequency hearing range of this species, which supposedly does not use ITDs for sound localization, but also to the extreme narrowness of its MSO, which renders experimental manipulations problematic.

To study the projections of the rat MSO, we have made small, single injections of the bidirectional tracer biotinylated dextran amine (BDA) into the MSO of this species and analyzed the trajectory, morphology and distribution of the labeled axons.

Our results are based on nine successful cases, whose injection site affected solely or almost exclusively the MSO. In all of them, dense plexuses of terminal axons were labeled ipsilaterally in the central portion of the dorsal nucleus of the lateral lemniscus and in the most dorsolateral portion of the central nucleus of the inferior colliculus, which are the regions of these nuclei where low-frequency sounds are processed. Minor projections, not previously described with anterograde tracers, were observed in the ipsilateral ventral nucleus of the lateral lemniscus and medial geniculate body of the thalamus.

Despite the widespread belief that the MSO of rats and mice is rather insignificant, our data demonstrate that it is hodologically similar to that of mammals with a lower auditory range, like the cat or the gerbil. They further suggest that MSO function is not limited to ITD coding.

ROTATIONS OF PREFRONTAL WORKING MEMORY REPRESENTATIONS TO PROTECT FROM TASK INTERFERENCE IN A DUAL-TASK PARADIGM

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Recent studies involving complex working memory (WM) tasks or tasks with distracting inputs have suggested that stimulus representations before and after distractors are orthogonal, thus allowing for the protection of stimulus information from interferences. However, whether ”orthogonalization” is a general mechanism for WM preservation remains an open question. Moreover, the specific network mechanisms that could allow for such an orthogonalization are unknown. Here, we investigated orthogonalization as an instrument of WM control on calcium imaging data from the medial prefrontal cortex (mPFC) in a novel olfactory dual-task - the ultimate WM interference condition - in behaving mice. The dual-task consists of an outer delay-paired association task (DPA) combined with an inner Go-NoGo task. We studied how the representation of the sample stimulus of the DPA was affected by presenting the Go/NoGo cue of the inner task. Specifically, we examined how sample information is transformed during the delay period of the dual-task, focusing on inferring low-dimensional coding directions to evaluate orthogonality between WM representations at different epochs of the delay period of the outer task. We found a significant change in the directions representing DPA sample information before and after the Go/NoGo cue. This result indicates a rotation of the representation of sample information in the early delay period into an orthogonal representation in the late delay. To probe that mPFC plays an essential role in this mechanism, we investigated how memory storage was related to animal behavior. We found that memory rotation strengthens with learning. Altogether, our results suggest that rotation of WM representation in mPFC is a fundamental mechanism for maintaining WM information in the face of interfering distractor tasks. Finally, we give a mechanistic account of mPFC WM rotations in a network model of strongly recurrent neurons with low-rank connectivity structure.

SECRETAGOGIN EXPRESSION IN THE MOUSE BRAIN

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Calcium-binding proteins such as calbindin, calretinin or parvalbumin are essential for the correct functioning of the brain. Their distribution has been widely studied in the central nervous system, mainly for neuroanatomical purposes since they provide a "Golgi-like" staining with noteworthy details of neurites. Secretagogin is another calcium-binding protein that also provides a high-quality immunostaining of specific neuronal populations, but whose distribution throughout the brain is poorly known.

Here, we analysed the expression of secretagogin in the brain of adult mice by immunohistochemistry, identifying positive cell populations in different areas and nuclei according to Paxinos mouse brain atlas. Combined immunofluorescence was used for studying colocalization between secretagogin and other common calcium-binding proteins.

We observed many secretagogin-positive cells throughout the brain. The staining was remarkable in some areas of the olfactory bulb, basal ganglia, thalamus and hypothalamus. Surprisingly, no labelling was detected in cerebellum, where other calcium-binding proteins are widely distributed. Secretagogin-positive cell populations were very heterogeneous, concerning both size and distribution: in some nuclei positive cells occupied all the analysed area, but in others few disperse positive cells were observed. Interestingly, in some cases such distribution did not fit with the nuclei traditionally described, suggesting either neurochemical subdivisions for partial-labelled regions or shared functions for adjacent nuclei. Regarding colocalization, results were also very variable. Depending on the region, secretagogin colocalized with either calbindin, calretinin or parvalbumin, but in other areas, even with high neuronal density, no colabelling was identified and they were independent populations.

We conclude that secretagogin is present in many different neuron populations in the mouse brain. The function of these cells seems to be heterogeneous, and this protein is expressed either independently or in combination with other calcium-binding proteins. Secretagogin can be a useful neuronal marker in different brain areas for specific populations and its knowledge advances the complex regulatory mechanisms of calcium levels in the central nervous system.

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SIMULTANEOUS ENCODING OF FEAR STATE AND THREAT IDENTITY IN PREFRONTAL CORTEX NEURONAL POPULATIONS

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In response to specific threats, mammals select a response among a repertoire of different defensive behaviors. The selection and the rapid execution of this response are crucial for animal survival and are determined not only by the nature of the threat but also by the contextual contingencies. Therefore, in order to survive a dangerous situation mammals have to integrate multimodal information regarding the threat, the context and its internal state to rapidly elicit the most adaptive fear response. The neuronal circuits and mechanisms allowing this rapid selection of appropriate defensive fear responses are still largely unknown. To address this question, we used a multi-level approach combining simultaneous electrophysiological recordings and optogenetic manipulations in a novel and unique behavioral paradigm allowing mice to select different defensive behaviors when facing different threats. Using this combination of techniques, we monitored the neuronal activity of dmPFC neurons of mice presented with different threats to demonstrate that at the population level, dmPFC neuronal activity encodes both a general fear state and more specific information about the identity of the threats. We further investigated the processes that allow for this simultaneous encoding of threat and fear information and the effects of manipulating the activity of the dmPFC in the selection of specific defensive responses.

SPATIAL PERIODIC FIRING IN THE SUBICULUM OF MICE

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Spatial cognition relies on a complex circuitry in which the hippocampal formation seems to be crucial. The subiculum is a region located at the core of this circuit, it receives inputs from grid cells located in the medial entorhinal cortex (MEC) and place cells from the CA1 area of the hippocampus. It integrates input from these two relevant spatial information sources and mediates the output from the hippocampus to cortical and sub-cortical areas also involved in spatial coding. Despite the potential relevance of the subiculum, its role in memory and spatial coding is still poorly understood. Previous work described a very heterogeneous population of spatial neurons in the subiculum, with evidence of its role in coding the geometry of the environment and in spatial navigation in darkness. However, its role in spatial coding remains to be unveiled. With the aim of understanding further the properties of spatial coding in the subiculum, we implanted mice with microdrives mounting tetrodes and multisite electrodes aiming at the CA1 and subicular area, and recorded neuronal activity across different behavioral paradigms. Our results indicate that place cells in CA1 present higher spatial resolution and sharper firing fields than those of subicular neurons. Also, place cells in the CA1 area seem to be differentially modulated by the local field potential. Interestingly, we found pyramidal neurons in the subiculum with periodic firing, the first evidence of this type of regular firing in subicular pyramidal neurons.

STUDY OF THE ANTIDEPRESSANT EFFECT OF NEW GENERATION DRUGS BASED ON GLUTAMATERGIC TRANSMISSION

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Depression is an extended pathology, with more than 300 millions of people affected. However, the most used antidepressants, based on the monoamine theory of depression, have several limitations. Hence, new therapeutic alternatives based on glutamatergic transmission have emerged. In order to optimize the use of these new therapies, it would be useful to describe biomarkers of their antidepressant effect and of other undesired but potential outcomes.

Therefore, the main objective of this work is to characterize the electrophysiological activity generated by two of these antidepressants, Ketamine and LY 404187, to propose potential electrical biomarkers of their effects.

To do so, two doses (5 and 30mg/Kg) of Ketamine and a dose of LY 404187 (3 mg/kg) were injected to different groups of CD1 mice, and local field potentials were recorded from their infralimbic cortex (IL), dorsal hippocampus (HPCd) and basolateral amygdala (BLA). Electrophysiological recordings were also performed during several days following drug administration to describe the duration of the effects. In other groups of animals, behavioural tests to measure antidepressant effects (Forced Swimming Test and Tail Suspension Test), psychotic symptomatology (Novel Object Recognition) or anxiety traits (O-Maze and Open Field) induce by each drug and dose were carried out. This allowed to better interpret the behavioural correlation of the electrical effect induced by each treatment.

Results showed that immediately to the administration of Ketamine there was an increase of the power of low theta and gamma oscillations in all nuclei and a decrease of that of beta oscillations into HPCd and BLA. Furthermore, animals showed psychomimetic behaviours. After LY administration, low theta oscillations in HPCd and BLA during the first 30 min as well as the expression of anxiety-related behaviours rose. Both drugs produce rapid and lasting antidepressant effects, so electrophysiological changes would also be predictive of these.

STUDY OF THE DISTRIBUTION OF □-TTP AND CALCIUM BINDING PROTEINS IN THE HIPPOCAMPUS OF A MURINE MODEL OF DELAYED AGEING, THE POL μ MOUSE

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Aging is a physiological and multifactorial process, where inflammatory mechanisms and oxidative stress (OS) play a fundamental role and leading to a gradual loss of the functionality of the different organs and tissues. Several neuron populations are selectively affected by the aging process. Interneurons play a key role in the maturation, function, plasticity, and organization of cortical circuits, as well as in the control of the activity of major or principal neurons. Disturbances in the hippocampal-inhibiting networks, which involve the loss of specific subpopulations of GABAergic interneurons (INs), could be an important factor in hippocampal aging. In recent years, the effects of antioxidant supplementation through the diet have been studied as it has been proved to reduce the redox imbalance and to decrease the effects of OS on aging. Vitamin E (VitE) is one of the antioxidants more widely used as an antioxidant therapy. Of all the isomers that constitute the VitE, the organism preferentially retains the isomer configuration α-tocopherol (α-T), introduced into neurons by a specific protein, the α-tocopherol transfer protein (α-TTP), widely distributed throughout the brain. This is one of the most affected organs in aging, and the hippocampus, mainly responsible for the generation and recovery of memories and spatial orientation, has been described as an especially vulnerable area. We have studied in detail the expression of α-TTP in all the regions and layers of the hippocampus along aging, as well as the presence of this transfer protein in different INs populations. The analysis was made in Pol □□mice, a delayed-ageing model, in animals of 4 and 24 months old. Our results suggest a specific distribution of α-TTP not only in the different layers of the hippocampus, but also a colocalization in certain INs populations. This suggest that the hippocampal INs could present a different susceptibility to redox imbalance according to their ability to use α-T as an external antio

SURPRISINGLY DENSE PROJECTIONS FROM THE VENTRAL NUCLEUS OF THE TRAPEZOID BODY TO THE DORSAL COCHLEAR NUCLEUS

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Animals integrate auditory and somatosensory stimuli because the perception of sounds depends not only on their position relative to the sound source, but also on the posture of the head and ears. This integration first occurs in the dorsal cochlear nucleus (DCoN). Key to the integration of different sensory modalities is the laminar structure of the DCoN, very similar to that of the cerebellar cortex. In the cerebellum, afferent information reaches mainly the deep layers and is subsequently transmitted to the superficial layers. However, the DCoN receives a presumably auditory afferent that, unlike what happens in the cerebellum, does not reach the deep layers: the projection from the ventral nucleus of the trapezoid body (VNTB).

Our goal was to carry out the first detailed morphological investigation of the projection from the VNTB to the DCoN of the rat. Using albino rats, we have performed two types of experiments. First, to analyze the distribution and morphology of the axons that innervate the DCoN, we injected the bidirectional tracer biotinylated dextran amine (BDA) into VNTB. Second, to characterize the neurons that give rise to this projection, we injected BDA into DCoN.

The VNTB-to-DCoN projection is very predominantly contralateral. VNTB axons form an amazingly dense terminal plexus restricted to the molecular layer and very rich in synaptic boutons. In this plexus, bands of higher density perpendicular to the pial surface alternate with bands of lower density. This projection is tonotopic.

VNTB neurons that innervate the DCoN possess medium-size multipolar cell bodies, occupy the central dorsoventral third of the nucleus and are distributed along the entire rostrocaudal length of the VNTB. They seem to belong to a hitherto unidentified neuron type.

These features suggest that the projection from the VNTB exerts an unknown, strong influence on the function of the DCoN.

TEMPORAL BINDING OF MULTISENSORY STEADY-STATE EVOKED RESPONSES

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Most events occurring in the external world concomitantly activate afferents from different sensory modalities. Building coherent representations of the environment requires integrating multisensory inputs. Some conditions such as autism spectrum disorder, schizophrenia, Parkinson's or Alzheimer disease exhibit sensory processing impairments and therefore, the perceptual experience of the world is altered.

Here we investigated the cortical and subcortical responses elicited by separated and concurrent auditory and visual inputs through steady state evoked potentials (SSEPs). Seven mice were implanted with electrodes in medial prefrontal cortex (mPFC), thalamic reticular nucleus (TRN) primary auditory (A1) and primary visual (V1) cortex and responses to only visual, only auditory and auditory-visual SSEPs at 10, 20, 40 and 80 Hz were obtained and analyzed through coherence and event-related estimates.

Across brain areas, concurrent and separated presentation of auditory and visual stimuli elicited evoked responses with different activation patterns across structures. Interestingly, the temporally congruent audiovisual condition elicited markedly enhanced auditory and visual SSEPs, that permeated to non-primary sensory areas (when compared to the only-visual or only-auditory SSEPs)

Taken together, these observations indicate that temporal congruency of audiovisual stimuli enhances the processing of multisensory inputs at sensory-specific stages of cortical processing, possibly through a dynamic binding of cortical and subcortical structures.

THE AMYGDALO-HIPPOCAMPAL PATHWAY: THE FIRST STEP TO THE WHO COMPONENT OF THE EPISODIC MEMORY

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One of the most complex issues in episodic memory is how the different types of information that contribute to an event are integrated. Within the hierarchy underlying memory formation, spatial (where) and temporal (when) memory must be integrated with social (who) memory to compose a complete episodic memory. Anatomical data suggest that vomeronasal information (encoding individual identity in mice) reaches the hippocampus indirectly via the lateral entorhinal cortex.

Since the primary vomeronasal cortex is the posteromedial cortical amygdala (PMCo), we hypothesize that it constitutes the first step of a putative pathway for integrating pheromonal signals into hippocampal-dependent memory in rodents. To test this hypothesis, simultaneous local field potentials (LFPs) in the PMCo and CA1 were recorded in awake head-fixed mice while exploring a virtual environment associated with olfactory and vomeronasal stimuli. The system consisted of testing corridors in which mice can navigate using a running wheel in a one-dimensional space, forcing exploration of experimentally controlled stimuli.

Active exploration was highly correlated with theta activity (5-12 Hz), not only in the hippocampal LFP but also in the PMCo. Consequently, high-coherence oscillations of both signals evidenced theta epochs, indicating plausible oscillatory theta co-activity. Furthermore, causal epochs could be detected during these active periods. A salient feature of this theta profile was its co-occurrence with gamma waves (30-200 Hz), a cross-frequency interaction related with phase encoding related to hippocampal formation.

We focused our analysis on the different spectral signatures profiled by gamma waves embedded in theta cycles. A similar set of five theta-nested spectral components was present in both PMCo and CA1 when vomeronasal signals were present, again suggesting coupled neural processing in the two areas, related to the incorporation of conspecific information into hippocampal memory

THE OLFACTORY PEDUNCLE IN HUMAN AND NONHUMAN PRIMATES

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The human olfactory system has historically been considered a system primarily involved in odor recognition. However, during the last decade, olfactory dysfunction has been studied as a preclinical and prodromic symptom related to Parkinson's and Alzheimer's diseases. The olfactory system appears to act as direct pathway to other cortical olfactory regions of the brain for some pathogens or prion-like diseases, and the anatomy of sensory neurons, directly exposed to the environment in the nasal cavity, allows this. In addition, most mammals generate new neurons during adulthood that are incorporated into a pre-existing olfactory circuit, presumably to keep their functionality intact. However, in adult humans this process has not been clearly elucidated and information regarding cytoarchitecture and cellular composition of different olfactory structures in the olfactory peduncle are still scarce.

In this work, we studied 28 olfactory nerves from humans aged 19-93 years and 5 cases from young adult primates (Macaca fascicularis). The olfactory tract of both species was dissected into the main olfactory bulb and anterior, medial and posterior regions of the olfactory peduncle. Then, morphology and cytoarchitecture were studied and compared. For this purpose, coronal section micrography atlas of the human olfactory tract has been developed. We observed an anatomic variation along the different portions and different cell distribution layers. Electron microscopy, immunohistochemical, and confocal techniques were performed to confirm the distribution of cell populations, such as glia, mature and young putative neurons and undifferentiated cells in the olfactory peduncle of both species. We also evaluated the density and distribution of blood vessels and corpora amylacea. Furthermore, in M. fascicularis, a rostral migratory stream (RMS) was identified in all cases along the peduncle and migrating neuroblast cell populations were stereologically quantified. These data aim to shed light on the differences in these olfactory structures between both species, the relationship between adjacent structures and the putative effect of aging on the human adult neurogenic process.

KEYWORDS: Olfactory system, adult neurogenesis, primates, humans, olfactory peduncle, doublecortin,

THE POWER SPECTRUM DETERMINES SUBTHALAMIC BETA BURSTS DYNAMICS IN PARKINSON'S DISEASE

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Excessive beta oscillations (13-35 Hz) in the basal ganglia are considered a hallmark of Parkinson's disease (PD). The intermittent dynamic of pathological beta oscillations has been recently characterized in terms of beta bursts, expanding the unitary perspective of beta power in the frequency domain to a dualistic view of burst amplitude and duration in the time domain. However, the possibility that at rest beta burst amplitude and duration may simply reflect the stochastic fluctuation of a noisy beta oscillator, defined by the power spectrum, has not been fully tested. To formally address this issue, here we recorded local field potentials (LFPs) from the subthalamic nucleus (STN) of PD patients at rest OFF and ON medication. We modeled these LFPs as noisy oscillatory processes with two signal modeling methodologies based on the power spectrum, using autoregressive methods and the autocorrelation function. We found that the dynamics of beta bursts – i.e. their average amplitude and duration – did not differ between recorded and simulated beta oscillations. Furthermore, beta burst amplitude correlated with the power of the beta peak in the power spectrum, whereas beta burst duration correlated with the sharpness of the beta peak. We thus clarified that beta burst dynamics in the time domain have a direct correspondence in the frequency domain. Overall, our results suggest that the shape of the power spectrum largely determines the dynamics of beta bursts.

THE PROJECTION FROM THE INFERIOR COLLICULUS TO THE POSTERIOR INTRALAMINAR NUCLEUS OF THE THALAMUS STUDIED WITH ANTEROGRADE TRACERS

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The relationship between acoustic stimuli and emotions is very intriguing. The posterior intralaminar nucleus of the thalamus (PIN) acts as an interface between the auditory system and the limbic system: it receives information from neurons of the most superficial layer of the inferior colliculus (IC) and innervates the amygdaloid complex. Despite this pivotal role, the projection from the IC to the PIN remains to be studied in detail.

To characterize the projection from the IC to the PIN, we have injected the anterograde tracers Phaseolus vulgaris-leucoagglutinin (PHA-L), biotinylated dextran amine (BDA) and tetramethylrhodamine-conjugated dextran (D-TMR) into the superficial layers of the external cortex of the rat IC (ICx) and analyzed the trajectory, morphology and distribution of the axons labeled in the PIN. To delimit the PIN, we followed neurochemical criteria, because this nucleus is immunonegative for parvalbumin and immunopositive for calretinin and calbindin (Márquez-Legorreta et al., 2016, Front Neuroanat 10:82).

All three tracers provided congruent results, which showed that ICx axons run caudorostrally in the brachium of the IC and innervate diffusely the entire ipsilateral PIN identified neurochemically. Within the PIN, ICx terminal axons are thin and scarcely ramified, and bear small, homogeneous en passant and terminal synaptic boutons. Many labeled ICx axons extend rostromedially, past the PIN, to innervate the ipsilateral subparafascicular nucleus of the thalamus. Given the near absence of inhibitory neurons in the most superficial layer of the IC, the ICx-to-PIN projection is most likely excitatory.

The present results refine the delimitation of the PIN and will serve as a basis for future morphological and functional studies.

THYROID HORMONE TRANSPORTERS MCT8 AND OATP1C1 ARE EXPRESSED IN NEURONS IN THE HUMAN AND MONKEY BASAL GANGLIA AND MOTOR THALAMUS.

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Thyroid hormone (TH) is essential for proper brain development, function, and metabolism. Monocarboxylate transporter 8 (MCT8) and organic anion transporting polypeptide 1C1 (OATP1C1) are highly specific TH transporters that facilitate TH to cross the plasma membrane to perform its bioactivity. Mutations of MCT8 and OATP1C1 affect inevitably the motor system in human, and so far it is unknown the nature of the neural cells in which these transporters are expressed in the adult monkey and human basal ganglia and motor related structures.
We performed immunohistochemistry, immunohistochemistry combined with NADPH-diaphorase histochemistry or double immunofluorescence of 30-50µm floating frozen brain sections from three cynomolgus monkeys and four adult humans. The immunolabeling results were plotted in distribution maps by means of the Neurolucida system (MicroBrightField Biosciences).
MCT8 and OATP1C1 are expressed in neuronal subtypes with different morphologies in the neostriatum both in human and monkey. MCT8 is expressed in medium-sized aspiny non-NOS expressing GABAergic interneurons. OATP1C1 and MCT8 are expressed in neurons in the globus pallidus and the motor thalamus. OATP1C1 and MCT8 distribution is similar in the human and monkey tissues, although their protein expression is stronger in the monkey brain. MCT8 is less abundant than OATP1C1 in general. In addition, we have noticed that both transporters are strongly expressed in substantia nigra in the monkey and in nucleus basalis of Meynert in human and monkey. MCT8 is expressed extensively in the endothelial cells of the various size vessels and capillaries in the basal ganglia and thalamus, while OATP1C1 is occasionally observed.
Our study provides the first evidence for the abundance of TH transporters MCT8 and OATP1C1 in the basal ganglia and thalamic neurons in the adult human and non-human primates, which suggests their important role in the motor system functionality.

USING 1D-CONVOLUTIONAL NEURAL NETWORKS TO DETECT AND INTERPRET SHARP-WAVE RIPPLES

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Sharp-wave ripples (SWR) are high frequency events recorded in the local field potential (LFP) of the hippocampus of rodents and humans. During SWR, the sequential firing of ensembles of neurons act to reactivate memory traces of previously encoded experience. SWR-related interventions can influence hippocampal-dependent cognitive function, making their real-time detection crucial to understand underlying mechanisms. However, existing SWR identification tools mostly rely on using spectral methods, which remain suboptimal.

Here, we introduce a 1D convolutional neural network (CNN) operating over high-density LFP recordings to detect hippocampal SWR both offline and online. The adapted architecture included seven convolutional deep layers composed of different filters to process 8-channel LFP inputs in increasing hierarchical complexity and one output layer delivering the probability of an occurring SWR. We report offline performance on several types of recordings (e.g. high-density probes, linear arrays, ultradense Neuropixels) as well as on open databases that were not used for training. By saturating the operation of different filters, we examine and interpret their optimal behavior associated to the ground truth versus a random selection. We then use dimensionality reduction techniques to visualize how the network evolve across learning. Finally, we show how by building a plug-in for a widely used open system such Open Ephys, our method detects SWRs in real time. We conclude with discussion on how this approach can be used as a discovery tool for better understanding the dynamics of SWR.

USING HIPPOCAMPOME.ORG TO INVESTIGATE HIPPOCAMPAL CIRCUIT DYNAMICS

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Understanding brain operation demands linking basic behavioural traits to cell-type specific dynamics of different brain-wide subcircuits. This requires a system to classify the basic operational modes of neurons and circuits. Single-cell phenotyping of firing behaviour during ongoing oscillations in vivo has provided a large body of evidence on hippocampal function, but data are dispersed and diverse. Here, we mined literature and obtained new data to update information on oscillatory dynamics of over 100 hippocampal neuronal types defined in Hippocampome.org. We integrate all current knowledge about the morphology, biophysics, genetic identity, connectivity, and firing patterns of a wealth of GABAergic and glutamatergic neurons to provide a comprehensive single-cell map of the hippocampal region. Finally, we show how using Hippocampome.org can provide knowledge-based classification of hippocampal neurons recorded with extracellular methods as well as provide additional resources for biologically realistic computational modelling to ease applications in artificial intelligence.

USING UNIFORM MANIFOLD APPROXIMATION AND PROJECTION (UMAP) FOR UNSUPERVISED SORTING OF SHARP-WAVE RIPPLES

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Sharp-wave ripples (SWR) are high frequency hippocampal events, which presumably play different cognitive roles in memory consolidation and planning. Understanding how SWR waveform variability relates to the underlying microcircuits remains elusive but is essential to dissect cognitive function. Here, we use topological data analysis to estimate the intrinsic structure of a wealth of SWRs recorded from head-fixed mice in vivo. We apply dimensionality reduction and visualization methods, such as Uniform Manifold Approximation and Projection (UMAP) to facilitate discovery of different SWR features. First, we show that a low number of intrinsic dimensions can explain waveform variability using a set of methods (Maximum Likelihood; Isomap; DanCo; Expected Simplex Skewness; PCA) tested against a ground-truth. Next, we apply UMAP to reduce dimensionality of SWR events and to visualize how different potential features (e.g. frequency, amplitude, slopes, etc…) accounted for waveform variability. Using cluster measures, we evaluate the distribution of these different features over the low dimensional manifold. We find different contribution of frequency, amplitude, slope and spectral entropy to the global variance of SWR, and confirmed some of these trends in synthetic datasets. Moreover, by projecting physiologically relevant measures over the UMAP manifold, we identify potential mechanisms associated to the expression of different features. Our study shows how topological analysis can be applied for unsupervised sorting of SWR events.

Topic 4

Cognitive and Behavioral Neuroscience

Posters

A NOVEL VISUOSPATIAL WORKING MEMORY TASK IN MICE

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Working memory (WM), defined as the ability to maintain and process information in our brain for a short time, is a cornerstone of cognition. It is involved in many cognitive processes and is impaired in multiple mental disorders. Despite decades of study, the neural circuit mechanisms underlying this key brain function remain debated.

Aiming to better understand limits of WM, we have developed a visuospatial WM task in mice inspired by classical work carried out in primates. Subjects are trained to look and memorize the location of a visual stimulus displayed in a touchscreen. After a variable delay period, mice had to report the remembered position by touching the screen.

In this task, animals made two types of errors: non-memory dependent, present in visually-guided trials; and memory dependent, they increase gradually with delay length. Part of these memory dependent errors are caused by idiosyncratic biases that increase as a function of delay. We hypothesize that idiosyncratic biases are induced by discrete attractor dynamics pulling memories towards a few stable representations in mnemonic space.

We also analyzed the repeating bias, defined as the excess of probability of making a particular response rt=X after the previous choice rt-1 is also X. We fit a linear mixed model to the repeating bias data and we observed a significant increase with delay length ($p=0.004$) suggesting that this type of choice bias could be a bias in WM caused by previous stored locations.

This novel task presents an opportunity to investigate visuospatial WM in mice, an animal model suited for circuit level electrophysiology, genetic and pharmacologic manipulations and models of mental disorders associated with a WM malfunction such as schizophrenia.

A ROLE OF 14-3-3ζ IN TRANSFORMATION OF LABILE SHORT-TERM OBJECT RECOGNITION MEMORY INTO STABLE LONG-TERM MEMORY

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The consolidation of new memories into long lasting memories is multistage process characterized by distinct temporal dynamics. However, our understanding on the initial stage of transformation of labile memory of recent experience into stable memory remains elusive. Here, with the use of rats and mice overexpressing a memory enhancer called regulator of G protein signaling 14 of 414 amino acids (RGS14(414)) as a tool, we will show that the expression of RGS14(414) in male rats perirhinal cortex (PRh), which is a brain area crucial for object recognition memory (ORM), is able to enhance ORM to the extent that it causes the conversion of labile short-term ORM (ST-ORM) expected to last for 40 min into stable long-term ORM (LT-ORM) traceable after a delay of 24 h and that the temporal window of 40 to 60 min after object exposure not only is key for this transformation but also is the time frame when a surge in 14-3-3ζ protein is observed. A knockdown of 14-3-3ζ gene abrogates both the increase in 14-3-3ζ protein and the formation of LT-ORM. Furthermore, this 14-3-3ζ upregulation increases BDNF levels in the time frame of 60 min and 24 h and 14-3-3ζ knockdown decreases the BDNF levels, and a deletion of BDNF gene produces loss in mice ability to form LT-ORM. Thus, within 60 min of object exposure, 14-3-3ζ facilitates the conversion of labile ORM into stable ORM, whereas beyond the 60 min, it mediates the consolidation of the stable memory into long lasting ORM by regulating BDNF signaling.

A SCALABLE AND PHYSICAL APPROACH TO THE STUDY OF SPATIAL NAVIGATION AND ITS COMPONENTS

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Spatial navigation is a very complex, multi-process function. In humans it is often studied using computer programs (via virtual reality or through a regular computer screen). Our goal was twofold: first, to create an apparatus to study spatial navigation in a more lifelike, less abstract approach, where subjects navigate through a real space. Second, to use this device to disaggregate and evaluate the components of spatial navigation through different tests.
We built a cylindrical structure (3 meters in diameter and height) with 5 computers connected to 4 led matrixes and a motorized laser. Everything is wirelessly controlled via WiFi. Two protocols were designed to specifically evaluate the different reference frames used during spatial navigation (i.e., egocentric and allocentric). The allocentric evaluation protocol consists of finding a goal's position (signaled by shining a laser on the floor) relative to spatial cues displayed through the led matrixes. The egocentric evaluation protocol is a scalable maze-like test simulating a grid of successive structures. This is accomplished by making the subject to enter and exit the structure through specific places to advance to the "next" position. Different positions are achieved by showing consequent combinations of images and cues on the led matrixes. Both protocols have a computerized version to (i) achieve a more reliable evaluation of the allocentric component, (ii) to compare the strategy and score differences between physical and virtual protocols, and (iii) to make a non-priming longitudinal evaluation.
We are currently validating the difficulty of the protocols for different scenarios, as we are planning to study how several pathologies (Alzheimer's disease, autism spectrum disorders, Parkinson's disease…) and variables (physical exercise, transcranial direct current stimulation, transcranial magnetic stimulation, age…) affect performance and strategies in each test.

A STUDY OF THE PAIN PATHWAYS IN THE CONTEXT OF FEAR MEMORIES

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Pain is a very effective teacher. Memories that derive from single painful events (like fear conditioning or taste aversion protocolos) usually show a remarkable strength: they are long-lasting, difficult to extinguish and have marked effects on behaviour. However, not all pains matter the same: the strength of the memory formed by a painful event directly depends on the intensity of the aversive stimulus experienced. This direct relation has been used to examine how the nervous system differentially encodes memories of weak and strong aversive stimuli. Most studies in this specific topic are focused in supraspinal structures that receive direct or indirect inputs from nociceptive ascending pathways. How spinal and ganglionic pain circuits contribute to the formation of fear memories of different strength has been scarcely studied, although a considerable complexity exists in these bottom levels. In the present work, we examine how fear conditioning with weak (0.3mA), middle (0.5mA) and strong (0.9mA) nocive electric stimuli differentially recruits ganglionic and spinal populations. The goal is to better understand how each learning experience is processed by these bottom levels of the nervous system and served to supraspinal structures to elaborate fear memories of different strength.

AGING EFFECTS ON EMOTIONAL REGULATION ARE SUPPORTED BY DIFFERENTIAL NEURAL NETWORKS AS PREDICTED BY MACHINE LEARNING PARADIGMS

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Aging is one of the primary health concerns in the entire world. Healthy seniors experience a cognitive decline that affects their daily functioning, yet their ability to process emotional information seems to be well-preserved. Aging modulations on this cognitive-emotional interrelation as well as the underlying neural networks remain unclear. Hence, we aimed to examine the neural underpinnings of joint emotional and memory processing in young and older individuals with electroencephalography (EEG). To this end, 34 young and 38 older individuals performed an memory task with emotional content while they were recorded with a 128-channel EEG system. Specifically, they performed an emotional recognition task with positive, negative and neutral pictures. Behavioural data was analysed under the frame of the Signal Detection Theory. We employed EEG machine learning paradigms to gain insight into the neural data. Specifically, we used linear (logistic regression) and nonlinear (multi-layer perceptrons) automatic models in order to classify subjects by age, just considering the neural information collected by EEG. Behavioural results reflect that older adults exhibit a reduced recognition ability for positive stimuli compared to young adults, suggesting that positive stimuli may generate higher interference than negative or neutral stimuli in emotional recognition with age. EEG results indicate that neural information alone can successfully classify young and older participants with high accuracy. In this regard, we are currently running brain-behavioural correlations that may help us fine-tune the interpretation of these results.

Key words: aging, cognitive-emotional interrelation, emotional recognition task, EEG, machine learning paradigms

ANALYSIS OF HIPPOCAMPAL PARTICIPATION IN SOCIAL INTERACTIONS IN A GENETIC MODEL OF AUTISTIC SPECTRUM DISORDER

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A large number of neurodevelopmental disorders associated with deficiencies in social interaction, language difficulties and repetitive behaviours are grouped under the name of autism spectrum disorders (ASD). Although the genetic causes of ASD are complex, one of the genes that have been associated with ASD is SOX5 (# 616803, LAMB-SHAFFER SYNDROME). Sox5 encodes a transcription factor with important functions in the control of neurogenesis (work in our laboratory) and in the correct specification of projection neurons of the cerebral cortex. However, alterations in regions of the brain other than the cortex such as the hippocampus have not been analysed yet.
Children with ASD present hippocampal morphology alterations associated with deficits in neuropsychological tests. Moreover, it has been described that the CA2 region of the hippocampus is fundamental in social behaviour in mice, a region where we have previously shown that Sox5 is expressed. Using conditional Sox5 mutant mice specific for the CA2 region (Amigo2-cre/Sox5fl/fl; Sox5Amigo2), we have determined that robust lack of Sox5 expression causes PCP4 level decrease in more than half of the pyramidal neurons in CA2. Using an extensive battery of mouse behavioural assays we have determined that Sox5Amigo2 mutant mice: i) exhibit normal basic reflexes, weight, and locomotion abilities; ii) males present a lower level of marbles burying activity and slight anxiety in open field test but not in elevated plus maze test; iii) exhibit a good performance in Morris water maze test; iv) present normal social preference and v) both males and females show an abnormal preference for a familiar animal over a stranger in social memory tests. Thus, we propose that Sox5Amigo2 mice could provide a new model of ASD, based on cellular and functional alterations of the CA2 region of the hippocampus that serves to understand the hippocampal component in the pathophysiology of ASD and for the testing of new therapeutic strategies.

ASSESSMENT OF SOCIAL BEHAVIORS IN C57BL/6 MICE EXPOSED TO CHLORPYRIFOS: AN ASSOCIATION WITH AUTISTIC-LIKE BEHAVIORS

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The balance between glutamate and GABA is essential for proper brain development and functioning. An imbalance between these two neurotransmitters is hypothesized to be associated with Autism spectrum disorder (ASD) symptoms. Moreover, a massive use of pesticides, in particular chlorpyrifos (CPF), has been shown to cause adverse effects, especially when the exposure takes place during development. In fact, recent literature has associated its exposure to neurodevelopment disorders including ASD. In this study, we aimed to assess social autistic-like behaviors and identify the association between the disorder and the time at CPF exposure in both sexes. For these reasons we assessed two developmental periods: i. Prenatal exposure experiment, where C57BL/6 pregnant mice were exposed to low doses of CPF through the diet, between gestational day (GD) 12 and 18. In this experiment, a positive control for autism (C57BL/6 mice exposed to valproic acid (VPA) on GD 12 and 13) was included; ii. Postnatal exposure experiment, where mice were exposed to the vehicle (corn oil) or low doses of CPF by oral gavage (from postnatal day (PND) 10 to 15). In both experiments, social behavior was evaluated during the adolescence by means of The Crawley three-chamber test. Then, at 45 days of age mice were sacrificed and brain samples collected to study gene expressions (related to glutamate and GABA signaling) in hippocampus samples. Results showed a preference for the social stimulus in all groups, while social recognition was altered in all treated mice, especially in males. Our findings suggest that males are more prone to CPF exposure, providing a plausible explanation for the sex bias in autism.

BEHAVIORAL MECHANISMS UNDERLYING VISUALLY-GUIDED CONTROL OF STEERING

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Goal-directed behaviour involves navigating in an environment in order to fulfill a particular objective. In order to attain their goal, animals, and agents in general, need to keep track of many variables critical in the system, such as location and heading direction for spatial navigation. In particular, processing of optic flow for visually guided navigation is crucial for the estimation of heading direction, taking part in a perception-action loop where heading informs the control of the observer's movement, which in turn modifies the pattern of optic flow at the next instant of time. Whereas human behavioral studies have extensively examined how optic flow contributes to goal-directed navigation, virtually nothing is known about the neural processing of optic flow that guides navigation. In addition, previous studies that have measured neural responses to optic flow during behavior have used forced-choice psychophysical tasks that are open-loop. In order to address these gaps, we introduce a simple navigation task in which a monkey needs to steer a joystick to align themselves to a cued target, which is not visible during steering, in a virtual environment that only provides noisy optic flow feedback that is a direct consequence of the monkey's actions. We develop a minimal and interpretable stochastic optimal control model that captures important features in the data such as urgency to reach the goal and reactive, closed-loop control in the presence of external perturbations. We also show, consistent with previous findings, that multiplicative control noise plays an important role in the reproduction of the monkey's control behavior. Identifying the dynamical variables that govern steering through our model is an important first step to study in future work how control-related signals might be used and represented in the brain and how the neural processing of optic flow guides realistic, closed-loop, navigation.

BEHAVIOURAL CHARACTERIZATION OF MOTOR AND COGNITIVE EVERYDAY LIFE HABITS IN PARKINSON'S DISEASE

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Humans can simultaneously and easily shift between automatic/habitual to voluntary/goal-directed modes of behavioural control. Habits (stimulus-response) allows us to perform well-practiced tasks with minimal or no conscious effort. In Parkinson disease (PD), there is a differential loss of dopamine (DA) along the caudal sensorimotor putamen initially transferring to more rostral regions as the disease progresses. The sensoriomotor putamen is primarily implicated in habitual control of behaviour therefore possibly explaining some motor deficits in PD such as arm swinging, walking and writing.

Our aim was to investigate with a more ecological approach whether early dopaminergic cell loss in PD (a) alters everyday life motor habits and (b) if as well cognitive everyday life habits are impaired in PD compared to controls.

We measured habitual behaviour in 36 early PD patients and 44 HC during performance of a natural habitual motor (handwriting, one of the most well-learned and automatic tasks) and cognitive tasks (PD = 14; HC = 14) (Go/noGo Associations Tasks GNAT on implicit bias). Tasks were developed to differentiate between habitual and goal directed components. In the writing task, we collected data from the most automatic (e.g. signature, Spanish) to more goal-directed conditions (e.g. Polish, Greek). In the GNAT, we used four kind of stimuli to create four possible Go conditions: two congruent and two incongruent associated to familiar and unfamiliar associations.

In the natural habitual motor task (handwriting), PD patients showed reduced automatism in the execution of habitual conditions (Spanish and signature writing) compared to controls. However, in the cognitive habitual task, results reveal similar habitual responses in both groups, showing faster responses for congruent conditions than incongruent ones.

Our results suggest larger differences in the motor domain of everyday life habits whereas the cognitive one remains unaffected in early PD, indicative of specific deficient use of motor habits.

BRIEF ASSESSMENT OF SOCIAL COGNITION IN "ATAXIA DA COSTA DA MORTE"

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SCA36 is a late-onset spinocerebellar ataxia affecting families from Costa da Morte (Galicia, Spain). It presents manifestatations consistent with the presence of cerebellar cognitive-affective syndrome. However, social cognition has not been evaluated in these patients. We used the “Reading Mind in the Eyes” Test in 21 patients of different stages of disease, matched in age, sex and educational level with 21 controls. Emotional processing of human faces, specifically those with negative and neutral valence, seems to be altered in preataxic stage, before first motor symptoms. Nevertheless, correct answers to negative stimuli continue decreasing as the disease progresses and correlate with the motor impairment measured with the SARA scale. This result supports the hypothesis that the change in the recognition of social emotions could be specifically related to an alteration in the cortico-cerebellar circuit.

CEREBELLAR INTERPOSITUS NUCLEUS ACTIVITIES UNDERLYING CLASSICAL EYEBLINK CONDITIONING IN RABBITS

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It is generally accepted that learning is a functional state of the brain that can only be fully understood during the very moment of its acquisition, storage or retrieval. We believe that learning and memory are distributed functional states (rather than localized, transient processes) requiring the participation of numerous neural structures and their proper and timed activation. The generation of new motor abilities is an essential component of the learning process. In this regard, we studied cerebellar interpositus nucleus (INTn) functioning by recording its unitary activity in behaving rabbits during an associative learning task (classical eyeblink conditioning). The reason is that the INTn has been related to the generation of the conditioned eyeblinks but is still unclear its specific role compared to other brain motor areas. We recorded INTn neurons in chronically implanted rabbits during classical eyeblink conditioning using a delay paradigm. We identified INTn neurons by their antidromic activation from the contralateral red nucleus and synaptic activation from the facial motor cortex. We have compared the activity of INTn neurons during classical eyeblink conditioning with those already collected in the same species from the contralateral somatosensory, motor and prefrontal cortices, as well as the contralateral red nucleus, and the ipsilateral facial motor nucleus. In this way, we will show for the very first time a complete picture of the firing activities of neurons located in the main brain motor areas during the performance of the same associative learning task.

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CHARACTERISTICS OF THE SPONTANEOUS BLINKING DEPENDING ON THE ATTENTIONAL CONDITIONS AND THE SENSORY NERVE ACTIVITY FROM THE OCULAR SURFACE

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Control of spontaneous blinking lies in a brainstem center that determines blinking pattern, which is influenced by the attentional state. Contrarily, reflex blink is produced in response to the increased activity of the sensory neurons innervating the eye surface and its annexes. We hypothesize that spontaneous blinking is also driven by sensory input. The aim of this work was to establish whether the pattern and characteristics of spontaneous blinking is modified or not by the activation or inhibition of ocular sensory nerves under different attentional conditions.

Orbicularis oculi muscle electrical activity (OOEmg) was recorded in 12 young healthy volunteers (7 women/5 men) using surface electrodes in three conditions (5min each): at rest (basal), and performing visual and non-visual tasks. Spontaneous blinking frequency (BF), inter-blink interval (IBI), and OOEmg amplitude and duration were analyzed in the three conditions. In a different day, OOemg recording protocol was repeated after receiving topically a drop of either perfluorohexyloctane (F6H8; that decrease the ocular surface temperature) or the local anaesthetic tetracaine/oxybuprocaine (1/4 mg/ml).

Compared with basal values, BF was lower during visual tasks and higher during non-visual tasks. During any task OOemg signal amplitude was larger and OOemg duration was shorter than at rest. BF increased significantly and IBIs were more regular after F6H8 treatment, in all three conditions, also increasing the amplitude and decreasing the duration of OOemg signals. Treatment with local anaesthetic did not significantly modify the parameters studied.

Present results confirm that the central blink generator controlling spontaneous blinking is regulated by higher brain centers, which reduce blink frequency and duration during visual attention tasks. Data also suggest that both the frequency and pattern of spontaneous blink is highly influenced by the increase of the sensory input from cold thermoreceptor endings innervating the ocular surface.

COACHING AND HUMAN BRAIN CREATIVITY MECHANISMS

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ABSTRACT

The coaching profession has been in a situation of both methodological and identity uncertainty for a long time, which causes a continuous misunderstanding regarding its practice and essence. The purpose of this study was, in the first place, to demonstrate, from the experimental point of view, how coaching of non-directive essence, with its competencies framework and its essence of non-transference of knowledge or judgment, is capable of enhancing the brain's creativity mechanisms of human beings. Secondly, it was necessary to provide psycophysiological evidence of the effects of non-directive coaching (NDC); and, thirdly, to frame coaching also within a specific field, such as creativity. This also allowed to identify coaching as a profession that helps to cover the current gap regarding the growing decrease of creativity in the human being that has been taking place for two decades; and, on the other hand, it contributed to react in an effective way to the challenges of this uncertain and complex world. For this purpose, an experimental methodology was developed, where the response of the subjects to three conditions (rumiative, directive and non-directive) was compared through electroencephalographic (EEG) measurement during problem solving and achievement of goals. For this, 16 subjects (8 men and 8 women) participated. The study allowed the detection of a series of differentiated and specific EEG patterns in the third condition (non-directive) related to the results found in previous studies on creative insight. Results showed significant changes in alpha and theta frequencies in the right temporal region, and alpha, theta and gamma in the right parietal region, compared to other experimental conditions. Thus, the application of the methodological framework of the NDC was related, in a specific way, to the creativity and the development of human knowledge.
Keywords: non-directive coaching, electroencephalography, insight, creativity.

COGNITIVE EFFECTS OF PHYSICAL EXERCISE ARE INHERITED BY THE SECOND GENERATION

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Physical exercise has a positive impact on brain and cognition. Previous results from our laboratory confirmed that these positive effects are not only present in animals that went through a forced protocol of exercise of moderated intensity (F0), but also in their sedentary male offspring (F1). Both exercised fathers and their litters had a cognitive advantage in behavioural tests that evaluate recognition memory and pattern separation abilities, and had increased levels of adult hippocampal neurogenesis. These results were replicated in three different experimental designs and the transmission via paternal lineage was confirmed. In the present work we tested if these effects are also inherited by a second generation (F2). Results indicate that the cognitive advantage reaches the F2, but are not accompanied by increased levels of adult hippocampal neurogenesis.

COGNITIVE NEURODYNAMICS DURING AUDIOVISUAL CUTS IN MEDIA PROFESSIONALS

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Professionalization requires long-time training. In the case of media professionals, they watch screens steadily over time (taking related decisions with high level of attention). Previous results from our group suggest that there is a media professionalization effect in visual perception of media contents.

We recorded electroencephalographic signals from 36 participants (18 media professionals and 18 non-media professionals) while they were watching audiovisual contents. We compared their brain activity and connectivity after the cuts. We analyzed event-related potentials (ERPs) for periods of 1.5 s (from 0.5 to 1 s). We approached brain connectivity from functional (phase-locking value, PLV) and effective (Granger causality, GC) connectivity analysis.

We identified substantial differences in the spontaneous blink rate (SBR) related to media professionalization. In media professionals, cuts have a greater impact with a decrease of SBR [$t(17) = -2.99$, $p = 0.008$, paired t-test], while cuts do not have such impact in non-media professionals [$t(17) = -1.14$, $p = 0.269$, paired t-test]. We found an effect of professionalization and scalp area in ERPs during the viewing of audiovisual cuts [$F(2,306) = 4.822$, $p = 0.009$]. Although we did not find statistical differences related to media professionalization in alpha band, we observed differences in functional connectivity (PLV) in all studied bands (theta, alpha, beta, low gamma) after the cut. We found a more dispersed GC index in non-media professionals, while media professionals' GC connectivity was much more concise since it was mostly concentrated in visual cortex, somatomotor, and frontal areas.

Cuts evoke an artificial interruption of the visual content in videos and movies. However, their impact varies depending on the media professionalization of viewers. Apparently, cuts start a similar activation of basic brain processing of the new visual information presented, but how that visual content is managed by the two groups differs afterwards.

CONDITIONAL DELETION OF THE CNTNAP2 GENE IN MICE: A PHENOTYPIC STUDY

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One of the most accepted theories about the pathogenesis of Autism Spectrum Disorder (ASD) is the alteration in the proportion of excitatory projection neurons (PNs) and inhibitory cortical neurons (cINs). Similar to findings in humans, the mouse model of autism, knockout for the Cntnap2 gene, displays migration abnormalities of PNs and reduced number of cINs and associated alterations in both the excitatory and the inhibitory networks.

Hence, the aim of our work is to decipher how each specific class of neurons contributes to the pathophysiology of ASD. For this, we have generated two conditional mouse models with specific forebrain deletion of Cntnap2 in either cINs or PNs by means of cre-lox technology.

Behavioral characterization of the models involves tests for the core domains of autism, including vocal communication, social behavior, and repetitive behavior, as well as tests for autism-related behaviors such as hyperactivity and sensory reactivity, shown to be altered in the full Cntnap2 KO mouse. Preliminary results in both conditional KO mouse models did not find a significant difference between the conditional mice and their controls in any of the tests.

These results suggest that the absence of Cntnap2 gene exclusively in cINs or in PNs do not seem to be enough to cause the ASD phenotype. Further studies are needed to identify the involvement of other neurons from different networks in the deficits observed in the KO full model.

DEALING WITH MOTHERHOOD: GENE EXPRESSION CHANGES INDUCED BY PREGNANCY AND LACTATION BUT NOT PUP STIMULI IN THE MOUSE MEDIAL AMYGDALA

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During lactation, male-derived chemosensory signals produced by males induce aggression in adult female mice. However, pup-sensitized virgin females, which share pup care with the dams, are not aggressive against male intruders. The genetic mechanisms underlying the switch from attraction to aggression are still unknown. In this work, we investigate whether gene expression in the medial amygdala, a key nucleus in the integration of chemosensory and hormonal information, differs between aggressive lactating females and non-aggressive pup-sensitized virgin females. To do so, we performed a RNA-sequencing study. Our data reveals that 197 genes are upregulated in dams, among which we find genes encoding hormones such as prolactin, growth hormone, or follicle-stimulating hormone, neuropeptides such as galanin, oxytocin and proopiomelanocortin, and genes related to catecholaminergic and cholinergic neurotransmission. By contrast, 99 genes are downregulated in dams, including those encoding inhibins and transcription factors of the Fos and early growth response families. The gene set analysis revealed numerous Gene Ontology functional groups with higher expression in dams than in pup-sensitized virgin females, including the group of genes related with the regulation of the Jak/Stat cascade and the negative regulation of the ERK1 and ERK2 cascade. Interestingly, we found several genes encoding olfactory and vomeronasal receptors expressed in the medial amygdala, although no differences in expression were observed between dams and virgins. Our results reveal many gene expression changes in the medial amygdala, previously unknown, which may underlie the behavioural changes observed in lactating females in relation to conspecific males.

KEY WORDS: vomeronasal amygdala, RNA-Sequencing, aggression, prolactin, transcriptome, maternal behaviours, females.

DREAM PROTEIN INHIBITION AS POTENTIAL TREATMENT AGAINST METABOLIC SYNDROME AND ITS ASSOCIATED NEUROLOGIC SIGNS

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High fat diet (HFD) chronic intake induces metabolic syndrome in mice, characterized by a body weight increase and insulin resistance (IR). Obesity is a risk factor in the development of neurodegenerative diseases, neuropsychiatric disorders and is associated with cognitive decline. DREAM/kchip3/calsenilin (DREAM) is a multifunctional protein that belong to neuronal Ca2+ sensors family. Previous studies have demonstrated the relevance of DREAM in nociception and in learning and memory processes. In the present study we characterize the consequences of DREAM, genetic or pharmacologic, inhibition in the metabolic and behavioural alterations caused by HFD intake in mice. Our results showed that chronic pharmacological and genetic DREAM inhibition block the metabolic syndrome development, and both of its neurologic comorbidity symptoms: the anxiety-related behaviour, and the cognition deficiency. Also, pharmacologic DREAM inhibition when metabolic syndrome is established did not affect metabolic parameter but improved metabolic syndrome-related neurologic alterations. Therefore, in this study we demonstrate: DREAM inhibition may be a potential treatment to restore neurological symptoms related with metabolic syndrome; and to block metabolic syndrome induced by HFD intake.

EFFECTS OF CHRONIC CB1 RECEPTOR AGONIST ACEA IN A MOUSE MODEL OF ALZHEIMER'S DISEASE.

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Alzheimer's disease (AD) is characterized by the brain presence of amyloid beta plaques, increased amyloid precursor protein (APP) and neurofibrillary tangles and courses with progressive behavioural alterations including cognitive and socio-affective dysfunctions. Although AD cause a huge impact on patient daily life representing the most common form of dementia, current treatments for AD only provide symptomatic relief. There is the need to better understand the brain mechanisms leading to this devastating disease. In this sense, alterations of the endocannabinoid system (ECS) have been found in animal models and human patients, and its modulation has been proposed as a new therapeutic avenue for the treatment of this disease.

In this study, we combined the APP/PS1 mouse model of AD with a pharmacological intervention at 3 months of age using the CB1 receptor cannabinoid agonist ACEA under a regimen of 21 consecutive days. At 4, 6, 9 and 12 months of age we performed a complete sex- and age-dependent behavioural characterization (cognition, social-related behaviours, depressive and anxiety-like states). In addition, we also performed an age-, sex-, brain-region- and cell-type specific characterization of the ECS and some related mechanisms.

APP/PS1 male mice showed cognitive deficits from 6 months of age that are maintained until 12 months of age. This cognitive impairment was not observed in APP/PS1 female mice. Interestingly, ACEA chronic treatment reversed the cognitive alterations observed in male mice. In addition, we have found sex- and brain region-dependent alterations on different ECS components at asymptomatic stages (3 months of age).

Altogether, this project provides compelling evidence on the importance of sex, age and brain locations when studying a complex brain disorder such as AD. Indeed, our results suggest the presence of gender differences in AD and reaffirm that cannabinoid drugs could be a beneficial therapeutic avenue for this brain disorder.

EVALUATION OF THE NEUROPROTECTIVE ACTIVITY OF THE ETHANOLIC EXTRACT OF MYRCIARIA DUBIA HBK McVAUGH "CAMU CAMU" IN A MURINE MODEL OF PARKINSON'S DISEASE

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Myrciaria dubia "camu camu" is an amazonian plant that is known to have high levels of antioxidants. The objective of this work was to determine if pretreatment with “camu camu” ethanolic extract reduces neurodegeneration and neuroinflammation caused by 6-hydroxydopamine (6-OHDA) in the nigrostriatal pathway. To prepare the extract, “camu camu” fruit flour was macerated in 70% ethanol for 24 hours, followed by drying the supernatant in a double boiler at 37 °C. The dry extract was dissolved in saline solution (NaCl 0.9%) to be administered to Sprague Dawley rats. This dissolved extract was administered orally (daily doses of 100 mg / Kg (ESC100 group) (n = 6) and 300 mg / Kg (ESC300 group) (n = 7)) for 15 days prior to bilateral intracranial injection in the striatum of 6-OHDA. In addition, there were two control groups not treated with the extract: the sham group (rats injected with vehicle solution) (n = 9) and the park group (rats injected with 6-OHDA) (n = 7). 21 days after the injection, the analysis of the hike pattern showed statistically significant differences between the ESC300 group and the park group in three variables. Through the histological immunofluorescence trials, it was found that the ESC300 group has a greater number of dopaminergic neurons in the black substance and greater axonal irrigation in the striatum, compared to the park group. Additionally, through immunohistochemistry, less neuroinflammation was found in the striatum of this latter group. This work concludes that pretreatment with ethanolic extract of “camu camu” at a dose of 300 mg / Kg protects neurons from the nigrostriatal pathway of 6-OHDA and reduces neuroinflammation in Sprague Dawley rats, as a consequence improves motor coordination. This project was supported by FONDECYT, Government of Peru, Contract number 109-2018-FONDECYT-BM-IADT-MU.

EXPLORING NETWORK CODING STRATEGIES THAT COULD BE ESSENTIAL FOR THE PROPER EXECUTION OF BEHAVIORAL SEQUENCES DURING AN OPERANT CONDITIONING TASK

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Humans remember events as they occur in a sequential order in time, such as before or after another event. Interestingly, patterns of behavior occur in a temporal sequence that determines the order in which behaviors unfold in time (i.e., a behavioral sequence). Behavioral sequences (also referred to as sequential learning) are arguably the most prevalent form of human and animal learning and they play a pivotal role in conventional studies of operant conditioning. The goal of this experimental-analytical study was to identify the conditions under which the activity-dependent changes in synaptic strength could be directly affected by an operant conditioning task, which is determined by several levels of expression of the conditioned response, different performances of the animal behaviors and also by different execution degrees of their behavioral sequences. To accomplish this goal, we recorded the field evoked potentials and exhaustively analyzed the synaptic-strength changes taking place at 13 selected sites of the cortical (hippocampal, medial prefrontal cortex) and subcortical (thalamus, amygdala, accumbens septi) circuits during the performance of 8 different behaviors related (to a greater or lesser degree) to the acquisition of an operant conditioning by alert behaving rats and also during the execution of specific temporal sequences of rat behaviors directly related to this type of associative learning task. Collected results allow to verify a large spatio-temporal diversity of synaptic-strength changes during the performance of different rat behaviors (locomotor, appetitive, consumatory, exploratory or stationary). In addition, the findings reported here seem to support a more selective repertory of underlaying rules based on modular integrations of the synaptic-strength changes and on spatio-functional propagation patterns of the evoked field potentials — i.e., two network coding strategies that could be essential for the proper execution of specific temporal sequences of animal behaviors during operant conditioning tasks.

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GALANIN AND NEUROPEPTIDE Y INTERACTIONS LINKED TO NEURONAL PRECURSOR CELLS OF THE DENTATE GYRUS IN THE HIPPOCAMPUS. ROLE IN DEPRESSION AND COGNITIVE IMPAIRMENT

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Galanin (GAL) interacts with Neuropeptide Y Y1 receptors (NPYY1R) in several regions of the central nervous system associated with mood and motivation, through GAL receptor 2 and NPYY1 receptor 1 (GALR2/NPYY1R) heterodimers. The current work is to evaluate GALR2 and NPYY1R interactions concerning newborn cell proliferation in the ventral and dorsal hippocampal Dentate Gyrus.

Rats (n = 6-8 per group) were randomly assigned to the groups. Each group received i.c.v. injections of artificial Cerebro Spinal Fluid (aCSF), GAL or NPYY1R agonist [Leu31,Pro34]NPY alone or in combination and 24 h later rats were subjected to a 5-min swimming session (test). A different set of rats received ip injections of BrdU 50mg/Kg at 2 and 4 hours after icv injections. 24 hours later brains collected for immunostaining to evaluate cell proliferation.

We observed that the icv injection of GAL and NPYY1R agonist significantly enhanced the decrease in the immobility and the increase in the swimming behavior compared with the NPYY1R agonist alone. Furthermore, GALR2 is involved in this GALR/NPYY1R interaction, since the presence of the GALR2 antagonist M871 counteracted all the parameters. In parallel, coadministration of GAL and NPYY1R agonist increased BrdU-labeled cells located in the SGZ compared with aCSF, GAL and the NPYY1R group. Similar results were observed in dorsal hippocampus.

Our results may provide the basis for the development of heterobivalent agonist pharmacophores, targeting GALR2/NPYY1R heteromers, especially in the neuronal precursor cells of the dentate gyrus in the hippocampus for the novel treatment of depression or cognitive impairments.

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GENOMIC BASIS OF DROSOPHILA SOCIAL MEMORY

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It is well known that isolation affects many different behaviors, such as sleep or aggressiveness. However, this raises the question of whether or not there is a "social memory": a memory specifically generated when individuals of the same species interact within a group. Interestingly, social behavior of Drosophila melanogaster has been demonstrated in recent years. In fact, the mushroom body (MB, functionally analogous to mammalian hippocampus) plays a role in such behavior, pointing towards the existence of a memory component.
We show that co-habitation increases the number of activated Kenyon Cells in the mushroom body (MB) when compared to isolated animals. This increase is also reflected in a differential behavior when a single fly is placed in a plate, which depends on its previous experience with other individuals. Both phenotypes rely on the MB and on cAMP levels, as expected if long-term memory formation does play a role. Surprisingly, memory-mutant animals behave closer to housed flies than to isolated ones, suggesting that the basal state of Drosophila is to be social.
In order to identify social memory-related genes we performed Targeted DamID in the MB comparing grouped, isolated and lack-of-memory mutant isolated animals. We show here how the epigenetic and transcriptional landscape of the MB changes in single flies, suggesting the existence of a loneliness-generated memory that affects to the final behavioral output. In summary, our work may shed light on a possible evolutionary-conserved genetic and epigenetic basis of social behavior.

GLOBAL HYPOPERFUSION MODEL OF BILATERAL COMMON CAROTID ARTERY STENOSIS INDUCES HIPPOCAMPUS-DEPENDENT MEMORY DEFICITS

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Chronic cerebral hypoperfusion due to carotid artery stenosis is one of the main causes of vascular cognitive impairment (VCI), the second cause of dementia behind Alzheimer's disease (AD; Iadecola 2013). Bilateral common carotid artery stenosis (BCCAs) in rodents is a well-established model of cerebral hypoperfusion in which most studies have focused on white matter pathology and subsequent cognitive deficit. Several reports have recently highlighted the importance of adult neurogenesis and neuroinflammation in the development of dementia (Hort et al. 2019, Moreno-Jimenez et al. 2019). However, so far, the implication of these processes in the BCCAS model and its relationship with cognitive hippocampal deficits have not been addressed.
To that aim, mice were subjected to global cerebral hypoperfusion by using the BCCAS procedure, and hippocampal memory and neurogenesis were assessed after 3 months. Cognitive function was evaluated using the Novel Object Location test (NOL). Hippocampal neurogenesis and neuroinflammation were evaluated by immunohistochemical methods, and cerebral cytokine expression was measured by RT-qPCR. Hypoperfusion was assessed by arterial spin labelling-MRI.
Our data demonstrate that hypoperfused mice displayed hippocampus-dependent memory deficit demonstrated by a lower recognition index in the NOL than sham control animals. Along with the cognitive deficit, neurogenesis assessed by the number of doublecortin-positive cells showed a significant decrease in BCCAS-mice and their analysis also suggest an altered morphology. Finally, our results also showed that hypoperfusion promotes a reduction in microglia branches.
Therefore, we can conclude that the hypoperfusion originated by BCCAS mouse model in brain lead to cognitive deficit concomitant to impaired hippocampal neurogenesis and morphological alterations in the microglia.

HUMAN BRAIN OSCILLATIONS AND REGION DISTRIBUTION IN TWO DIFFERENT FATIGUING TASKS

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Fatigue is one of the symptoms of many diseases such as multiple sclerosis, spinal cord injury and Parkinson's disease. It is a very disabling symptom that has a very significant detrimental impact on the quality of life of patients. The mechanisms underlying this symptom are not yet well understood. It is necessary to establish neurophysiological biomarkers and to know differs between patients and healthy people. Understanding these mechanisms could help optimize symptom treatments. The objective of this study is to describe how brain oscillations behave in two different fatiguing tasks and which brain regions are involved in the fatigue developed by the two different fatiguing tasks.
This study was carried out in healthy volunteers and was divided into two different experiments.
In Experiment 1, subjects participated in a rs-fMRI study after completing a fatiguing task inside RM. The participants were randomized to completed two different fatiguing tasks, an isometric task or a finger tapping task during two minutes. In Experiment 2, participants completed a crossover fatiguing-EEG study, with an isometric task and a finger tapping task during two minutes.
Results showed different brain regions involved in the two different tasks mechanisms of fatigue triggered by isometric contraction and repetitive movements. The consideration of these differences might help to optimize the study of fatigue in physiological conditions and neurological disorders.

IMMUNE RECEPTOR TLR4 MEDIATES COGNITIVE DEFICIT INDUCED BY HIGH SODIUM DIET

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It has been recently proposed that high sodium diet (HSD) triggers an immune response in the gut, characterized by an expansion of Th17 lymphocytes and increased IL-17 levels, which suppresses resting cerebral blood flow, leading to cognitive impairment (Faraco et al. 2018). Several studies have highlighted the importance of adult neurogenesis and hippocampal neuroinflammation in the development of dementia (Hort et al. 2019, Moreno-Jimenez et al. 2019). However, these processes have not been studied in the context of HSD. Here, we hypothesize that the immune receptor TLR4, due to its participation in both neurogenesis and immune response, could be involved in the development of cognitive impairment associated with HSD.

For investigating this, eight-week-old male wild type C57Bl/6 (WT) and TLR4-deficient B6.B10ScN-Tlr4lps-del/JthJ (TLR4-/-) mice were fed either a normal diet (ND, 0.4% NaCl) or a high sodium diet (HSD, 4% NaCl). Hippocampus-dependent memory deficits were evaluated after 5 weeks of diet using the Contextual Fear Conditioning test (CFC). Neurogenesis and neuroinflammation were evaluated by immunohistochemical studies and cytokine levels were assessed in brain and plasma.

Our data demonstrate that, in WT mice, HSD-induced memory deficits in the CFC shown by a lower freezing response compared to those mice fed with ND. On the contrary, this deficit was not present in TLR4-/- HSD animals. In addition, HSD in WT mice also promoted a reduction in hippocampal neurogenesis (demonstrated by the quantification of doublecortin+ cells), which positively correlated with cognitive impairment. Finally, WT HSD animals showed an increase in plasma levels of IL-17A, which was not observed in TLR4-/- HSD animals.

In conclusion, HSD in rodents produces a peripheral immune response characterized by increased IL-17A levels, which promotes a decrease in hippocampal neurogenesis and cognitive deficits in hippocampus-dependent memory. This immune response is not present in TLR4-/- animals, suggesting that TLR4 plays a crucial role in the development of cognitive impairment associated to HSD-induced IL17-dependent pathways in this model.

INDIVIDUAL VARIATION IN DROSOPHILA MELANOGASTER IMPACTS FEEDING BEHAVIOR

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In front of a stimuli organisms from the same species tend to behave apparently similar, tending to show similar types of choices, but some aspects of the behavior can display huge variability among individuals. That phenotypic variation emerges from the combination of gene function and developmental factors, including experience, that may impact on the survival of the organisms in the environment. A behavior of particular interest is the ability of animals to differentiate properly the nutritious from the poisonous food as it is key for their survival. Interestingly, feeding behavior shows great variability between individuals and in addition, the behavioral responses are clear and easy to measure, making it an ideal model where to study the genetic basis of animal personality and stochastic behavior. For the present study we have used a collection of inbred lines, Global Diversity Lines (GDL), to address the genetic basis of feeding variation among individuals by analyzing the feeding microstructure at individual and collective context with different behavior assays. We have found that variation among GDL lines from the same population is lower than between GDL lines from different populations. Further, there are specific lines that show higher sensitivity to sucrose measured with Proboscis Extension Reflex (PER) which is also correlated with higher feeding activity according to two-choice feeding assays (FlyPad). These findings suggest that there is a positive correlation between sensitivity to sucrose and two-choice activity, and that specific genetic background is responsible for this behavior, which vary more between populations than within lines from the same population.

INTRANEURONAL β-AMYLOID BUT NOT TAU ACCUMULATION ENHANCES FEAR AND ANXIETY IN ALZHEIMER'S DISEASE TRANSGENIC MICE

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Progressive cognitive decline and neuropsychiatric symptoms are common clinical features of Alzheimer's disease (AD). Emotional disturbances, including anxiety and fear, occur very early during clinical AD, when individuals meet criteria for mild cognitive impairment. The mechanistic link between the classical cerebral disease pathological features, amyloid-β (Aβ) and tau, and amygdala-dependent emotional symptoms in AD is largely unclear. Here we show that anxiety and fear symptoms are associated with Aβ accumulation but not tau pathology in emotion-related brain regions of AD transgenic (Tg) mice. By generating and analyzing littermate control, APP, Tau and APP/Tau Tg mice we demonstrate an age-dependent increase of Aβ and phospho-tau accumulation in the hippocampus and basolateral amygdala (BLA) of APP/Tau Tg mice. Both males and females APP and APP/Tau Tg mice, but not Tau Tg mice, displayed enhanced innate and conditioned fear symptoms and a deficiency in extinction fear memory consolidation coinciding with enhanced accumulation of Aβ in β-aminobutyric acid (GABA)ergic neurons of the BLA. This behavioral alterations occur in parallel with decreased activity of hippocampal neurons as assayed in APP/Tau; cfosp-EGFP reporter mice. Overall, these results suggest a novel pathogenic role of intraneuronal Aβ in GABAergic interneurons on anxiety and fear symptoms in AD. This study clarifies the relationship between amyloid and tau pathologies, and it provides a useful mouse model to delineate the neurobiological and pathological mechanisms underlying neuropsychiatric symptoms in AD.

IS YOUR GAZE YOUR AIM? EYE POSITION IN REWARD GAMBLING AND THE ROLE OF ORBITOFRONTAL CORTEX IN ENCODING THE VALUE OF VISUALLY CUED OFFERS

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A wealth of studies has revealed how cells in frontal brain areas are involved in cognitive control functions. Of crucial interest is the understanding of how the activity of neural cells relates to the processing of external stimuli features bound in abstract entities of behavioral relevance, thus playing a functional role in cognitive control tasks requiring working memory and decision making. For decision making tasks with sequential reward offers presentation, neurons in the orbitofrontal cortex (OFC) have been associated with the coding and maintenance of the estimated value of a firstly presented offer so that it can be later compared with the estimated value of a later presented one. Importantly, it is yet to be assessed what is the role of perceptual features of visually presented offers such as the order of presentation and their spatial location. Our research aims to investigate the role of task variables in eye movement behavior and the role of neural activity in OFC during the execution of a two-alternative gambling task with sequential visual offer presentation. Interestingly, we report that eye movements consistently fell within the visual screen side with best offer expected value, thus showing how eye position can be used as a marker of readout of the actual best guess. In addition, despite the subjects were left with blank screen and free to direct gaze at their will, we found that they most frequently reached the side of best offer at each time during task execution. We find evidence for this behavior soon after the first offer was presented and, very consistently, soon after the second offer presentation. Lastly, we investigated the role of cells in OFC, revealing how a significant portion of cells shows linear tuning in their firing rate with respect to offer features. In particular, we report spatial selectivity to the side of presentation, to the order of presentation of offers with different values, and to the value of rewards achieved in previous trials.

LEARNING CONDITIONS INFLUENCE HIPPOCAMPAL-DEPENDENT MEMORY AND CONTEXT DISCRIMINATION

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In contextual fear conditioning (CFC), learning occurs when an aversive foot-shock (unconditioned stimulus, US) is presented within a context (conditioned stimulus). Hippocampal circuits, in particular CA1 and dentate gyrus (DG), integrate sensory and spatial information and contribute to the formation of the associative memory. Learning is expressed in the form of a conditioned response (CR, freezing), which can be restricted to the conditioning context (discrimination) or extended to a similar neutral context (generalization). Although mice discriminate contexts efficiently, they show a wide range of CR persistence in both conditioning and neutral contexts. The objectives of this work are: 1) To identify behavioural traits and learning conditions that influence this variable discrimination capacity and 2) to elucidate the impact of those behavioural traits and learning conditions on the activity of CA1 and DG neurons using fibre photometry.

We first analysed several behavioural traits (locomotion, centre/periphery exploration, shock reactivity, freezing) throughout the CFC training and test sessions. Correlation analysis showed multiple significant associations of behavioural traits with discrimination suggesting that individual variation in natural behaviours may predict discrimination capacity. Regarding learning conditions, we found a significant correlation between the position within the cage (centre or periphery) where mice received the US and discrimination. Based on this, we designed an experiment to control shock reception zone. This manipulation resulted in differences in mice discrimination, suggesting that learning conditions influence context discrimination. Fibre photometry revealed that locomotion, US and freezing differentially modulate the activity of CA1 and DG. Importantly, we observed different levels of neuronal activity depending on the cage position (centre/periphery) occupied by mice.

In conclusion, our results show that discrimination capacity relies on learning conditions that affect the hippocampal circuits responsible for memory encoding. This suggests that learning conditions regulate discrimination through modulation of neural networks involved in associative learning.

LEARNING OF ALLOCENTRIC AND EGOCENTRIC STRATEGIES IN AN AUTOMATIZED MAZE

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The hippocampus and related structures play an important role in learning behavioral responses that are best suited for particular situations. Understanding how these structures map the environment through allocentric and egocentric representations is crucial for understanding cognition, but the processes that allow the use of these representations to generate goal directed behaviors are equally important and have received much less attention. Testing for the mechanisms underlying how mice learn under different contingencies requires maze-based tasks while recording neural activity, as well as interventional tools such as optogenetics. We used an Arduino-based automatized pizza-shaped maze (P-maze) that allows clamping spatial and temporal components of different tasks while changing reward contingencies in order to force learning of different navigational strategies. The maze consists on a plus sign and a circular track platform with four potential reward chambers equipped with water ports. Multiple linear trajectories can be implemented to force navigation based on different set of rules. We ran a variety of reference and working memory tasks requiring egocentric and allocentric learning strategies. Our results show how some strategies are more demanding than others and illustrate the potential mechanisms underlying adaptive behavior.

MATERNAL SEPARATION ALTERS WORKING MEMORY AND BRAIN FUNCTION OF MALE WISTAR RATS

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Early life stress increases the risk of anomalous development of several brain areas and it could lead to diverse cognitive impairments related to learning, mnesic and executive functions, such as working memory (WM). Maternal separation is an established animal model of early life stress that produces changes in brain development. The aim of this study was to evaluate the effect of maternal separation on the WM function and on the metabolic activity of adult Wistar rats. We employed 24 rats divided into a control group (AFR, n=12) and an experimental group (MS, n=12) subjected to maternal separation for 4h a day for 21 consecutive days. In adulthood, we tested spatial WM of both groups using the Morris water maze, and brain metabolic activity was determined using the histochemical technique of cytochrome c oxidase (CCO). Results showed that MS subjects acquired the WM task with a significant delay. MS subjects increased their WM-related CCO activity in the cingulate cortex, anterior thalamus and supra mammillary areas along with a decrease in the medial-medial mammillary nucleus. These findings could contribute to the long term effects of early stress on executive functions but further studies should be necessary to explore other behavioural and brain alterations after a period of maternal separation.

Keywords: early stress, maternal separation, working memory, cytochrome c oxidase, brain development.

MECHANISMS OF POST-STROKE COGNITIVE IMPAIRMENT: HIPPOCAMPAL INVOLVEMENT

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Stroke produces a progressive impairment of hippocampus-dependent memory in ~40% of mice exposed to the ischemic insult and stimulates neurogenesis in adult rodent dentate gyrus. Recent studies suggest that newborn neurons after stroke show an aberrant morphology and may therefore incorrectly integrate into the pre-existing hippocampal circuits, leading to the cognitive impairment observed (Cuartero et al., 2019). The mechanisms by which these alterations occur are unknown. Based on these findings, our main objective is to explore the possible alterations of the neurogenic niche components, which could be related to a deterioration of hippocampal-dependent memory after stroke.

To perform this study, stroke was induced by occlusion of the middle cerebral artery (MCAO) in 2-month old C57Bl/6 male mice. Different hippocampal metabolites and neurotransmitters were analysed by magnetic resonance spectroscopy and episodic memory was evaluated using the contextual fear conditioning test. The number of microglia, astrocytes, parvalbumin+ and somatostatin+ interneurons was analysed by immunofluorescence and confocal microscopy.

Our results show that the levels of some metabolites and neurotransmitters changed after ischemia, including Glutamate + Glutamine, N-acetylaspartylglutamic acid + N-acetylaspartate, glycerophosphocoline (GPC), inositol and GABA. GPC, which is usually associated with cellular turnover, was increased after stroke; this increase might be reflecting the changes in neurons at the population level including the cycle of proliferation and maturation of the newborn neurons. Also, ischemia induced gliosis and astrogliosis with astrocyte activation and an increase in inositol, which is considered a glial marker. Interestingly, higher hippocampal GABA levels at 14 days after ischemia correlated with worse memory at 35 days ($n=15$; $P<0.05$); in agreement, there was an increase in the number of hippocampal immunoreactive somatostatin+ interneurons.

In conclusion, our results suggest that experimental stroke by MCAO in mice induces hippocampal alterations which may account for the neurogenic alterations associated with the cognitive deficit observed.

MICROGLIA REGULATE LEARNING AND MEMORY THROUGH NF-κB

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Microglia, the resident immune cells of the CNS, have been implicated in brain plasticity and function. However, the mechanisms remain largely unknown. Here, we show that Cre-dependent removal of the RelA subunit of the NF-κB transcription factor from adult microglia results in impaired learning and long-term potentiation. Depletion of RelA elicits changes in chromatin accessibility and transcriptome landscapes of microglia associated with specific gene regulatory programs driving the activation of specific microglia phenotypes. Our findings suggest that NF-κB gene products drive specific microglia phenotypes modulating neuronal circuits for learning and memory.

MicroRNAs SIGNATURES FOR VULNERABILITY TO FOOD ADDICTION

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Food addiction is characterized by a loss of behavioral control over food intake leading to obesity and eating disorders. We used a mouse model of food addiction to classify extreme subpopulations of vulnerable and resilient phenotypes to identify differential miRNA signatures. Subsequently, we choose three candidate miRNAs down-regulated in addicted mice and performed a functional validation to recapitulate the vulnerable phenotype. The validation was performed by a Tough-Decoy inhibitor delivered by an adeno-associated viral vector through a stereotaxic surgery into the mPFC. Interestingly, we demonstrated that the inhibition of two candidate miRNAs recapitulated the vulnerable phenotype, increasing the persistence or the compulsivity, respectively, underlining the specificity of their effect in the resulting phenotype. The manipulation of the third candidate gene did not have any significant effect in the vulnerability to addiction. In addition, we also characterized other phenotypic traits more subtle but also related to the vulnerability towards addiction. The elucidation of the epigenetic mechanisms underlying these behavioral alterations provides new advances toward innovative and effective interventions for this disorder.

MODULATION OF GUT MICROBIOTA AS A THERAPEUTIC APPROACH TO IMPROVE BEHAVIOURAL DEFICITS IN A MOUSE MODEL OF DOWN SYNDROME

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Proper social interaction with others, conserved cognitive abilities and a good motor coordination are crucial functions for our everyday life. Deficits in all these behavioural processes have been described in many neurodevelopmental disorders such as Down syndrome (DS). Although most of these abnormalities have a more profound impact in the daily functioning, more attention should be given to find potential therapeutic strategies to diminish their consequences. Several studies point the gut microbiota to play an active role in cognitive functions and social domains through the regulation of the gut-brain-axis (GBA). For this reason, we hypothesize that some cognitive and social alterations in DS individuals may be modified by modulating the GBA. In this work, we supplemented Ts65Dn mice, an accepted mouse model of DS, with a synbiotic treatment before gestation and, at 8 weeks old mice, we performed several behavioural paradigms to assess motor coordination (beam walking), cognitive functions (novel object recognition) and social functions using the three-chamber task and the affective state discrimination test (ASD). Our results suggest that Ts65Dn mice present sex-dependent changes in sociability and that a synbiotic treatment is able to reverse these phenotypes. Interestingly, we also show for the first time that Ts65Dn mice present a deficit in emotional recognition in the ASD test, since they are not able to discriminate between a negatively affected mouse and a neutral mouse. Strikingly, this phenotype can be rescued with synbiotic supplementation only in male mice. Overall, this study showed novel behavioural phenotypes in a mouse model of DS and suggests that the dietary modulation of the GBA could emerge as a promising therapeutic strategy to improve behavioural disturbances in DS.

NAVIGATION TO A VIRTUAL PLATFORM TO EVALUATE ENCODING AND RETRIEVAL OF "EVERYDAY MEMORIES"

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The study of behaviour allows us to contextualize and provide biological meaning to experimentally acquired physiological and molecular data. Properly designed, behavioural tests represent a powerful tool for disambiguating neurophysiological processes that operate in parallel or sequentially. Encoding and retrieval of declarative memory are the two sides of the coin with respect to the neural mechanisms of learning [1]. Often, loss- and gain-of-function experiments to study the mechanisms underlying specific memory processes focus on misleading learning curves during manipulations, which likely reflect their effect on encoding, storage, consolidation, and/or retrieval, without discriminating relative contributions, nor identifying possible confounding factors due to performance alterations (i.e., attention and motor effects). Here we present a modification of the delayed-matching to place protocol of the water maze [2] to investigate "everyday memory" formation. The test is based on spatial navigation in a large arena and the finding of a virtual (invisible) "platform". Online video tracking of the animals is used to operate the maze, decreasing the light intensity in the room, and opening the access door to a home location when the animal crosses the target virtual platform location. The task requires remembering the location of the previous day platform and encoding the current location, to be tested on the next day. We show that, in a 3-day protocol with experimental interventions in the second day, this procedure allows dissociation between memory encoding, retrieval and consolidation, measured in the behavioural performance. Furthermore, the same animals can be tested repeatedly, increasing the statistical power in a longitudinal design. The implementation in a dry maze, facilitates concomitant electrophysiological recordings and brain network manipulations based on deep brain electric or optogenetic stimulation.

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NEURAMINIDASE-INDUCED NEUROINFLAMMATION CAUSES ANXIETY AND MICROGLIOSIS IN THE AMYGDALA

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An intracerebroventricular (ICV) injection of neuraminidase (NA) within the lateral ventricles originates an acute event of neuroinflammation, which is solved to a great extent after two weeks. Recently, neurological problems or behavioral alterations have been associated with neuroinflammation. Although the majority of them fade along with inflammation resolution, the possibility of long-term sequelae should be taken into consideration. Thus, we aimed to explore if NA-induced neuroinflammation provokes behavioral or neurological disturbances at medium (2 weeks) and long (10 weeks) term. Initially, rats were ICV injected with NA or saline. Two or 10 weeks later they were made to perform a series of neurological tests and behavioral evaluations (open field test). The neuroinflammation status of the brain was studied by immunohistochemistry and qPCR. While no neurological alterations were found, the open field test revealed an increased anxiety state 2 weeks after NA administration, which was not observed after 10 weeks. In accordance with this behavioral findings, an overexpression of the molecular pattern receptor TLR4 was revealed by qPCR in hypothalamic tissue in NA treated animals after 2 weeks of ICV, but not after 10 weeks. Moreover, histological studies showed a microgliosis in the amygdala of NA injected rats 2 weeks post-ICV, as well as a slightly activated state evidenced by morphometric parameters of these cells. These histological findings were not present 10 weeks after the ICV injection. These results suggest that NA-induced neuroinflammation might cause anxiety, with no neurological manifestations, in the medium term, along with a mild microglial activation in amygdala. Such symptoms seem to revert, as they were not detected 10 weeks after NA administration.

NON-CONVENTIONAL GluN3A EXPRESSION GATES MEMORY FORMATION BY LIMITING SYNAPTIC mTOR SIGNALING IN JUVENILE AND ADULT MICE

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GluN3A-containining NMDA receptors are key regulators of postnatal neuronal development. Thought to be downregulated after postnatal stages, remnants of GluN3A expression have been recently identified in specific areas of the adult brain (Murillo et al., Cereb Cortex, 2021), though the role is still unknown. Transgenic mouse studies show that synapses that express GluN3A are resistant to the induction of long-lasting functional and structural plasticity; and memories fade more quickly in mutant mice with prolonged GluN3A expression. In line with this work, humans with low levels of GRIN3A (human gene encoding GluN3A) perform better in cognitive tasks. Here we investigated the impact of GluN3A expression in cognitive functions and explored the underlying mechanisms. We found that developmental or genetic loss of GluN3A enables synaptic mTORC1-dependent translation and facilitates memory consolidation in spatial and associative tasks. Notably, unlike the memory enhancement seen after global manipulations of translation, GluN3A deletion does not compromise memory flexibility or extinction. The memory enhancement is evident since early postnatal ages and can also be achieved by adult deletion of GluN3A in excitatory neurons. These findings identify GluN3A as regulator of synaptic translational control during memory encoding, and offer a potentially selective target for cognitive modulation.

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PERCEPTUAL DECISIONS RESULTS FROM THE ACCUMULATION OF UNPREDICTED SENSORY EVIDENCE

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Accumulation of evidence (AE) and predictive coding (PC), two major frameworks for understanding perception, offer contrasting views on how incoming stimuli are integrated with beliefs about the current state of the environment. According to AE, sensory evidence is added to the current belief, allowing reliable temporal evidence integration in face of ambiguous stimuli. By contrast, PC suggests that predictions formulated from current beliefs are subtracted from novel sensory information, allowing rapid responses to changes in unambiguous environments. Here, we show that this apparent discrepancy can be reconciled within a bayesian framework that we call Accumulation of Unpredicted Evidence (AUE). In AUE, current belief is updated with the prediction error conveyed by each stimulus (unpredicted evidence) and not the raw stimulus evidence. AUE (but not AE) is the normative approach to perceptual decision-making when sequential dependencies exist between sensory information. We tested the AUE model in an auditory accumulation reaction-time task where we introduced sequential correlations between pairs of successive tones within a stimulus sequence. The AE model predicts that first and second tones in a pair (unpredictable and predictable tones, UT and PT) should have equal impact on perception. By contrast, in AUE, PTs impact on current belief is smaller, because part of PT evidence can be predicted from the previous tone. In agreement to AUE, UTs preceding subject choice had a larger impact on choices than PTs, as the decision threshold was more frequently reached after UT rather than PT presentation. Moreover, a central late positivity EEG signal, previously associated with the accumulation of evidence, showed a much stronger response to the UT than to PT evidence. This signal contrasted with a negativity EEG signal similar to the Mismatch Negativity, which scaled with the degree of sensory surprise associated with each tone. Overall, behavior and neuroimaging results confirm that perception relies on the accumulation of unpredicted evidence, combining the predictive component of PC with the integration properties of AE.

PHYSIOLOGY OF HORMETIC EFFECTS OF EXERCISE ON COGNITIVE ENHANCEMENT: miRNA AND MICROBIOTA INVOLVEMENT

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Physical exercise has well-known effects on the body and brain both at a physiological and behavioral level. Previous laboratory work showed that physical exercise has a positive effect on cognitive level of laboratory mice, measured by a better performance in hippocampal-dependent tests and an increase in different neurogenesis markers. Nevertheless, little is known about the mechanisms underlying the cognitive benefits mediated by exercise (in our case using a treadmill as a protocol of forced exercise).

To achieve this, we (i) maked a time course of these benefits and as these effects are dependent on the type of exercise, we (ii) developed different exercise protocols, with a moderate intensity group, and a stronger intensity group (either for the exercise duration or for running speed) to see the timing when these positive effects take place.

Two of the most promising candidates mediating these mechanisms are miRNAs and the microbiota (miRNAs as a mechanism of host-microbiota communication). Recent evidences have pointed out the relevance of both mechanisms as an important element involved in the exercise effects on cognition and on the intergenerational inheritance of its effects.
Exercise changes microbiota but there is no evidence of a direct relationship with cognitive improvement due to exercise. That is why the main objective of this work has been to correlate exercise-induced cognitive improvement with the expression of miRNAs and the microbiota abundance or diversity.
We also analyzed adult hippocampal neurogenesis, blood vascularity, astrocyte levels, and both learning-memory- and mood-related behaviors.

PSICOICTUS: EVALUATION AND PROGNOSIS OF AFFECTIVE AND COGNITIVE DISORDERS AFTER MINOR STROKE

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The neurovascular unit (NVU) represents the structural and functional multicellular relationship between the brain and blood vessels. The NVU is vital for autoregulation of cerebral homeostasis and control of cerebral blood flow (CBF). Minor stroke (MS), a type of ischemic stroke, is defined as an episode of focal neurological symptomatology lasting more than 24 hours, and mild functional outcomes. Despite the neurological outcomes, a wide range of affective and/or cognitive disorders often occurs after MS. How MS would interrogate the NVU, as a disbalance, and how the simultaneous presence of both (MS and NVU) can affect the clinical outcome and determine long-term poor outcomes and dementia is still unknown. For that, evaluating the NVU in patients is essential to understand the pathophysiology of MS and define the clinical and cellular components. Psicoictus project aims to characterize the affective and cognitive profiles in MS patients by a neuropsychological battery, neuroimaging studies and serum biomarkers by metabolomics and lipidomics approach. Psicoictus is an observational, longitudinal and prospective study that includes patients with MS's diagnosis from January 2018 to March 2020. Patients are evaluated by a screening battery (MoCA, MADRS and AES-C) within five days from stroke minor diagnose. Subsequently, patients with affective and/or cognitive impairments in the screening battery are followed up at 15 days, 6 months and 1-year post-stroke. 178 patients were recruited and 118 patients were included: 31 (26%) had depression, 25 (21%) apathy and 28 (24%) cognitive impairment on the first screening. Patients with cognitive impairment were further evaluated by a complete neuropsychological battery, being executive functions and attention the most affected domains. The untargeted metabolomics and lipidomic defined a set of molecules on plasma samples obtained at 3-5 days post-stroke. The current study would validate a new diagnostic and prognostic strategy for MS patients and it brings evidence that a light disbalance of the NVU would have a potential long-term clinical outcome, being essential for interpretation of its physiopathology.

RELEVANCE OF METALLOPROTEINASE-9 IN DEPRESSION: A STUDY IN TRANSGENIC ANIMAL MODELS

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Major depressive disorder (MDD) is one of the main causes of disability worldwide. Its etiopathology is still unknown, but several hypotheses have been proposed. The matrix metalloproteinase 9 (MMP-9) is overexpressed in plasma of depressive patients and its levels are restored after chronic treatment with antidepressant drugs. Therefore, the aim of this work is to characterize the behavioural phenotype of transgenic MMP-9 mice in tests related to anxiety/depression-like behaviour.

In this work, we used MMP-9 knockout (MMP-9 KO) and MMP-9 overexpressing (MMP-9 OE) male and female mice, and their corresponding wild types (WT) counterparts, 2-3 months old. To characterize the transgenic mice phenotype, a battery of depressive- (social interaction and tail suspension test) and anxiety- (open field and novelty-suppressed feeding test) like behavioural tests were assessed.

On the one hand, MMP-9 KO male mice spent more time, both, in the centre of the open field test and the interaction zone of the social interaction test than the WT male mice. MMP-9 KO female mice spent less time in the centre of the open field and presented less immobility time in the tail suspension test. On the other hand, MMP-9 OE male and female mice had an increased latency to feeding than their WT counterparts in the novelty-suppressed feeding test. Moreover, MMP-9 OE female mice spent more time in the centre of the open field test.

In conclusion, the levels of MMP-9 had a different impact in the behavioural phenotype sex-dependent. MMP-9 KO mice do not present a depressive-like phenotype. Nevertheless, MMP-9 KO male mice have less innate anxiety, while females are more anxious. In contrast, MMP-9 OE mice have a depressive-like phenotype, while MMP-9 OE male mice present increased innate anxiety and MMP-9 OE females show less innate anxiety.

RETRIEVAL UNDER DIFFERENT CONDITIONS: IT IS ALWAYS EASY TO RECOVER THE SPATIAL INFORMATION?

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Introduction. Spatial navigation is an indispensable cognitive function. It makes possible to find a path to reach a goal location. When retaking routes, the original visual stimuli that allowed us to establish cognitive mapping using an allocentric strategy during the acquisition phase may not remain physically identical at the time of retrieval, as a consequence of environmental changes. In the standard experimental paradigms to assess spatial memory, the cues are typically maintained constant, obviating the mentioned issue. Material and methods. In order to deepen into cue availability during the retrieval phase in comparison with learning, we trained rats on a reference memory protocol with five cues placed on black curtains that surrounded the pool, and seven days later, we tested memory retrieval under different conditions: maintenance of the five cues, removal of two and four of them, and the addition of three extra ones. Rats subjected both to the maintenance or the removal of some of the original visual distal cues during retrieval achieved it adequately, whereas those exposed to extra cues failed to retrieve the spatial memory. Then, we assessed brain oxidative metabolism through cytochrome c oxidase (CCO) histochemistry. Results: Under full- and partial-cue conditions, there is an enhancement of the hippocampal, prefrontal, retrosplenial, parietal, and rhinal cortex metabolism. However, rats that failed to retrieve spatial information in the extra cues condition showed similar or lower CCO activity than controls across many limbic areas. Conclusions: The presence of a partial portion of visual stimuli from learning makes it possible to reactivate the entire memory trace, but extra spatial information makes difficult to disengage the novel information from the older knowledge and establish a contextual generalization.

Keywords: cytochrome c oxidase; brain metabolism; retrieval; cue availability; spatial memory.

ROLE OF ASTROCYTE-NEURON SIGNALING IN MAJOR DEPRESSIVE DISORDER

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Major depressive disorder (MDD) is a severe and debilitating mental illness with a very large socioeconomic impact worldwide (1). The neurobiology of this disease has been studied for a long time, focused on neuronal alterations; however, the underlying etiology is not yet fully understood. Astrocytes, a glial cell type, have been shown to play relevant roles in synaptic transmission and plasticity, with significant impact on behavioral responses (2). Evidence collected during the past two decades have shown that astrocytes might contribute to the pathophysiology and pathogenesis of MDD (3). Therefore, we aim to investigate the role of astrocyte-neuron signaling in this mental disease.

Here, we used a corticosterone treatment approach as depressive-like mouse model to evaluate the role of astrocyte-neuron signaling in medial prefrontal cortex (mPFC) from naïve and MDD mice. Ca2+ imaging techniques in vivo and ex vivo, and behavioral test have been performed.

Results: 1- In vivo spontaneous and behaviorally-related astrocyte calcium signaling was altered in MDD mice.

2- Serotonin-evoked astrocyte calcium dynamics were reduced in mPFC slices from MDD mice.

3- Selective chemogenetic activation of astrocytes by Gq-DREADDs in mPFC was able to restore the behavioral deficits of MDD mice.

Although additional experiments are required, these results reveal the potential impact of astrocyte signaling in the pathophysiology of MDD.

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ROLE OF THE GALANIN N- TERMINAL FRAGMENT (1-15) IN THE MESOLIMBIC DOPAMINERGIC SYSTEM

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Role of the galanin N- terminal fragment (1-15) in the mesolimbic dopaminergic system

We have recently discovered that Galanin(1-15) [GAL(1-15)] induced depression-like behaviour in the Forced Swimming Test and Tail Suspension Test. Since anhedonia is a core feature of depression, we have analyzed in rats GAL(1-15) actions in anhedonic-like behaviour tests: Saccharin self-administration test, Novelty Suppressed Feeding (NSF) and Female urine sniffing test (FUST). To investigate the areas involved in GAL(1-15) effects, we have analyzed transcriptional changes in the VTA and NAc. We have also studied the impact of GAL(1-15) on other reinforcers, specifically on the effect of alcohol.

In the saccharin Self-administration, a dose-response curve of GAL(1-15) 1nmol, 3nmol was performed. In NSF and FUST, we have analyzed GAL(1-15) 3 nmol effects in the latency of the first feeding episode and the female urine sniffing duration. The VTA and NAc were dissected from the NSF and FUST experiments, and RT-qPCR measured the mRNA expression of C-Fos, Dat and, Vmat2.
In the two-bottle choice test, groups of rats received i.c.v. GAL (1-15) 1, 3nmol or vehicle 2hours before the measures.
One-way ANOVA followed by Fisher´s least significant difference test was used.

GAL (1-15) 3nmol significantly increased the latency of feeding ($p<0.001$) in the NSF and significantly decreased sniffing duration ($p<0.001$) in the FUST. In the VTA, GAL(1-15) 3nmol produced a significant decrease in the mRNA levels of Dat and Vmat2 ($p<0.05$). In NAc, GAL (1-15) induced a significant reduction in the expression of C-Fos mRNA.
GAL (1–15) 3nmol significantly decreased the ethanol intake ($p<0.05$) and preference ($p<0.05$).

These results suggest the participation of the mesolimbic dopaminergic system in action mediated by GAL(1-15) on anhedonia and ethanol consumption, paving the way for its use in other drugs of abuse.
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SENSORY INDEPENDENT HISTORY CHOICE BIASES IN AUDITORY CATEGORIZATION TASKS IN RATS

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To make adequate decisions, animals need to evaluate not only the current sensory information but also recent actions and outcomes. In trial-based perceptual categorization tasks, both humans and rats can develop three main history choice biases: (1) the win-stay/lose-switch bias, which reflects a tendency to repeat previous rewarded actions and avoid unrewarded ones; (2) a repulsive aftereffect caused by previous stimuli and related to sensory adaptation; and (3) the transition bias, a tendency to repeat or alternate the previous response based on an internal estimate of the repeating probability of the sequence of events. While the first is known to be an action-reward bias, the last two are assumed to be sensory-related, but few studies have validated this hypothesis. To do it, we trained rats on two tasks which only differ in the existence of sensory information.

First, to test if the Aftereffect bias is affecting the perception of upcoming stimuli, we trained rats in an auditory categorization task (Sound task) with a 10% of Silent catch trials. With this design, animals could generally use sounds to guide their choices. We found that animals still exhibited an Aftereffect bias in Silent trials, suggesting the bias does not require the perception of a stimulus to impact consequent choices. Second, to elucidate which is the contribution of sensory stimuli to generate the Transition bias, we trained rats in a free-choice task (Foraging task) in which there were no acoustic stimuli to guide their choices, and animals had to rely solely on the serial correlations of the sequence of previous choices to obtain reward. We found that rats developed the same Transition bias as in the Sound task, suggesting that it reflects the prediction of the rewarded response rather than an expectation of future stimuli.

These results suggest that the three more prevalent history biases observed in rats performing a decision-making task -win-stay/lose-switch, transition and aftereffect- can all be manifested in the absence of a sensory stimulus.

SPATIAL MEMORY EVALUATED BY LOW ANXIOGENIC BARNES MAZE IS PRESERVED IN THE 3xTg-AD MICE MODEL OF ALZHEIMER'S DISEASE FOLLOWING A CANNABINOID TREATMENT

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Alzheimer's disease (AD) is characterized by a cognitive decline associated with a selective vulnerability of basal forebrain cholinergic neurons (BFCN), under the modulation of the endocannabinoid (eCB) system.

In this work, we assessed cognitive function using low anxiogenic Barnes Maze (BM) in the triple transgenic mice model of genetic AD (3xTg-AD), following a sub-chronic five-day treatment with 0.1 mg/kg (i.p.) of WIN55,212-2 (7-month old male mice, WIN-treated group, n=9). Their performances were compared with control mice for treatment (vehicle, n=8), and genotype (WT mice, n=6). Mice performed a five-day BM protocol with 4 daily trials for learning acquisition and spatial memory test on the fifth day.

Both Vehicle and WIN-treated mice showed delayed learning compared to WT mice, as shown by their longer learning latencies throughout the first two days (Two-way Repeated Measures ANOVA; post-hoc test Bonferroni, $p<0.01$ for Vehicle vs. WT, and WIN-treated vs. WT). Vehicle and WIN-treated groups reduced their latency from day 1 to day 4 (Paired t-test; $p<0.001$ for Vehicle, day 4 vs. day 1; $p<0.01$ for WIN-treated, day 4 vs. day 1), but no differences in acquisition were observed between both 3xTg-AD groups. On the probe day for spatial memory, mice showed target quadrant preference regardless of treatment (Kruskal-Wallis test; post-hoc test Dunn's, $p<0.001$ for Vehicle, and $p<0.05$ for WIN-treated).

3xTg-AD mice showed spatial memory impairment in more anxiogenic tests, such as Morris water maze, but we did not observe that in BM. Moreover, previous results from our group showed high levels of anxiety in 3xTg-AD mice, measured as an increase in acquisition latency to an aversive stimulus, which were ameliorated following this same cannabinoid treatment. Anxiety, rather than cognitive impairment, might explain these distinct behaviors. To avoid anxiety-related bias in the evaluation of cognitive dysfunction in 3xTg-AD mice, low anxiety behavioral tests are recommended.

STRESS RESEARCH AND IMPLICATIONS FOR THE NEUROPSYCHIATRIC CLASSIFICATION OF EMOTION RELATED BRAIN FUNCTIONING

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Introduction: My research of the effect of stress on adolescent brain functioning has validated the positive therapeutic impact of the fluid teaching interaction, based on a dimension of personality called fluidity (empathy, sincerity and positive interaction). Therefore, I propose a new hypothesis about a quadruple brain functioning classification (supercold, cold, hot and superhot brain) that has implications from the neuropsychiatric perspective of establishing a diagnostic and of an efficient therapeutic intervention.

Materials and methods: I administered empathy, depression, anxiety questionnaires and my fluidity questionnaire (Cronbach α=0.8). Comparing the psychological database (N=103) and the stress test database (N=12), I constructed four neuropsychological types of brain functioning by emotion-cognition criteria. The cognitive coefficient used was my own self-cognition coefficient.

Results: The supercold brain that approximates a psychopathic personality has fifteen times more cognitive bias than the normal hot brain group. The superhot brain that approximates an anxious and depressive personality has a lower cognitive bias. I also validated the superhot brain type with two subjects that have a clinical diagnostic.

Conclusion: The four brain types capture the variation of cognitive processes by the variation of empathy and anxiety/depression in strong correlation with DSM taxonomy, proving to be a useful instrument to describe brain emotion-cognition activity. I present some criteria and elements of each category and at the same time I argue that the proposed classification of brain functioning from the emotion-cognition interaction perspective surpasses the linear and clear-cut partition and offers arguments for the four types of brain functioning as a psychiatric classification on a continuum but also, mutatis mutandis, as four modes of everyday brain functioning in an active-adaptive strategy to a changing social context.

SUB-CHRONIC PERIPHERAL CANNABINOID TYPE-1 RECEPTOR BLOCKADE ENHANCES COGNITIVE PERFORMANCE IN NAÏVE MICE AND IN A MODEL OF FRAGILE X SYNDROME

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Memory is a physiological function that allows encoding and storing information over time, to be later retrieved for specific purposes. Intellectual disability disorders such as fragile X syndrome, show in most cases deficits in learning and memory performance. Fragile X syndrome is a rare disorder derived from the repression of the FMR1 gene in humans, which has been modeled in mice by knocking out the Fmr1 gene (Fmr1 KO). Our group has previously demonstrated that the peripheral cannabinoid type-1 receptor (CB1R) participates in memory modulation. Thus, peripheral CB1R activation occurs under acute stress conditions reducing memory persistence, while acute peripheral CB1R blockade enhances memory persistence. We now evaluated the behavioral, cellular and molecular outcome of a sub-chronic blockade of peripheral CB1Rs in naïve mice and in Fmr1 KO mice. We found that sub-chronic (7 d) administration of the peripherally restricted CB1R antagonist AM6545 shows in naïve mice a mnemonic effect measured in the novel object-recognition memory test (NORT). No changes in the proliferative marker Ki67 were observed in the subgranular zone of the dentate gyrus, while AM6545 sub-chronic treatment induced changes in hippocampal dendritic spine morphology driving an enhancement of mature spines. Sub-chronic peripheral CB1R blockade also occluded long-term potentiation in CA3-CA1 hippocampal synapses and increased the hippocampal expression of Bdnf and Ngf neurotrophic factors. Interestingly, executive function facilitation was observed after sub-chronic AM6545 administration in naïve mice. In Fmr1 KO mice, sub-chronic AM6545 treatment prevented NORT deficits and normalized aberrant mGluR5-dependent long-term depression and dendritic spine alterations. Altogether, our results suggest that the peripheral CB1R contributes to the modulation of memory persistence and hippocampal synaptic plasticity both in naïve mice and in a model of fragile X syndrome, mimicking the effects previously observed of systemic approaches reducing CB1R function.

SUBJECTIVE COGNITIVE COMPLAINTS ARE RELATED TO COGNITIVE DISPERSION AND RESTING-STATE NETWORKS SEGREGATION IN A MIDDLE-AGED HEALTHY POPULATION

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Cognitive dispersion index (DI) is defined as individual variability in performance across cognitive tests, which has been proposed as an independent predictor of cognitive decline and it has been associated with alterations in brain functional integrity. Our aim was to study the interactions between DI and cognitive complaints defining their cerebral functional correlates using System Segregation (SyS), a graph metric that quantifies how resting-state networks are segregated from each other.

A total of 619 healthy volunteers (age range: 45-66 years; 301 female) from the Barcelona Brain Health Initiative (https://bbhi.cat/en/) cohort with available neuropsychological assessment and functional Magnetic Resonance Imaging (fMRI) acquisitions were included in our analyses. The sample was stratified into high (N=285) and low (N=333) cognitive complaints reported by Neuro-QoL. Then, we computed DI for episodic memory (EM-DI), executive functions (EXE-DI) and speed of processing (SP-DI), as well as for global cognition (C-DI). Individual SyS was estimated based on Schaefer fMRI atlas resting-state networks. All the analyses were adjusted by age, gender, and years of education.

Those subjects with low cognitive complaints exhibited higher performance than those with high complaints regarding SP (t=3.455, p=0.001), EXE (t=2.785, p=0.006) and global cognition (t=2.724, p=0.007). However, there were no group differences for DI measures, neither for SyS. Taking into account each group independently, those subjects with high cognitive complaints evidenced negative correlations between almost all DI measures and the corresponding performance (EM-DI and EM: r=-0.131, p=0.027; EXE-DI and EXE: r=-0.136, p=0.022; C-DI and global cognition: r=-0.148, p=0.013). Interestingly, SP was associated with lower EM-ID performance (r=-0.158, p=0.008) and higher SyS (r=0.154, p=0.017). No significant correlations were identified for the low-complains group.

Segregation of the brain's connectome into distinct functional networks was associated with speed of processing among subjects with higher rates of cognitive complaints.

SYNCHRONIZED EYE BLINKS PREDICT NARRATIVE CONTENT IN VIDEOS

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On average, humans blink 8-21 times per minute while resting, but this unconscious rate changes with activities such as talking, listening, or watching screens. Apart from the primary physiological function of wetting the cornea, blinks are linked to attention and vary according to the cognitive processing of visual activities. Previously, we found that the style of edition of videos affected viewers' eye blink rate.

We presented three videos with the exact same narrative but different styles of editing and recorded the blink rate of 40 participants (age: 43.97 ± 8.07 years). We compared the eye blinks of participants while watching the actions within the three styles of edition.

Blinks were distributed into 40 bins of 4.95 s each for visual presentation in a histogram and observed that blink evolution across bins was very similar, regardless of the style of edition presented to participants. We found a significant effect of Time [$F(39,3041) = 5.199$, $p < 0.001$] and a significant Time × Style interaction [$F(78,3041) = 2.004$, $p < 0.001$], while no main effect of Style was found. In addition, we found six actions in the narrative content when special synchronization of viewers' blink happened. Three actions corresponded with a decrease of the blinks, the rest with an increase. The moments of increased blinks corresponded to those when the actor leaves the scene and when the movie repeats the same action for a while. The moments of decreased eye blinks corresponded to actions where visual information was crucial to proper understanding of the scene presented.

According to our results, viewers' attention is more related to the narrative content presented on videos than to the edition style. We conclude that in the context of managing viewers' attention, content overrules the style.

TAKE CARE OF YOUR BABIES! MOUSE PUPS PRODUCE PHEROMONES THAT INDUCE MATERNAL BEHAVIOUR

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Maternal behaviour is a social behaviour characterized by conducts undertaken with the objective of increasing the survivability of the progeny. Female mice show several well-described behaviours when exposed to pups; however, they change depending on the stage the female is in, i.e. mother or virgin. We believe that the innate responses shown by mothers when exposed to pups, are triggered by a series of stimuli released by pups, specifically, we think that vomeronasal (VNO) related stimuli are essential for this kind of behaviours.

We have shown that pups activate the VNO in females compared with a non-social stimulus. Moreover, virgin and late-pregnant females show differential pup-induced activation of the vomeronasal amygdala, thus indicating hormone-induced changes in the response of females to pup chemosignals.

In our study, we aim to:

Find if pup-related volatiles are attractive for females (mothers and virgins), and explore if these have reinforcing properties over them.

Identify pup-derived volatiles that could act as pheromones triggering maternal behaviours.

First, we performed a conditioned place preference test, where females were exposed to anesthetized pups on one side and glass marbles on the other. Both stimuli were placed inside a stainless steel infuser so that females only had access to the volatiles released by pups. The results showed that while mothers found pup stimuli attractive and reinforcing, virgins did not. This suggests that pups produce volatile compounds acting as attractive pheromones for post-partum females.

Next, we extracted the volatolome (set of volatiles) of neonatal pups and youngsters at the age of weaning (week 4). Combining GC-MS with untargeted metabolomics we identified 10 volatiles present exclusively in the volatolome of neonatal pups. One of them, durene, has been shown to activate VNO neurons of adult mice. This and other identified compounds are good candidates for pup pheromones in mice.

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THE ASSOCIATIVE STRIATUM MEDIATES FLEXIBLE EXPECTATION-BASED CHOICE BIASES

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Recent actions and their outcomes can generate expectations that guide upcoming choices, but the underlying neural circuits have yet to be specified. We hypothesized that the associative striatum (i.e. DMS) is well positioned to support such cognitive function, and hence designed a task and manipulation that would allow us to differentiate it from the role of striatum in motor control. To promote the use of expectations, we used a two-alternative auditory discrimination task with serial correlations in the stimulus sequence. A generalized linear model confirmed that rats leverage on the recent history of rewarded responses to estimate the next correct choice when evidence is ambiguous, referred to as the transition bias. We virally expressed the opsin stGtACR2 in DMS projection neurons to silence their somata in the millisecond scale. Bilateral photostimulation during the inter-trial interval (ITI; ~ 20% trials), i.e. before stimulus onset, markedly reduced the transition bias compared with light-off trials. In contrast, ITI inhibition had no effect after an error response when rats temporarily waived trial history, consistent with a cognitive effect of inhibition. Notably, ITI inhibition increased or decreased response accuracy depending on the congruence of the trial with the serial correlation, but independently of response time. Strikingly, unilateral ITI inhibition was equally effective at ablating transition bias for ipsi- and contralateral responses, clearly departing from the ipsiversive effect of unilateral inhibition during action selection. Together, our new findings change the current view of the DMS, and provide a starting point to dissect the circuits subserving rule-based expectations.

THE HOT BRAIN HYPOTHESIS AND A NEW TYPE OF INTERACTION. A RESEARCH ON STRESS

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Introduction: The impact of emotions in education is a common fact not always transformed in a pedagogical principle and less in a research topic for validating a pedagogical method. The method y propose is based on the fluid (empathic, sincere and positive) interaction in a democratic encounter group. To explain how it works I constructed the Hot Brain Hypothesis that brings the perspective of an emotionally fueled cognitive process and states that emotions are fundamental for cognition, my self-cognition coefficient changing with the emotional involvement. The fluidity equation (N=103) and the empathy coefficient across different groups prove that fluidity is strongly connected with the emotional involvement in group interaction, that is, intensity of emotion is what counts.
Materials and methods: The experimental design consisted in a social stress test followed by an encounter group interaction, comparing salivary cortisol levels after each one of them (N=12). The psychological analysis was done using five types of questionnaires: depression, anxiety, empathy, stress and my fluidity questionnaire (Cronbach α=0.8).
Results: Cortisol levels decreased after the group interaction, proving that fluidity is reducing participant's stress. The fluidity equation that describes the connection between my fluidity and the fluidity of the participants and of the group captured the impact of stress.
Conclusion: As the HPA axis in the stress test experiment is reflecting the limbic system reaction, especially amygdala's reaction, but by prefrontal cortex modulation through empathy, this allows the Hot Brain Hypothesis to predict the impact of emotions on cognitive processes in line with the regression analysis results and to explain the impact of an empathic interaction in the reduction of stress, decreasing cortisol levels, proving that the fluid interaction is therapeutic.

THE ROLE OF AKKERMANSIA MUCINIPHILA AND ENVIRONMENTAL ENRICHMENT IN REVERSING COGNITIVE IMPAIRMENT ASSOCIATED WITH HIGH-FAT HIGH-CHOLESTEROL CONSUMPTION IN RATS

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Non-alcoholic steatohepatitis (NASH) is one of the most prevalent diseases globally. A high-fat, high-cholesterol (HFHC) diet leads to an early NASH model. It has been suggested that gut microbiota mediates the effects of diet through the microbiota–gut–brain axis, modifying the host's brain metabolism and disrupting cognition. Here, we target NASH-induced cognitive damage by testing the impact of environmental enrichment (EE) and the administration of either Lacticaseibacillus rhamnosus GG (LGG) or Akkermansia muciniphila CIP107961 (AKK). EE and AKK, but not LGG, reverse the HFHC-induced cognitive dysfunction, including impaired spatial working memory and novel object recognition; however, whereas AKK restores brain metabolism, EE results in an overall decrease. Moreover, AKK and LGG did not induce major rearrangements in the intestinal microbiota, with only slight changes in bacterial composition and diversity, whereas EE led to an increase in Firmicutes and Verrucomicrobia members. Our findings illustrate the interplay between gut microbiota, the host's brain energy metabolism, and cognition. In addition, the findings suggest intervention strategies, such as the administration of AKK, for the management of the cognitive dysfunction related to NASH.

THE SOCIAL COMPONENT OF ENVIRONMENTAL ENRICHMENT IS A PRO-NEUROGENIC STIMULUS IN ADULT C57BL6 FEMALE MICE

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In rodents, the hippocampal dentate gyrus gives rise to newly generated dentate granule cells (DGCs) throughout life. This process, named adult hippocampal neurogenesis (AHN), converges in the functional integration of mature DGCs into the trisynaptic hippocampal circuit. Environmental enrichment (EE) is one of the most potent positive regulators of AHN. This paradigm includes the combination of three major stimulatory components, namely increased physical activity, constant cognitive stimulation, and higher social interaction. In this regard, the pro-neurogenic effects of physical activity and cognitive stimulation have been widely addressed in adult rodents. However, the pro-neurogenic potential of the social aspect of EE remains unexplored.

Here we tackled this question by studying the effects of a prolonged period of social enrichment (SE) in adult female C57BL6 mice. To this end, 7-week-old mice were housed in groups of 12 per cage for 8 weeks. These mice were compared with others housed under control housing (2–3 mice per cage) or EE (12 mice per cage plus running wheels and toys) conditions during the same period. We analysed the number and morphology of Doublecortin-expressing (DCX+) immature DGCs. Moreover, we used RGB retroviruses, which allow labelling three populations of newborn DGCs of different ages in the same mouse, to study DGC maturation, and performed and behavioural determinations.

Both SE and EE similarly increased the number and morphological and dendritic maturation of DCX+ immature neurons and newborn DGCs of different ages. Moreover, both manipulations increased exploratory behaviour in the Social Interaction test. Therefore, our data revealed the potent neurogenesis stimulating potential of SE in the absence of any further cognitive stimulation or increase in physical activity. Given that therapies based on increased physical activity may be strongly discouraged under certain pathological circumstances, our findings may be relevant in the context of enhancing AHN via physical activity-independent mechanisms.

THE STEROID SULFATASE INHIBITOR STX64 IMPROVES AGE-ASSOCIATED COGNITIVE DEFICIENCIES

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Aging produces in brain a progressive oxidative process and neuroinflammation that deteriorates cognition. These brain dysfunctions provoke high economic and social cost to developed societies. For this reason, the search for treatments to prevent cognitive deficits associated with aging is a priority. Previous results suggest that STX64, a steroid sulfatase (STS) inhibitor, improves cognition in neurodegenerative diseases. Here we have designed cellular (immunohistochemistry), molecular (qPCR) and behaviour (mainly, object recognition and passive avoidance for the study of learning and memory) assays to know if subchronic oral treatment with STX64 can rescue cognitive dysfunction present in aging mice. Our behavioural results show that STX64 treatment improved cognition in aging mice, reflected as an improvement in object recognition and in passive avoidance tests. At cellular level, aging mice treated with STX64 showed an increase in microglial response and an increase in hippocampal adult neurogenesis. By last, preliminary qPCR assays indicated that hippocampus from aging mice treated with STX64 express lower levels of inflammatory factors mRNA compared with untreated aging mice. Then, our results indicate that STX64 may be a drug with potential therapeutic interest for the treatment of cognitive deficits associated to aging.

TRANSCRANIAL MAGNETIC STIMULATION REVEALS THE EXPERIENCE-DEPENDENT ROLE OF THE PREFRONTAL CORTEX IN MAKING DECISIONS BASED ON ABSTRACT RULES

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Recent evidence suggests that the brain substrate of executive function changes with experience, with the prefrontal cortex (PFc) assuming the control in the initial phases and more posterior areas taking over with training. To assess this experience-dependent involvement of the PFc on working memory and decision-making, we designed a complex behavioral task in which performance depends on the participants' ability to learn abstract rules and apply them to compare the visual properties of two stimuli. The task began with the simultaneous presentation of two visual stimuli (static gratins) that varied in two dimensions (frequency and orientation). After a short delay, the participant was presented with a cue that indicated what stimulus and dimension were relevant. After a second delay, one stimulus was presented in the center of the screen and the participant had to indicate, with an eye movement, whether this last stimulus was the same as the relevant stimulus regarding the relevant dimension. The participants (n=16) were tested in two sessions; half received transcranial magnetic stimulation (TMS) over the left dorsolateral prefrontal cortex (2 pulses at 7 Hz immediately after the cue, 100% resting motor threshold) during the first session and over the vertex (same stimulation protocol) in the second session, and the other half received the TMS in the inverse order. Our results show that the order in which the TMS was applied had significant effects on performance. When the participant received the TMS over the PFc in the first session, its performance was significantly worse than when the stimulation was applied in the second session. We did not find significant differences between stimulating the vertex in the first or second sessions. This suggests that the PFc is required to learn the rules of the task but, once this knowledge is acquired, this area plays a minor role in performance.

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UNDERSTANDING THE POTENTIAL ROLE OF SIRTUIN 2 ON AGING: CONSEQUENCES OF SIRT2.3 OVEREXPRESSION IN SENESCENCE

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Sirtuin 2 (SIRT2) has been associated to aging and age-related pathologies. Specifically, an age-dependent accumulation of isoform 3 of SIRT2 in the Central Nervous System has been demonstrated; however, no study has addressed the behavioral or molecular consequences that this could have on aging. In the present study, we have designed an adeno-associated virus vector (AAV-CAG-Sirt2.3-eGFP) for the overexpression of SIRT2.3 in the hippocampus of 2 month-old SAMR1 and SAMP8 mice. Our results show that the specific overexpression of this isoform does not induce significant behavioral or molecular effects at short or long term in the control strain. Only a tendency towards a worsening in the performance in acquisition phase of the Morris Water Maze was found in SAMP8 mice, together with a significant increase in the pro-inflammatory cytokine Il-1β. These results suggest that the age-related increase of SIRT2.3 found in the brain is not responsible for induction or prevention of senescence. Nevertheless, in combination with other risk factors, it could contribute to the progression of age-related processes. Understanding the specific role of SIRT2 on aging and the underlying molecular mechanisms is essential to design new and more successful therapies for the treatment of age-related diseases.

WHITE MATTER HYPERINTENSITIES AND COGNITIVE RESERVE AFFECT WORKING MEMORY STATUS AND TRAJECTORY: A TASK-BASED FUNCTIONAL MAGNETIC RESONANCE IMAGING STUDY

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Cognitive reserve (CR) theory hypothesizes that some lifestyles such as years of formal education may modulate the relationship between cognition and age-related brain changes. The aim of this study was to characterize the longitudinal (2-year follow-up) functional magnetic resonance imaging (fMRI) changes and cognitive trajectories among healthy older participants stratified at baseline according to their education level (as a proxy of CR) and the degree of white matter hyperintensities (WMHs) exhibited (i.e., degree of atrophy).

Eighty-six participants (aged: 63-75 years at baseline) were included. At the two time-points, we acquired MRI data in a 3T Siemens scanner: T1-weighted 3D MPRAGE, FLAIR and fMRI-EPI scans during an N-back task. FLAIR images were used to compute the WMH volume using LST toolbox from SPM and fMRI scans were analyzed using FEAT-FSL software, statistical significance was set at $p<0.05$ and $z>2.3$ (cluster wise corrected).

We found that education contributed positively to a higher cognitive level in the intercept, rather than modulate the slope. However, WMH burden mediated the relationship between CR and cognition. Among the high educated participants, those with high WMHs seemed to resist the increase in WMH volumes through the over-activation of task-related areas and the recruitment of additional brain regions. In contrast, those participants with low WMH showed a young-like activation pattern and cognitive stability. Regarding the low educated participants, the increase in WMHs induced a decrease in cognitive performance, and the fMRI analyses suggested an unsuccessful attempt of compensation through the recruitment of non-task-related areas.

Our findings demonstrate that education is related to a better cognitive progression in cognitively normal older adults, but it does not predict cognitive trajectory on its own since WMHs load has an impact on brain activation, resulting in distinct cognitive profiles.

Topic 5

Theoretical and Computational Neuroscience

Posters

A COMPUTATIONAL MODEL OF SLOW WAVE OSCILLATION PROPAGATION ACROSS CORTICAL AND STRIATAL NETWORKS

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The activity of every neural circuit is limited by anatomical and functional constrains, which will restrict its repertoire of activity patterns. Therefore, the knowledge obtained from the study of the brain spontaneous activity is a valuable resource to understand how those circuits operate during behaviour.

We present a computational model of cortical and striatal populations under the Slow Wave Oscillation (SWO) regime. This spontaneous brain state is characterized by periods of high spontaneous activity (Up states) intermingled with silent periods (Down states) at the frequency of ~ 1 Hz. It originates in the cortex and from there propagates to the striatum, among other brain regions. While traditional SWO models are focused on single and isolated cortical areas. our study aims to provide a theoretical understating of the contribution of local and long range connections to the properties of the SWO in multiple connected neuronal populations. This includes the study of the neuronal and circuit substrates behind the different Up state attributes across cortical regions and the rostrocaudal preferential directionality of the SWO propagation. With this, we will provide an explanation to the differences in the SWO recorded in slices or brain slabs compared to the intact brain. In addition, we will use it to investigate the neural mechanisms that differentiate the dorsolateral and dorsomedial striatal regions, which were demonstrated to be two different functional circuits, based on their activity (Alegre-Cortés et al. Elife, 2021)

In conclusion, we have designed a model of SWO propagation along cortical and striatal populations. With this tool, we studied the neuronal and circuit substrates of the propagation of this wave along these networks, unifying the previous knowledge based on in vivo and ex vivo recordings. Together with this, we will predict and ultimately test the key components that differentiate dorsal striatal circuits.

BUMP ATTRACTOR DYNAMICS UNDERLYING STIMULUS INTEGRATION IN PERCEPTUAL ESTIMATION TASKS

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Perceptual decision and continuous stimulus estimation tasks involve making judgments based on accumulated sensory evidence. Network models of evidence integration usually rely on competition between neural populations each encoding a discrete categorical choice. By design, these models do not maintain information of the integrated stimulus (e.g. the average stimulus direction in degrees) that is necessary for a continuous perceptual judgement. Here, we show that the continuous ring attractor network can integrate a stimulus feature such as orientation and track the stimulus average in the phase of its activity bump. We reduced the network dynamics of the ring model to a two-dimensional equation for the amplitude and the phase of the bump. Interestingly, these reduced equations are nearly identical to an optimal integration process for computing the running average of the stimulus orientation. They differ only in the intrinsic dynamics of the amplitude, which affects the temporal weighting of the sensory evidence. Whether the network shows early (primacy), uniform or late (recency) weighting depends on the relative strength of sensory stimuli compared to the amplitude of the bump and on the initial state of the network. The specific relation between the internal network dynamics and the sensory inputs can be modulated by changing a single parameter of the model, the global excitatory drive. We show that this can account for the heterogeneity of temporal weighting profiles observed in humans integrating a stream of oriented stimulus frames [1,2]. Our findings point to continuous attractor dynamics as a plausible mechanism underlying stimulus integration in perceptual estimation tasks.

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CONTROL LIMITATIONS SHAPE PERCEPTUAL DECISION MAKING

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Adaptive behavior in a perceptual decision making (PDM) task involves a trade-off between the performance deficit of responding too soon and the cost of time associated to gathering evidence. The optimal solution to this problem has been understood using the Partially Observable Markov Decision Process framework (POMDP). However, these kinds of policies assume perfectly rational agents and therefore ignore constraints that real agents have to confront. Here, we generalize ideas from optimal control theory to derive optimal policies that solve a categorical PDM task in the presence of a "cost of control", which penalizes policies that deviate from the agent's default actions.
In particular, we consider the effect of impulsivity, a spontaneous tendency to respond (consistent with the concept of exploration).

We provide semi-analytical solutions for the probability that the agent will choose an option at a given time with a given belief, deriving predictions on measurable observables: choice, reaction time (RT) and decision confidence. We show that when the cost of control is significant, the behavior of decision confidence departs from the POMDP solution and resembles predictions of signal detection theory. Moreover, this regime also provides a natural account of "lapses" dictated by the default dynamics, as opposed to the standard descriptions that rely on an ad-hoc independent guessing process. Finally, we showcase the model's ability to generate history effects through a biased default policy, which can be modulated through the cost of control and contrasts with the effects generated by reward asymmetries or typical history-dependent heuristics.

Overall, our results clarify the link between the observed phenomenology of decision confidence and different notions of optimality, and also provide a more general notion of normative behavior that includes both task contingencies as well as unavoidable costs faced by real organisms.

EPILEPTIC SEIZURE PREDICTION WITH A LSTM NETWORK

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Over the years, epilepsy research has focused on discovering its aetiology, designing appropriate treatments and improving patients' quality of life. One of the key elements for the latter two goals is to know when an epileptic seizure episode is going to occur, since knowing the window in which an attack will occur well in advance makes it easier to control it with the necessary therapeutic measures and reduces the uncertainty and stress suffered by the patient.

Predicting an epileptic seizure requires an artificial intelligence algorithm that integrates a series of data in real time.

Predicting an epileptic seizure requires an artificial intelligence algorithm that integrates a series of real-time data. This data can be from motion, heart rate or even optical sensors, but the best predictor to date is the patient's own brain signals.

In this study, an artificial neural network has been developed with the aim of predicting epileptic seizures in a dataset obtained from multi-electrode arrays. For this purpose, a data processing software has been programmed to obtain the most relevant spectral and morphological characteristics of the signals, namely: spectrogram, Welch power estimate, spectral entropy, zero-crossing rate, total signal area, mean, variance, skewness and kurtosis. This neural network has been trained on graphics processing units to speed up computation times and facilitate the parallelisation of the process.

The resulting neural network is able to predict with attacks in time windows of 15 minutes with an accuracy of 87.38%. Based on this network, future work will adapt the system to the EEG data and try to optimise the architecture to improve the results.

EPILEPTOGENIC BIOMARKERS BASED ON COMBINED POWER ACTIVATION AND CONNECTIVITY OF iEEG SIGNALS

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The only effective treatment of pharmacoresistant epileptic patients relies nowadays on surgery. Over the last decade many computational approaches have attempted to characterize the epileptogenicity of brain signals recorded with either scalp or intracranial EEG (iEEG) to localize the seizure focus and improve pre-surgical planning. Most of these contributions with iEEG have estimated seizure-driven power activations, while connectivity measures have been applied in scalp EEG studies and shown to improve localization in this modality. However, the interplay between power and connectivity features in iEEG with regard to seizure focus localization has not been thoroughly addressed. As a result, a few questions arise in this context: How redundant are connectivity measures with respect to power activations of iEEG signals during pre-seizure and seizure periods? How much information do linear connectivity measures add to power activations to improve seizure focus localization?

For the same cohort of patients analyzed in [1], we characterized the level of redundancy of linear connectivity measures with respect to power activations during seizures and pre-seizure periods at different frequency bands. For almost all patients, the connectivity strength (measured via Pearson correlation) was in general highly redundant with respect to the power activations when evaluated across recording sites during seizure epochs. This level of redundancy was always manifested at the broad frequency spectrum (1-150Hz) and was localized at specific frequency bands for each patient. The level of redundancy was significantly higher during seizure than pre-ictal epochs. This indicates that basic non-directional linear connectivity is highly influenced by power coactivations that might be caused by either physiological mechanisms and/or by statistical biases of the connectivity measures. Overall, these preliminary results question the addition of linear connectivity measures to improve current power-based algorithms for seizure focus localization.

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EVOKED NEURAL POPULATION ACTIVITY DURING STATIC AND DYNAMIC VISUAL STIMULI RECOGNITION: A COMPARATIVE STUDY BASED ON INTRACRANIAL EEG

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It takes a fraction of a second to recognize a person even when seen under strikingly different conditions. However, how such a robust, high-level representation is achieved by neurons in the human brain is still unclear. In particular, the way that neurons encode different percepts is one of the most intriguing questions in neuroscience and has to a large extent been addressed in poorly realistic experimental conditions. In this work we study the activity of neural populations in humans during conscious perception of realistic stimuli. To this aim, we evoke brain responses to static and time-varying visual representations of consciously perceived faces and characterize the differences between their evoked brain responses.

To address the above question, we designed task paradigms and developed a methodological pipeline based on human intracranial recordings (iEEG) to localize and quantify time-varying brain responses to consciously perceived stimuli of static and dynamic modality. Our initial analysis in 3 subjects during static face recognition revealed significant brain responses in regions from occipital, temporo-parietal and frontal lobe, thus characterizing the sequential engagement of key areas along the visual processing pathway. Moreover, when faces were viewed within a dynamic context, known high-frequency responses to recognized concepts [1] significantly diminished their intensity in specific regions of the medial temporal lobe, suggesting that (more) ecological stimuli might be differentially processed in brain areas traditionally associated with episodic memory.

Overall, this work systematizes existing methodology to localize neural population activity measured with intracranial EEG during cognitive tasks and explores the specificities of these neural activations when conscious recognition takes place under a more realistic setting than that of static object viewing.

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FINDING USEFUL BIOMECHANICS MARKERS AS FUNCTIONAL CORRELATES OF THE EYELID MOVEMENTS

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Most studies on eyelid movements focus on the determination of physiological variables and parameters such as latencies, peaks, or areas. Nonetheless, very few are dedicated to revealing significant information on the anatomical-functional conglomerate (active and passive contributions of muscle fibers, ligaments, and other connective tissues) that support the biomechanics of the eyelid motor system and, therefore, the palpebral kinematics. This study aims to design and develop an analytical-experimental approach that integrates in a single formulation the phenomenological and computational modeling of eyelid movements, in the general context of a paradigm of classic conditioning of the blink reflex. A relevant aspect of our approach was that modeling of the angular displacement of the eyelid was carried out in relation to the force component of the orbiculari oculi muscle mainly in the closing phase and in the first stage of the opening phase, which informs us of a series of biomechanical parameters useful for the exhaustive control of the eyelid kinematics. By simulation, the model proposed here also characterized the dynamics of the antagonist levator palpebrae muscle, resulting on a more realistic modelling of the second stage of the opening phase. The results showed that there were a significant number of optimization trials (search of verisimilitude between the observed experimental recording and the simulated theoretical solution for the palpebral position) which were classified as good or excellent (67.2%), according to our Versatile Interface for Optical Fitting of Eyelid Kinematics (VIOFEK). Finally, it is important to point out that there are several possible clinical applications of this analytical-experimental approach. This model could help a more objective clinical exploration of neuromuscular disorders (facial nerve paralysis), pathologies with prevalence of eyelid dysfunctions (ptosis, blepharospasm and hemifacial spasm) and follow-up after eyelid surgery.

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GENE EXPRESSION PATTERN OF HNS1 HUMAN NEURAL STEM CELLS IN DIFFERENTIATION TO APPLICATIONS IN NEUROSCIENCE

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hNS1 are multipotent Neural Stem cells (NSC) which differentiate to neurons and glial cells. Several molecular mechanisms and signalling pathways involved in NSC differentiation have been previously reported, however due to their complexity are still not fully understood. The aim of this study was to identify and characterize key molecular mechanisms involved in proliferation and differentiation of hNS1 as an experimental model to research applications in neuroscience.

To analyze gene expression alterations of hNS1 in differentiation versus proliferation we conducted RNA sequencing and in silico pathway analysis. We found 1900 genes up regulated in differentiation and 3341 genes up regulated in proliferation. hNS1 in differentiation has increased the expression of genes in the signaling pathways of axón guidance, Wnt, Notch, Sonic hedgehog (Shh), and Neurotrophins. Gene Ontology and Gene set enrichment analysis (GSEA) report genes involved in axonogenesis and synaptogenesis. The number of genes associated to extracellular matrix (ECM) is also important in neural differentiation of hNS1. Systematic search for regulatory targets from ChEA and ENCODE ChIP-seq found a significant number of genes in differentiation of hNS1 associated with REST and EZH2. We also compare the transcriptomic data obtained in this study with data from neural databases.

This poster discuss challenges and potential future directions using hNS1 as a model to approach in vitro research in neuroscience, for example enabling the research to elucidation of mechanisms of human neurodevelopmental processes.

INFORMATION TRANSMISSION IN DELAY-COUPLED NEURONAL CIRCUITS IN THE PRESENCE OF A RELAY POPULATION

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Synchronization between neuronal populations is hypothesized to play a crucial role in the communication between brain networks. The binding of features, or the association of computations occurring in spatially segregated areas, is supposed to take place when a stable synchronization between cortical areas occurs. While a direct cortico-cortical connection typically fails to support this mechanism, the participation of a third area, a relay element, mediating in the communication was proposed to overcome this limitation. Among the different structures that could play the role of coordination during the binding process, the thalamus is the best placed region to carry out this task.

We studied how information flows in a canonical motif that mimics a cortico-thalamo-cortical circuit composed by three mutually coupled neuronal populations (called V-motif), Through extensive numerical simulations, we found that the amount of information transferred between the oscillating neuronal populations is determined by the connection delay and the mismatch in their oscillation frequencies (detuning). While the transmission from a cortical population is mostly restricted to positive detuning, transmission from the relay (thalamic) population to the cortical populations is robust for a broad range of detuning values, including negative values, while permitting feedback communication from the cortex at high frequencies, thus supporting robust bottom-up and top-down interaction. Interestingly, the addition of a cortico-cortical bidirectional connection to the V-motif (C- motif) expands the dynamics of the system with distinct operation modes. While overall transmission efficiency is decreased, new communication channels establish cortico-thalamo-cortical association loops.

Switching between operation modes depends on the synaptic strength of the cortico-cortical connections. Our results support a role of the transthalamic V-motif in the binding of spatially segregated cortical computations, suggesting an important regulatory role of the direct cortico-cortical connection

JOINT REPLAY OF CORRELATED PLACE MAPS IN HIPPOCAMPUS

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Place cells in the mammalian hippocampus show tuning to location. During rest and sleep, when immobile, the hippocampus replays place cell sequences which represent recent trajectories taken through the environment. Such replay is enabled by attractor dynamics in the CA3 area of the hippocampus, where bursts of excitation push the network towards recently active states characterized by newly strengthened synapses between excitable place cells. Hippocampal replay is believed to play an important role in memory consolidation, generalizing across episodes, and more.

On constrained, linear paths, place cells also exhibit directional tuning, which means that trajectories in different directions are encoded by different activity patterns. Replayed trajectories can therefore be decoded to one direction only, or both directions; however, models and analysis of replay typically consider just one direction at a time.

To address this, we re-analyzed previously published single-unit recordings from CA1 in five rats during exploration of novel linear tracks. Place cells quickly developed directionally modulated tuning, forming two correlated place maps corresponding to runs in opposite directions. During brief rest periods between runs, we could detect offline reactivation of the place maps, whose Bayesian decoding often revealed a clear trajectory along the track. Joint replay, reflecting replayed patterns which are decodable to both place maps at once, appeared more than could be predicted by chance, suggesting that they form mixed attractors in the hippocampal network.

To test whether mixed attractors can explain the prevalence of joint replay, we extended a previous computational model of CA3 to produce spontaneous replay of correlated place maps. We found that only a small overlap between place maps is sufficient to produce attractor dynamics that reflect coherent replay in both place maps at once. This has implications for how hippocampal replay enables generalization between distinct episodes for flexible navigation.

LONG-TERM TURNOVER DYNAMICS IN AREA CA1 OF HIPPOCAMPUS ARE CONSISTENT WITH PLASTICITY OF NON-SPATIAL INPUTS

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Recent chronic imaging experiments in mice have revealed that the hippocampal code exhibits non-trivial turnover dynamics over long time scales [1]. Specifically, the subset of cells which are active on any given session in a familiar environment changes over the course of days and weeks. While some cells transition into or out of the code after a few sessions, others are stable over the entire experiment. The mechanisms underlying this turnover are unknown. Here we show that the statistics of turnover are consistent with a model in which non-spatial inputs to CA1 pyramidal cells readily undergo plasticity, while spatially tuned inputs are largely stable over time. The heterogeneity in stability across the cell assembly, as well as the decrease in correlation of the population vector of activity over time, are both quantitatively fit by a simple toy model with Gaussian input statistics. In fact, such input statistics emerge naturally in a network of spiking neurons operating in the fluctuation-driven regime. This correspondence allowed us to map the parameters of a large-scale spiking network model of CA1 onto the simple statistical model, and thereby fit the experimental data [2] quantitatively.
Our model suggests that the internal representation of space in the hippocampus evolves over time mainly due to changes in non-spatial inputs, which may represent changing contextual cues, or simply the passing of time. It also suggests that the locus of plasticity underlying turnover may be in the inputs from the entorhinal cortex, and not necessarily CA3.

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META-ANALYSIS ON NEURAL DATA: A COMPARISON BETWEEN DIFFERENT APPROACHES TO SPIKE-SORTING ON CLAUSTRUM MULTI-UNITARY ACTIVITY

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Recent advances in signal acquisition systems which allow neuronal recordings up to hundreds of channels simultaneously results into substantial time consuming and basic signal processes limitations. Within spike-sorting, the accuracy of the overall analysis is dependent on every step of the procedure. Spike clustering alongside feature extraction are critical steps after an appropriate data preprocessing. Some methods developed during the past few years have been focused on reducing the amount of computation required for spike-sorting by a mathematical simplification of the raw data. Often, these algorithms trend over use mathematical entities to predict waveforms rather than use physiological features, which results in the loss of interpretability due to a misapplication of extracted features. As far as we know, meta-analysis (i.e., examine and combine results from several analytical-experimental approaches) applied to neuronal spike-sorting are uncommon. In this work, we chose Kilosort, the latest and most influential algorithm for spike-sorting, as the perfect counterpart to VISSOR, an approach based on shape, phase, and distribution features of each spike, which reveals functional information of the neural events under study. Kilosort needs from voltage measures at closely-space sites which enable the algorithm to use together spatial and temporal shape features. The key aim is to compare the two aforementioned approaches applied to Claustrum (CL) multi-unitary activity during classical eyeblink conditioning: a tone as conditioned stimulus (CS) and an air-puff as unconditioned stimulus (US). Neurons were recorded using 16 channels proves with the aim of reaching the whole length of the CL. According to our results, Kilosort is functional for high-density recordings due to its novel spike-sorting framework, but its performance was not as enlightening as expected, when the inter-stimulus interval (CS-US), in which the neural correlates should be determined, is very short (250 milliseconds) and the number of electrodes is limited (e.g., 16 channels).

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NEURAL NETWORK DYNAMICS UNDERLYING THE ADJUSTMENT OF TEMPORAL EVIDENCE WEIGHTING IN PERCEPTUAL DECISIONS

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During perceptual decision making, sensory information can be accumulated using distinct strategies; weighting some stimulus epochs more heavily than others. A recent study has shown that primates can flexibly adapt their temporal weighting strategy to the stimulus statistics (Levi et al., Eneuro 2018). Sensory stimuli with more information at the beginning of the trial produced early weighting, while stronger sensory evidence in later stimulus epochs caused a switch to a late weighting strategy.

To shed light on how this flexible adjustment can be mechanistically implemented at a neuronal level, we used a two-area firing rate model composed of a sensory and a decision circuit with bottom-up and top-down connectivity (Wimmer et al., Nat. Commun. 2015). We included a time-varying modulation signal, ("choice commitment" signal), that altered the attractor dynamics of the decision circuit. This modulation signal affected the decision circuit in two ways. Firstly, it initiated the decision process by pushing the network into a competition regime. Secondly, it changed the decision dynamics by accelerating or delaying the choice, similar to an urgency signal.

The model could reproduce the experimentally observed primacy weighting for early and flat stimulus statistics and late weighting for late stimulus conditions when the time-course of the modulation signal reflected the stimulus statistics. We reasoned that the modulation signal may be related to the subject's task engagement, which we measured as the time needed to execute a successful fixation at the start of the trial. Consistent with the model, we found that the subject's engagement was higher (faster fixation) in the early weighting condition and lower (slower fixation) for the late condition.

Preliminary analysis of neural data recorded from areas MT and LIP indicated that the neurons' pre-stimulus activity was correlated with task engagement, providing further evidence for the modulation signal.

ONE-SHOT LEARNING IN RECURRENT NETWORKS USING BEHAVIORAL TIME-SCALE PLASTICITY

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The formation of episodic memory requires fast and potent plasticity mechanisms which allow for one-shot association of events on a time-scale of seconds or longer. Traditional Hebbian plasticity rules rely on the concurrence of pre- and post-synaptic spiking and typically lead to plasticity windows of only tens to hundreds of milliseconds. Furthermore, such rules tend to lead to instabilities unless the learning occurs slowly, making them inappropriate for one-shot learning.
However, recent in-vivo experiments in area CA1 of mouse hippocampus have revealed a new form of plasticity, dubbed Behavioral Time-scale Plasticity (BTSP), which leads to the rapid formation of place fields in previously silent cells, or the shifting of place field location in place cells. BTSP relies on the coincidence of pre-synaptic firing and a global, dendritic-wide post-synaptic signal consisting of a broad Ca2+ spike, which results in a plasticity window of several seconds or more. These plasticity effects are quantitatively fit by a computational model in which the amplitude and direction of plasticity of a given synapse depend on presynaptic firing, the current state of the synapse, and a global signal. However, this model is dynamically complex and is not amenable to analysis, making it difficult to investigate the role of such a plasticity rule in memory formation in large recurrent networks.
Here we propose a simple one-dimensional map for synaptic plasticity which qualitatively captures all relevant features of BTSP. Specifically, we can reproduce rapid place-field formation and shifting as well as the dependence of place-field width on the velocity of motion of the animal. Our map can furthermore be straightforwardly extended to recurrent networks, allowing for the analytic derivation of memory capacity for one-shot learning. We find that the weight-dependence of the rule leads to the classical stability-plasticity trade-off in learning by which older memories decay through overwriting.

SPECIALIZED PREFRONTAL CIRCUITS EXPLAIN POPULATION DYNAMICS DURING WORKING MEMORY ENCODING AND MAINTENANCE

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Neuronal population activity recorded from primate prefrontal cortex (PFC) carries information about the presented stimulus during working memory tasks. During cue presentation and in the beginning of the delay period the population code is dynamic [1], before it stabilizes and remains stable throughout the rest of the delay period. The circuit mechanisms underlying this dynamic-to-stable transition in the code are not yet understood. Here, we show that a spiking network model composed of three specialized attractor circuits can explain these experimental observations. Each conceptual ring circuit represents a specialized PFC sub-population (encoding, storage and readout neurons). Based on experimental data [2] we structured the model such that the stimulus input excites the encoding population while it suppresses the storage population. Since the recurrent connections in the encoding ring are not strong enough, the activity fades upon stimulus removal. However, at the same time the strongly connected storage units are released from stimulus suppression and form the stable bump which will maintain the memory. The readout circuit receives input from the other two populations and is thus active during cue and delay periods even without strong recurrency. Cross-temporal decoding analysis in the model fails to generalize across cue and delay periods because different neuronal subsets are most informative during the respective epochs. We validated the specific decoding pattern in PFC recordings from a visual and memory task [2] Finally, from a functional point of view, the network model predicts increased robustness to distractors once the activity bump has formed in the storage population. In sum, our findings suggest that the presence of a highly dynamic cue to delay transition originates mainly from different neuronal subpopulations. After this initial transient, a stable state is reached, and memory maintenance is achieved through attractor dynamics.

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SYNAPTIC EXTENSION OF THE BUMP ATTRACTOR MODEL PREDICTS TARGET-DISTRACTOR ONSET ASYNCHRONY EFFECTS

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Distractor filtering is fundamental to achieve an efficient management of working memory (WM). The capacity of a distractor to impair WM performance depends on its time separation with the target stimulus, the target-distractor onset asynchrony (TDOA). Distractors are more disruptive for short than long TDOA. This fact suggests a period of relative memory instability at early stages of the WM delay period, but its exact mechanisms remain elusive. The bump attractor model of WM explains memory maintenance through elevated firing activity during the delay period, and it can explain the TDOA effect by assuming a slow, gradual formation of the attractor in the delay. Here, we propose an alternative model based on the interplay of persistent activity and short-term synaptic plasticity. The combination of synaptic depression and facilitation at the onset of sustained activity induces a transient dip in the firing rate of memory-selective neurons in the early delay period. We tested this specific prediction by reanalyzing single-neuron recordings in macaque dorsolateral prefrontal cortex (dlPFC) and lateral intraparietal area (LIP) while performing a visuo-spatial WM task with distractors presented with TDOAs of 100, 200, 300 and 900ms [1]. Consistent with the model, we found that neurons selected based on their target stimulus selectivity at the end of the delay displayed a short drop in firing rate following elevated firing in the cue period. We also tested the validity of the model in human participants performing a more complex task where, besides manipulating the TDOA, distractors were presented not just after but also before the target. Behavioural, modeling and electrophysiological results point towards a dlPFC population that combines circuit-reverberation and short-term synaptic plasticity mechanisms to achieve distractor-resistant memory maintenance.

[1] Suzuki & Gottlieb (2013) Nat. Neurosc. 16, 98–104

THE INVERSE PROBLEM IN INTRACEREBRAL FIELD POTENTIALS: A REAPPRAISAL OF VOLUME-CONDUCTED AND LOCAL FIELD POTENTIALS

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Finding the deep sources that compose an EEG at the skull is termed the inverse problem, and cannot be solved without spatial information of the sources. However, the problem affects even further to intracranial recordings, which is usually neglected, but recent technical and theoretical developments have begun to address it adequately. The mixing of potentials in the volume causes the original temporal motifs to distort each other and vary at different sites. Thus the time-frequency parameters of brain waves (amplitude, phase, duration) may become stripped of physiological meaning. Here we explore how the FPs of one source modify the waveform parameters of others. Experimental data shows a conspicuous presence and far reach of FPs generated by primary structures as the cortex and hippocampus, which not only contaminate each other, but impose their temporal traits on large portions of the brain. Both contain several sources whose activation is state, region, and time-dependent. Feed forward simulations show that the reach of FPs is determined by the source's geometry. We noticed that discriminating potentials by distance to the source (volume-conducted vs local field potentials) is inadequate, particularly for large sources. For example, it is often forgotten that LFPs themselves are formed by mixing multiple nearby sources that blur each other's time course in the FP mixture, or that large sources can be near and far from an electrode. Altogether, the risks of assuming a "locality" and/or a single source origin for intracranial recordings are exposed, and we propose a broader view that prioritizes the geometry and the position of the sources over the distance to the electrodes. Obtaining the spatial demarcation of the active sources should be a primary objective together with their full disentangling to guide the correct treatment toward physiologically meaningful time courses of the activities in the neural networks.

Topic 6

Disorders and Nervous System Repair

Posters

A CLOSER LOOK AT CUX1 HETEROZYGOSIS IN THE NEOCORTEX, WHEN ONE COPY IS NOT ENOUGH

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Neurodevelopmental disorders can emerge due to abnormal neuronal function and/or connectivity. Cortical wiring requires a variety of neuronal subtypes which identity is defined by specific transcription factors (TF). Some of them are dosage-dependent, meaning that a single functional copy of the gene cannot maintain the phenotype. In the neocortex, upper layer neurons, which participate in the most complex and evolved circuits, are defined by the expression of Cux1 TF. This gene is involved in their dendritogenesis, synaptogenesis, and the establishment of interhemispheric projections as shown by knock-down experiments. Moreover, heterozygous patients carrying a mutant Cux1 allele have neurological diseases such as epilepsy, intellectual disability, and autism spectrum disorder. However, the overall cortical development and brain structures seem normal in heterozygous condition. Thus, a deeper characterization of the heterozygous scenario is needed. For this purpose, we have studied Cux1 expression in different functional areas of the cortex during development by immunostaining; performed a battery of neonatal motor tests in Cux1 heterozygous mice; analysed their susceptibility to kainate-induced seizures, and investigated a possible axonal dysfunction in these neurons. Our results show a significant reduction of Cux1 expression in the neocortex at postnatal day (P) 10, specially in the barrel field of the somatosensory area. These differences are attenuated in adulthood (P30), suggesting a possible rescue mechanism that leads to a catch-up phenotype. We also demonstrate that Cux1 heterozygosis increases predisposition to develop seizures in mice. Taking together, our findings highlight the relevance of Cux1 levels of expression during cortical development and point to the necessity of investigating the consequences of Cux1 haploinsufficiency at a molecular level.

A NEW NON-AGGREGATIVE SPLICING ISOFORM OF HUMAN TAU IS DECREASED IN ALZHEIMER'S DISEASE

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Tauopathies, including Alzheimer's disease (AD) and frontotemporal lobar degeneration with Tau pathology (FTLD-tau), are a group of neurodegenerative disorders characterised by Tau hyperphosphorylation. Post-translational modifications of Tau such as phosphorylation and truncation have been demonstrated to be an essential step in the molecular pathogenesis of these tauopathies. In this work, we demonstrate the existence of a new, human-specific truncated form of Tau generated by intron 12 retention in human neuroblastoma cells and, to a higher extent, in human RNA brain samples, using qPCR and further confirming the results on a larger database of human RNA-seq samples.

Furthermore, diminished protein levels of this new Tau isoform are found by Westernblotting in Alzheimer's patients' brains with respect to non-demented control subjects, suggesting that the lack of this truncated isoform may play an important role in the pathology. This new Tau isoform exhibits similar post-transcriptional modifications by phosphorylation and affinity for microtubule binding, but more interestingly, is less prone to aggregate than other Tau isoforms. Finally, we present evidence suggesting this new Tau isoform could be linked to the inhibition of GSK3β, which would mediate intron 12 retention by modulating the serine/arginine rich splicing factor 2 (SRSF2).

Our results show the existence of a potentially relevant new isoform of Tau and suggest that further research on this less aggregation-prone Tau may help to develop future therapies for Alzheimer's disease and other tauopathies.

ACTIVATING EPIGENETIC MODIFICATIONS ARE UPREGULATED IN THE POST-MORTEM BRAIN OF SCHIZOPHRENIA SUBJECTS: EFFECTS OF ANTIPSYCHOTIC TREATMENT

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Susceptibility to develop schizophrenia (SZ) is determined by complex interactions between genes and environment. Environmental risk factors may exert their negative effects at critical stages of brain development, possibly via epigenetic mechanisms. While most studies evaluated gene-selective epigenetic modifications, few studies reported SZ-associated alterations affecting epigenetic mechanisms globally. Here, we evaluated the global expression of histone posttranslational modifications (HPTM) in a case-control cohort of SZ subjects.

Dorsolateral prefrontal cortex samples of SZ subjects and age-, sex-, and post-mortem delay- matched controls were obtained at autopsies performed at the Basque Institute of Legal Medicine. SZ subjects were divided into antipsychotic treated (AP-treated) or antipsychotic free (AP-free) groups, according to blood toxicological data at the time of death. For this study, cortical amounts of gene expression activating (H3K9ac, H3K27ac, H3K4me3 and H4K16ac), and repressing (H3K9me3, H3K27me3 and H4K20me3) HPTM were quantified by Western Blot.

H3K9ac (+42%, $p<0.01$), H3K27ac (+28%, $p<0.05$) and H3K4me3 (+24%, $p<0.05$) were significantly increased in SZ subjects, compared to matched controls. Cortical immunodensities of all other HPTM did not show significant differences between SZ subjects and controls. Subgroup analyses in AP-free and AP-treated SZ subjects revealed that H3K27ac and H3K4me3 were specifically increased in AP-treated group. Pearson's correlation analyses showed overall positive associations between activating and, separately, between repressing HPTM. The expression of activating H3K4me3 also correlated with that of repressing H3K27me3.

In conclusion, upregulation of H3K9ac, H3K27ac and H3K4me3 may be associated with globally increased gene expression in SZ. AP treatment might enhance H3K27ac and H3K4me3 levels. Future studies should clarify whether the observed upregulation of cortical H3K27ac and H3K4me3 might be part of the mechanism of action of AP drugs, or due to a specific mechanism in SZ brain. Finally, the overall correlations between the HPTM species suggests that epigenetic mechanisms are tightly regulated

ACTIVATION OF SGK1.1 UP-REGULATES THE M-CURRENT IN PRESENCE OF EPILEPSY MUTATIONS

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In the central nervous system, the M current plays a critical role in regulating the subthreshold electrical excitability of neurons, determining their firing properties and responsiveness to synaptic input. M-channel is mainly formed by subunits Kv7.2 and Kv7.3 that co-assemble to form a heterotetrametric channel. The relevance of their functionality is demonstrated by the negative effects of mutations on their encoding genes. Mutations in KCNQ2 and KCNQ3 have been associated with a long list of hyperexcitability phenotypes including benign familial neonatal epilepsy (BFNE) and neonatal epileptic encephalopathy (NEE). SGK1.1, the neuronal isoform of the serum and glucocorticoids-regulated kinase 1 (SGK1), is a M-current modulator described by our group able to increase M-current density in neurons, leading to reduced excitability and protection against seizures. Herein, using two-electrode voltage clamp on Xenopus laevis oocytes, we demonstrate that activated SGK1.1 is able to up-regulate the M-channel in presence of two different epilepsy mutations found in Kv7.2 subunit, R207W and A306T. In addition, proximity ligation assays on N2a cell line allowed us to address the effect of these mutations on Kv7-SGK1.1-Nedd4 molecular associations, a proposed pathway underlying the M-channel up-regulation by SGK1.1

ACUTE COCAINE ENHANCES DOPAMINE D2R RECOGNITION AND SIGNALLING AND COUNTERACTS D2R INTERNALIZATION IN SIGMA1R-D2R HETERORECEPTOR COMPLEXES

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The current study was performed to establish the actions of nanomolar concentrations of cocaine, not blocking the dopamine transporter, on dopamine D2 receptor (D2R)-sigma 1 receptor (δ1R) heteroreceptor complexes and the D2R protomer recognition, signaling and internalization in cellular models. We report the existence of D2R-δ1R heteroreceptor complexes in subcortical limbic areas as well as the dorsal striatum, with different distribution patterns using the in situ Proximity Ligation Assay. Also through BRET these heteromers were demonstrated in HEK293 cells. Furthermore, saturation binding assay demonstrated that in membrane preparations of HEK293 cells coexpressing D2R and δ1R, cocaine (1nM) significantly increased the D2R Bmax values over cells singly expressing D2R. CREB reporter luc-gene assay indicated that coexpressed δ1R significantly reduced the potency of the D2R like agonist quinpirole to inhibit via D2R activation the forskolin induced increase of the CREB signal. In contrast, the addition of 100nM cocaine was found to markedly increase the quinpirole potency to inhibit the forskolin induced increase of the CREB signal in the D2R-δ1R cells. These events were associated with a marked reduction of cocaine induced internalization of D2R protomers in D2R-δ1R heteromer containing cells vs D2R singly expressing cells as studied by means of confocal analysis of D2R-δ1R trafficking and internalization. Overall, the formation of D2R-δ1R heteromers enhanced the ability of cocaine to increase the D2R protomer function associated with a marked reduction of its internalization. The existence of D2R-δ1R heteromers opens up a new understanding of the acute actions of cocaine.

ACUTE EFFECTS OF INTERMITTENT THETA-BURST STIMULATION IN THE REMEDIATION OF IMPULSIVITY IN HYPERSEXUAL PARKINSON'S DISEASE

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Hypersexuality in Parkinson's Disease (PD+HS) is a form of impulse control disorder (ICD) characterized by excessive and hard to resist urges to engage in sexual-related activities that can arise in up to 7% of patients under dopamine agonists. Disruption of corticolimbic circuitry including pre-supplementary motor area (pre-SMA) has been linked to impaired inhibitory control in ICD. To date, clinical management for reducing ICDs-related behavior consists of reducing dopaminergic drugs at the expense of worse motor functioning. We aimed to use a repetitive transcranial magnetic stimulation protocol with excitatory effects over targeted circuits known as intermittent theta-burst stimulation (iTBS) to modulate the corticolimbic circuitry and consequently improve behavioral control using an erotic stop-signal task. A single-blind randomized controlled trial consisting of two stimulation sessions (real/sham) was designed. We stimulated over the pre-SMA in 19 PD+HS patients. Additional neurophysiological and neuropsychological variables were collected to characterize the cortical signature in ICD and their cognitive profile. We designed a modified version of a stop-signal task that included erotic and non-erotic stimuli to elicit sexual desire. In a single trial, after the presentation of visual stimuli, patients were asked to either press a key to a right of a left-pointing arrow or to withhold their response upon presentation of a cross. In total, 16 PD+HS were included in the final analysis. After real iTBS stimulation, PD+HS patients responded significantly faster in go signal reaction times ($p = 0.01$). Interestingly, improvements in stop-signal reaction times (SSRT) only reached significance for erotic stop trials ($p = 0.03$). Excitatory neuromodulation of pre-SMA using iTBS might prove beneficial for remediating impulsivity-related failures to control unwanted actions in PD. Further studies are necessary to clarify the potential therapeutic role of iTBS for treating hypersexuality and other subtypes of ICDs in PD and other neurocognitive and neuropsychiatric disorders.

AGE-DEPENDENT MULTISYSTEM PARKINSONIAN FEATURES IN A NOVEL NEUROMELANIN-PRODUCING TRANSGENIC MOUSE MODEL

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Parkinson's disease (PD) is characterized by a preferential degeneration of neurons that accumulate with age the pigment neuromelanin, especially neurons from substantia nigra (SN) and locus coeruleus (LC). We aim to characterize the consequences of age-dependent intracellular neuromelanin accumulation in catecholaminergic neuronal populations to understand the relationship between this process and the vulnerability of these cells in PD, as well as its impact on healthy brain aging. We previously generated a rat model exhibiting progressive unilateral SN production of neuromelanin that showed parkinsonian-like neuropathology and motor deficits1. Here, we generated a new neuromelanin-producing rodent model, based on the tissue-specific constitutive expression of human tyrosinase (hTyr) under the tyrosine hydroxylase (TH) promoter (Tg-TH-hTyr), that mimics the bilateral distribution of pigmentation within the aging human brain (i.e. catecholaminergic groups A1-A142). In parallel to neuromelanin intracellular buildup, Tg-TH-hTyr mice exhibited major PD features, including motor and non-motor behavioral alterations, inclusion body formation and degeneration of specific catecholaminergic neuronal groups. Genome-wide transcriptomic analysis of neuromelanin-laden neurons revealed alterations in PD-related biological pathways that correlate with human PD postmortem studies. Our results show that modelling human neuromelanin accumulation in rodents leads to age-dependent catecholaminergic dysfunction and molecular alterations resulting in motor and non-motor deficits, which is relevant to PD pathology and brain aging.

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AhR DELETION REDUCES AMYLOID PLAQUE ACCUMULATION AND AXONAL DYSTROPHY IN THE APP KI NL-F ALZHEIMER'S MOUSE MODEL

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An interaction between intrinsic and environmental factors probably contributes to the molecular processes that drive Alzheimer Disease (AD). Although variation in specific genes increases the risk of AD, one of the main risk factors is age. However, how molecular processes of aging predispose to, or become deregulated in AD, still remains to be understood. Studies in different organisms from invertebrates to humans show that the Aryl Hydrocarbon Receptor (AhR), that integrates environmental stimuli (from pollutant to diet components with agonist properties) into transcriptional changes, is implicated in the aging process and therefore, we decided to investigate its role in age-associated neurodegeneration.

To that aim, we crossed the APPNLF knock-in mouse model of AD with an AhR knockout mouse (AHR-/-). Histological characterization of plaque development, soluble and insoluble Aβ loading, and tandem mass tagging (TMT)-based quantitative proteomics analysis of cortex samples were carried out for investigating the potential role of AhR in AD development.

Our results demonstrate that the absence of AhR reduces amyloid plaque formation, Aβ load and plaque-associated dystrophic neurites. Importantly, correlation network analysis and functional enrichment from proteomic data identified a set of pathways associated with mitochondrial metabolism, neuron projection and synaptic vesicles among others.

Therefore, we can conclude that AhR plays a pivotal role in the development and progression of AD and suggests that the AhR pathway and/or its modulation by exogenous or endogenous agonists can be explored for AD therapy.

ALPHA-SYNUCLEIN INTERACTS WITH NEUROMELANIN TO ENHANCE LEWY BODY FORMATION AND NEURODEGENERATION IN NEUROMELANIN-PRODUCING PARKINSONIAN RODENTS

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Parkinson's disease (PD) is characterized by a preferential loss of neurons that contain the pigment neuromelanin, especially dopaminergic neurons of the substantia nigra (SN), and the presence in affected neurons of alpha-synuclein (aSyn)-containing insoluble cytoplasmic aggregates termed Lewy bodies (LB). While aSyn aggregation is considered a central pathogenic event in PD, the mechanisms and significance of LB formation remains unknown. In PD brains, LBs appear in close physical association with neuromelanin within affected neurons. In addition, it has been reported that aSyn redistributes to the lipid component of neuromelanin at early PD stages and that aSyn is entrapped within neuromelanin granules extracted from PD, but not control, brains. The increased concentration of neuronal aSyn and neuromelanin pigment in SN neurons may predispose these neurons to LB formation and cell death. However, it has not been possible yet to experimentally assess in vivo a potential pathological interaction between aSyn and neuromelanin because, in contrast to humans, neuromelanin is absent in common experimental animals such as rodents. We recently developed the first rodent model of human-like neuromelanin production based on the viral vector-mediated nigral expression of melanin-producing enzyme tyrosinase (AAV-hTyr). This has revealed that neuromelanin can trigger PD pathology when accumulated above a specific pathogenic threshold. Here we assessed the potential interaction between aSyn and neuromelanin by combining aSyn overexpression with hTyr-induced neuromelanin production in rodents. Compared to regular non-melanized animals, AAV-mediated nigral expression of human aSyn in melanized hTyr-expressing rodents resulted in an increased formation of aSyn oligomeric species within melanized neurons, as assessed by proximity ligation assay (PLA), an enhanced and continuous production LB-like inclusions and an aggravated nigrostriatal denervation. Our results indicate that increased levels of aSyn, as it occurs in PD patients, may accelerate and enhance neuromelanin-linked PD pathology.

ALUMINUM PROFILES IN THE CEREBROSPINAL FLUID DURING ALZHEIMER'S DISEASE DEVELOPMENT. RELATION TO PATHOLOGICAL BIOMARKERS

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Alzheimer´s disease (AD) is a complex neurodegenerative disease characterized by progressive destruction of mainly brain cortical areas related to cognitive and memory performances. Most identified forms of AD are idiopathic and the precise pathogenic mechanisms remain yet unknown.
Aluminum is considered a neurotoxic metal for humans. Some previous studies have revealed the accumulation of aluminum in the cerebral parenchyma of postmortem brains of patients suffering AD. In this study, we show that aluminum is present in significant amounts in the cerebrospinal fluid (CSF) of control healthy individuals and that its concentration increases in individuals with AD. Aluminum in CSF is also elevated in patients with mild cognitive impairment (MCI), considered to be a prodromal stage of AD. Aluminum contents correlate with classical biomarkers of AD (particularly with phosphorylated tau and amyloid β) following complex association patterns which vary depending on the stage of the AD spectrum,
and dismiss a direct relationship. Associated with increased aluminum in AD patients, indicators of oxidative stress, namely Iso-PG2 and MDA, are also increased in the CSF of AD and MCI individuals, which also correlate with aluminum concentration. We hypothesize that aluminum content in CSF is a significant factor which, in synergy with other variables, may favors the initiation and progression of AD.
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AMYLOID PROPAGATION IN A SPORADIC MODEL OF ALZHEIMER DISEASE

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- Most age-associated neurodegenerative disorders involve the aggregation of specific proteins within the nervous system, as occurs in Alzheimer's disease (AD). Recent evidence indicates that Aβ can misfold and aggregate into seeds that structurally corrupt native proteins, mimicking a prion-like process of template protein corruption or seeding. In fact, studies in FAD-based animal models show that Aβ deposition and cerebral amyloid angiopathy may be induced by intracerebral infusion of brain extracts from AD patients or from aged APP-transgenic mice. These studies have shown that the characteristic of both the seeding agent and the host influence the pathologic signature of the Aβ seeds. In this regard, the majority of the Aβ-seeding studies have been done in APP-transgenic animal models that overproduce APP and/or Aβ. However, it remains to be elucidated whether Aβ deposition can be induced by Aβ seeds in an animal model that does not overexpress APP and produces wild type human Aβ and if these aggregates are similar to the human condition.
- Here, we used an innovative animal model to better understand the amyloidogenic events that occur in the sporadic form of the disease. Our model, termed hAβ-KI, expresses wild-type human Aβ under the control of the endogenous mouse APP gene. Thus, amyloid seeds from AD patients (stage C for amyloid) from the Alzheimer's Disease Research Center (ADRC) at UCI were administered into 7-8-month-old hAβ-KI and as positive controls 3xTg-AD mice were employed.
- Our findings demonstrated that amyloid seeds differentially occur in 3xTg-AD and hAb-KI mice and these aggregates are developed earlier in the familial model, 3xTg-AD mice.
- These results suggest that multiple factors such as the seed, recipient model and time are critical factors that can modulate the amyloid pathology onset and progression. Thus, more profound understanding these factors will provide key insight on how amyloid pathology progress in AD.

AN IN VIVO REPROGRAMMING MODEL TO STUDY GLIOBLASTOMA FORMATION

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Glioblastoma multiforme (GBM) is the most frequent and aggressive primary tumour developing in the Central Nervous System. Within GBM, a group of cells with stem cell features has been described, the glioma stem cells (GSCs). GSCs have the potential to give rise to a complete GBM by themselves and have been postulated to be responsible for the origin of the tumour. In the adult mammalian brain, a pool of multipotent neural stem cells (NSCs) with the ability to self-renew and give rise to differentiated neural lineages is maintained throughout life. NSCs share several features with GSCs, making them excellent candidates for being the origin of GBM. Our group has developed a murine model of GBM formation based on the in vivo reprogramming of NSCs in the adult brain. These mice carry a polycistronic cassette encoding Yamanaka's factors (Oct4, Sox2, Klf4 and c-Myc), specifically induced in glial fibrillary acidic protein (GFAP) cells after doxycycline administration. Our results show that GFAP+ cells, when reprogrammed, are capable of giving rise to tumoral masses in the brain with high efficiency, some of them sharing many features with GBM. We have also traced the origin of these tumours specifically targeting quiescent NSCs (qNSCs) by in vivo electroporation and found that qNSCs are one of the possible cells of origin of GBM.

ANTIDEPRESSANT ACTIONS OF KETAMINE ENGAGE CELLULAR MECHANISMS OF ENDOPLASMIC RETICULUM STRESS BY THE eIF2α PATHWAY

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Chaperone proteins and folding enzymes in endoplasmic reticulum (ER) perform a key role in proteostasis, which can be disrupted by numerous factors leading to an accumulation of unfolded proteins in the lumen. It results in ER stress and unfolded protein response (UPR) is elicited to restore homeostasis. However, under prolonged cellular stress, the UPR pathway can lead to cell dysfunction/loss. Impaired ER mechanisms are responsible for neurodegeneration in numerous human diseases and there is also growing evidence that ER stress is implicated in the neuronal dysfunctions of neuropsychiatric disorders. The present study was aimed to check the hypothesis that ER stress and UPR pathway over-activation in the serotonin (5-HT) neurons are involved in the cellular pathological mechanisms of anxiety and depression by causing an impaired proteasome function. ER stress was induced by a single local application of tunicamycin (200 μg/μl) in dorsal raphe nucleus of mice. We examined tunicamycin effects on proteins related to ER stress, UPR, and apoptosis, on serotonin function as well as on anxiety- and depression-like behaviors. Tunicamycin rapidly induced ER stress in 5-HT neurons, leading to a time-dependent increase in GRP78 protein levels. Furthermore, CHOP protein, which triggers apoptosis pathways, was also increased 7 days after tunicamycin infusion. ER stress led to an increased eIF2α and eEF2 phosphorylation, suggesting the activation of PERK pathway in 5-HT neurons. Tunicamycin-treated mice exhibited an anxious-depressive phenotype and showed altered 5-HT neurotransmission in medial prefrontal cortex. A single dose of ketamine (10 mg/kg, ip) reversed the depressive phenotype 30 minutes post-administration, which is linked to reduced levels of phosphorylated eIF2α and recovery of proteostasis. The results strongly indicate that ER stress and UPR may represent cellular pathogenic mechanisms in the development of mood disorders and the eIF2α pathway is central for antidepressant activity of ketamine.

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ApTOLL: A NOVEL REMYELINATING MOLECULE IN A MODEL OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a degenerative, autoimmune and chronic disease of the central nervous system (CNS) that constitutes the second cause of neurological disability in young adults. It is characterized by the loss of oligodendrocytes and, therefore, myelin, both in the white and in the gray matter. On the other hand, the autoimmune component that underlies the pathology of MS is the promoter of the processes of inflammation, demyelination, and damage to the axonal network, where the Toll-like type 4 receptor (TLR4) and proinflammatory signaling that triggers its activation plays a crucial role. In this sense, the innovative ApTOLL molecule has been developed with aptamer technology and seeks to antagonize the TLR4 in order to achieve an immunomodulatory and anti-inflammatory effect. ApTOLL is a single chain DNA aptamer that supposes a novel strategy, both for its molecular nature and for its mechanism of action, for the treatment of diseases with an important inflammatory component such as MS.

In this research, the immunomodulatory and remyelinating effect of four doses of ApTOLL (0.45 mg/kg, 0.91 mg/kg, 1.82 mg/kg, and 3.6 mg/kg) has been determined for the study of the therapeutic dose of this compound in the Experimental autoimmune encephalomyelitis (EAE) model of MS. A clear reduction in the clinical score of animals treated with ApTOLL with respect to the vehicle group is observed, as well as a greater area of myelin and neurofilaments in the spinal cord. Furthermore, this molecule seems to have a direct effect on the biology of oligodendrocytes precursors cells (OPCs) by promoting their proliferation and differentiation towards myelinating phenotypes. This effect combined with other possible neuroprotectors could be a highly innovative strategy that would cover all aspects of the ideal therapy for MS.

ASSESSMENT OF THE INTEGRITY OF THE ENDOTHELIAL JUNCTIONS AND BLOOD-BRAIN BARRIER DISRUPTION IN MCT8 DEFICIENCY

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Thyroid hormones (TH) are essential for the proper development of the brain. TH need transmembrane transporters to access their specific tissues and target cells. The monocarboxylate transporter 8 (MCT8) exclusively transports TH and is crucial for the maintenance of adequate contents of TH in the brain.
Allan-Herndon-Dudley Syndrome (AHDS) or MCT8 deficiency is a rare X-linked disease due to inactivating mutations in the SLC16A2 gene, which encodes for the MCT8 transporter. AHDS is characterized by peripheral hyperthyroidism and by cerebral hypothyroidism due to impaired transport of TH across the blood-brain barrier (BBB). Patients with AHDS present symptoms such as delayed neurological development and severe psychomotor disorders.
The aim was to assess the integrity of the BBB by analyzing the ultrastructure of the neurovascular unit in an animal model of MCT8-deficiency: the Mct8/Dio2 KO mouse. We used transmission electron microscopy) to evaluate the organization of the tight junctions and the pericyte and astrocyte end-feet coverage of the blood vessels. We also analyzed the expression of tight junction proteins by western blot, to confirm the ultrastructural defects of the neurovascular unit observed by electron microscopy. The functionality of the BBB was also assessed by the quantification of the infiltration of sodium fluorescein dye in the Mct8/Dio2 KO brain compared to controls. Finally, the blood-vessel density of the whole brain was studied by angiography neuroimaging.
Our results could provide new therapeutic targets and biomarkers associated to the neurological symptoms of the AHDS and can contribute to the generation of new therapeutic strategies to improve the quality of life of MCT8-deficient patients.

ASTROCYTIC CALCIUM DYNAMICS IN MULTIPLE SCLEROSIS: REGULATION BY CB1 RECEPTORS

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Multiple sclerosis (MS) is a chronic demyelinating disease initiated by pathogenic immune responses against myelin followed by a broader inflammatory and neurodegenerative process. Astrocytes physiologically respond to endocannabinoids and other synaptically released neurotransmitters with cytosolic Ca2+ elevations that engage intracellular signaling and fine-tune intercellular communication. MS induces a pronounced transformation of astroglial cells whereby they acquire a variety of disease-promoting functions. In particular, accumulating evidence supports the existence of a subset of neurotoxic reactive astrocytes that exhibit transcriptional programs destructive to synapses and oligodendrocytes in response to pro-inflammatory signals. Aberrant Ca2+ signals in reactive astrocytes are closely related to disease severity in a number of neurological disorders. However, the Ca2+ handling properties of astrocytes in the context of autoimmune demyelination remain to be investigated.

In this study we analyzed astrocyte Ca2+ dynamics and their modulation by CB1 receptors in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS as well as in neurotoxic astrocytes induced in vitro. Systemic administration of Δ9-THC increased the amplitude of astrocytic Ca2+ transients in the somatosensory cortex of freely moving mice carrying astroglial GCaMP6. Cannabinoid-induced increase of astroglial Ca2+ levels was mediated by the population of CB1 receptors present astrocytes as determined using GFAP-CB1-KO conditional mutant mice. EAE induced a shift in spontaneous astrocytic Ca2+ activity that correlated to disease symptomatology and attenuated Ca2+ responses mediated by Δ9-THC. Astrocytes purified during acute EAE exhibited deregulated gene expression of several membrane receptors coupled to intracellular Ca2+ regulation as well as changes affecting Ca2+ signaling/homeostatic toolkits. These observations were partially mimicked by neurotoxic activation of cultured astrocytes and associated to aberrant cytosolic Ca2+ responses by ATP and glutamate. Our results suggest deficits in spontaneous and pharmacologically induced astrocytic Ca2+ activity during autoimmune demyelination that may reflect and/or contribute to MS disease severity.

AUDITORY EVOKED OSCILLATIONS ARE ALTERED IN UBE3A KNOCK-OUT RAT MODEL OF ANGELMAN'S SYNDROME

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Angelman's syndrome (AS) is a severe neurodevelopmental disease caused by a lack of function of a segment of chromosome 15. Most of the time it is related to a deletion or mutation of the UBE3A gene on that chromosome. The electroencephalogram (EEG) in AS is usually abnormal and may be used as a quantitative biomarker for differential diagnosis, to track progression, and as clinical outcome estimate.

Here we investigate the effect of UBE3A deletion on the spontaneous activity and auditory-evoked responses by using a UBE3A knock-out rat model of AS. To do that, we explored the ongoing activity (qEEG), the auditory steady state responses (ASSR, oscillatory responses to rhythmic auditory stimuli) and the chirp-EP responses (responses related to a tone modulated in amplitude at increasing frequencies) under open-field, freely-moving conditions.

Spectral analyses suggest a decrease in gamma oscillatory activity at the frontal cortex in knock-out rats when compared to wild-type. Interestingly, robust changes in the ASSR and chirp-EP were found. The 40-Hz ASSR evoked activity and intertrial coherence were increased for transgenic animals. Similarly, the chirp-EP evoked activity and intertrial coherence were increased in a region at around 60 Hz. These differences are found bilaterally in the frontal cortex but not in the parietal cortex, suggesting a region specific disruption of neuronal synchronization mechanisms in the UBE3A knock-out rats. If replicated in patients, the ASSR and chirp-EP could constitute a useful biomarker for AS, guiding pharmacological development of therapies that target aberrant neuronal function.

BCAS1 DEFINES A HETEROGENEOUS POPULATION IN OLIGODENDROGLIOMA AND GLIOBLASTOMA

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Glial-derived tumors such as oligodendrogliomas (OGs) and glioblastomas (GBs) account for the majority of CNS tumors. OG is a type of diffuse glioma, characterized by IDH1/2 mutations and 1p/19q co-deletion. OGs are subclassified into grade II and III according to malignancy. GBs, on the other hand, are the most aggressive type of glioma (grade IV). The cell of origin of these neoplastic entities is still elusive. However, different state-of-the-art studies point towards immature oligodendrocytes as a possible source of gliomagenesis. Therefore, studying markers that identify oligodendrocyte precursors has become of great interest. Breast carcinoma amplified sequence 1 (BCAS1) has emerged as a novel marker that defines an immature oligodendrocyte population. This marker has been associated to non-CNS tumors. In this study we analyzed the expression of BCAS1 in a series of surgically removed OGs (n=17) and GBs (n=58). To study the distribution and proliferative status of the different cell subpopulations within OG and GB, we co-stained BCAS1, with EGFR, Vimentin and Ki-67. Additionally, we analyzed the ultrastructure of BCAS1+ cells by immunoelectron microscopy. We performed stereological quantification of the cell density and proliferation of BCAS1+ cells and compared them to EGFR+ proliferative cells. Our results depict that BCAS1+ cells constitute a heterogeneously distributed population in glial-derived tumors. This population displays two different morphologies: stellate or spherical cells. In particular, stellate cells can form tightly packaged nodules and present a high proliferative rate when compared to EGFR+ cells. Statistical analysis show that BCAS1+/Ki67+ cells increase according to the malignancy of the tumor. Nevertheless, the density of BCAS1+ cells decreases in more aggressive tumors. This suggests that BCAS1 is a marker defining a specific cell subpopulation within diffuse gliomas, which could correspond to a state of transient amplification, thus contributing to tumor malignancy.

BILE ACIDS REDUCE GLYCOLYSIS IN PROINFLAMMATORY MACROPHAGES

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Glycolysis is the metabolic pathway that converts glucose into pyruvic acid. CNS pathologies, such as spinal cord injury (SCI) and ischemia, increase the glycolytic pathway in the damaged areas as part of the inflammatory response.
Pyruvate kinase is a key glycolytic enzyme that converts phosphoenolpyruvate and ADP to pyruvate and ATP. In glial cells, pyruvate kinase has two isoforms, PKM1 and PKM2, originated from the same gene. PKM2 has less pyruvate kinase activity than PKM1, but as a monomer or homodimer PKM2 acts as a transcription factor that regulates the expression of target genes involved in glycolysis (e.g. Glut1, LDHA) and inflammation (e.g. IL-1β).
After an injury, both resident (microglia) and hematogenous macrophages are key inducers of the inflammatory response with deleterious effects. We have previously shown that the bile acid tauroursodeoxycholic acid (TUDCA) has anti-inflammatory effects in microglia cells involving the inhibition of NF-κB pathway.
In the present study we have investigated whether bile acids affect the expression of glycolytic enzymes and their regulation by PKM2 in rat microglia cultures and after rat SCI.
Lipopolysaccharide induced the expression of PKM1, PKM2 and its target genes in cell cultures. SCI caused an increase of PKM2 IR in macrophages after SCI. Pretreatment with TUDCA or taurolithocholic acid (TLCA) reduced the expression of PKM2 and its target genes in cell cultures. Similarly, TUDCA treatment reduced the expression of PKM2 in the injured spinal cord.
These results confirm the importance of PKM2 in the inflammatory response in CNS pathologies and indicate a new mechanism of bile acids as regulators of glycolysis.

CALCIUM TRANSPORT AT ER-MITOCHONDRIA CONTACT SITES IS MODULATED BY PYK2

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Mitochondria-associated membranes (MAMs) are specialized compartments where ER and mitochondria closely interact. These regions are known to enable calcium effluxes from the ER to the mitochondria via the IP3R3-VDAC1 complex, reinforced by Grp75. Interestingly, genes encoding for IP3R3 and Grp75 have been suggested as genetic risk factors for schizophrenia. Moreover, DISC1, a schizophrenia related protein, has been shown to localise at MAMs and to regulate calcium transport.

On the other hand, Pyk2 is a non-receptor tyrosine kinase protein enriched in the hippocampus and has also been proposed as a genetic risk factor for schizophrenia. Pyk2 can be activated by calcium-dependent mechanisms and can translocate to mitochondria upon neuronal activation. Furthermore, we have previously observed that Pyk2 modulates mitochondrial dynamics in hippocampal neurons.

In the current work, we tested whether Pyk2 could have a role in the regulation of MAMs calcium transport. First, we observed by electron microscopy that Pyk2 is present both in mitochondria and in MAMs. Next, we used full Pyk2 knockout mice (Pyk2-/-) to analyse levels of MAM-resident proteins in brain tissue by Western Blot. Moreover, we obtained Pyk2-/- neuronal cultures to evaluate ER-mitochondria contact sites and calcium homeostasis. Cell live imaging experiments showed that Pyk2-/- neurons present an impaired calcium retention, suggesting that both ER and mitochondria compartments are affected. Finally, we explored the implication of MAMs in the pathology of schizophrenia by biochemical analysis of post-mortem brain samples of schizophrenic patients.

Taken all together, our results point out that Pyk2 could be highly relevant in the modulation of ER-mitochondria calcium efflux, hampering mitochondrial function.

CERKL, A RETINITIS PIGMENTOSA GENE, IS INVOLVED IN THE REGULATION OF MITOCHONDRIAL DYNAMICS IN RETINAL AND HIPPOCAMPAL NEURONS

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The retina is the specialized region of the central nervous system that transduces light into neural signals. It is endowed with an active metabolism and displays a particular vulnerability to genetic and environmental alterations causing mitochondrial dysfunction. Mitochondrial alterations make photoreceptors and retinal ganglion cells (RGCs) more susceptible to cell death.
CERKL mutations cause Retinitis Pigmentosa in humans, a visual disorder characterized by photoreceptors and retinal neurons degeneration and progressive vision loss. Preliminary evidences indicate that CERKL is a sensor of photoreceptor stress by contributing to the formation of RNA stress granules. Both in human and mouse, CERKL produces a wide range of mRNA isoforms that translate into proteins displaying different domains. Depending on the domains of each protein isoform, CERKL subcellular localization and functional role may be different.
Although CERKL function has been mostly related to the retina, we have detected CERKL expression in hippocampus, where its intracellular behaviour in control and stress conditions is similar to that in RGCs. Hence, we use hippocampal cells as an additional suitable tool to study mitochondrial dynamics through live imaging.
Here we describe a pool of CERKL isoforms that localize at mitochondria in RGC and hippocampal cell primary cultures. Using Cerkl KD/KO mouse models, we studied the impact of CERKL downregulation on the mitochondrial network organization and dynamics. Our results show that the depletion of Cerkl causes alterations in mitochondrial size, distribution, and trafficking. In addition, we analysed the expression of proteins regulating mitochondrial dynamics (Mitofusin2, Opa1 and Drp1), and observed specific changes.
Overall, our studies indicate that Cerkl is a neural gene involved in the regulation of mitochondrial dynamics, thus becoming a new player in the multiple pathways that control mitochondrial health in the mammalian retina.

CHRONIC PAIN INDUCES PLASTICITY INTO LOCUS COERULEUS OVER TIME: ROLE OF THE LOCUS COERULEUS - DORSAL RETICULAR NUCLEUS PATHWAY.

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Chronic pain triggers maladaptive brain and spinal remodelling leading to emotional disorders. Locus coeruleus (LC) is the main source of noradrenaline in the CNS and although its role in the descending inhibition of acute pain is well established, its contribution to pain facilitation has been also reported. One of the nuclei that receives noradrenergic projecting is the dorsal reticular nucleus (DRt) which has a pronociceptive role. Thus, we hypothesized that the LC→DRt pathway is involved in the pathological nociception associated with chronic pain. Using the chronic constriction injury of the sciatic nerve (CCI) as a model of neuropathic pain in, we evaluated time-dependent plasticity (from 2, 7 and 30 days after surgery) of the ipsilateral (LCipsi) and contralateral (LCcontra) LC through pharmacologic and chemogenetic approaches. Microinjection of lidocaine intra-LCipsi only increased cold and mechanical hypersensitivity in the CCI-2d group and not later. By contrast, microinjection of lidocaine intra-LCcontra reduced cold and mechanical hypersensitivity only in the CCI-7d and CCI-30d groups and not earlier. Additionally, lidocaine blockade of the LCipsi or LCcontra reversed pain-induced depression in the CCI-30d group. Furthermore, we observed an enhanced pCREB expression in the DRtcontra but not in the DRtipsi of CCI-30d animals. In this line, we evaluated the inhibition of the LCcontra→DRtcontra pathway using chemogenetics approaches in CCI-30d. The results showed a robust analgesia in evoked and spontaneous pain. However, the inhibition of the LCcontra→DRtcontra pathway did not relieve the depressive-like behavior in CCI-30d animals. Interestingly, the inhibition of this pathway induced depressive-like behaviour in sham animals. Overall, we demonstrated that unilateral nerve injury activates the LCipsi in the short-term, which temporally dampens the neuropathic phenotype. However, long-term pain triggers a bilateral LC activation and specifically, to the contralateral LC→DRt ensemble, contributing to chronic pain and the associated depressive-like phenotype.

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CHRONICALLY INCREASING CORTICOSTRIATAL ACTIVITY PRODUCES STRIATAL ASTROCYTOSIS IN MICE

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In Parkinson's disease, the neurodegenerative process of nigrostriatal cells involves the activation of striatal astrocytes. However, the possible causal relationships between striatal astrocytosis and nigrostriatal degeneration remain unclear. Here we hypothesized that striatal astrocytosis may be directly induced by excessive corticostriatal activity.

To test this hypothesis, we performed descriptive and causal experiments in wild-type mice. Our primary outcome measure was the immunohistochemical expression of glial fibrillary acidic protein (GFAP), which is a sensitive marker of striatal astrocytosis in animal models of nigrostriatal degeneration.

First, we found that the normal antero-posterior and medial-lateral organization of GFAP in the dorsal striatum corresponds to the known topography of corticostriatal projections from the sensorimotor cortex. Second, by chemogenetically manipulating corticostriatal activity with designer receptors exclusively activated by designer drugs (DREADDs), we observed that chronically increasing corticostriatal activity for 3 weeks increases GFAP expression in the receiving striatal areas.

These results suggest that excessive corticostriatal activity by itself can produce striatal astrocytosis, thus representing a possible top-down stressor in Parkinson's disease.

COMBINED CELL AND GENE THERAPIES STOP MITRAL CELL DEATH IN PCD MICE

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The Purkinje Cell Degeneration (PCD) mutant mouse suffers the postnatal death of the mitral cells of the olfactory bulb. Previous results demonstrated that a transplant of healthy bone marrow slows down the degeneration of these neurons, improving the olfaction of PCD mice. Besides, IGF1 is a growth factor with neuroprotective properties that is also defective in PCD mice. Then, we combined both cell and gene therapies by over-expressing the Igf1 gene in transplanted cells for optimizing the neuroprotection for PCD's bulbar degeneration.

Hematopoietic stem cells from healthy green fluorescent protein (GFP) donors were cultured during 9 days in vitro (DIV). At DIV 7, the cells were infected with lentiviruses carrying the Igf1 gene under the EF1a constitutive promoter, and the cyan fluorescent protein (CFP) as reporter molecule. In parallel, the bone marrow of PCD mice was ablated by an irradiation of 7.5 Gy at postnatal day 19 (P19). At P20, irradiated animals received an intravenous transplant of 7.5 million of unfractioned healthy bone marrow stem cells, supplemented with genetically modified hematopoietic cells (ratio 1:1). At P150, animals were sacrificed and their olfactory bulbs were analyzed by immunofluorescence techniques and quantitative PCR.

Our results showed that the transplantation of a genetically modified healthy bone marrow virtually stopped the mitral cell loss of PCD mice. Interestingly, it also allowed the survival of certain Purkinje cells, a practically inexistent neuronal population in PCD mice at P150. Both standard and genetically modified transplants activated PCD's microglia also changing its inflammatory pattern, which resembled a wild-type one. Additionally, the genetically modified transplant prevented DNA damage, which seems to be the most plausible mechanism that underlies its higher protection. Therefore, the combination of cell and gene therapy supposes a remarkable strategy to achieve neuroprotection.

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COMPARING ASTROGLIAL REACTIVITY IN TWO TRANSGENIC MOUSE MODELS OF TAUOPATHY

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Astrocytes are becoming crucial players in the pathology of neurodegenerative disorders, such as Alzheimer's disease (AD). Astrocyte responses have been mainly analyzed in the context of amyloid-beta (Abeta) pathology, highlighting their role in the development/progression of amyloidosis and their relationship with the microglial response. Regarding tau pathology, some studies have reported that astrocytes respond to hyperphosphorylated tau (phospho-tau) and suggested their implication on tau transmission/elimination. Here, we aimed to analyze the astroglial reactivity to tau pathology in the hippocampus of two transgenic mouse models of tauopathy, ThyTau22 and P301S. Proteinopathy was assessed by western-blotting and immunohistochemistry using phospho-tau antibodies (AT8). Inflammatory markers (GFAP, Iba-1, CD45, TREM2) were analyzed by qPCR and immunohistochemistry for bright-field microscopy; glial-phospho-tau relationship was analyzed under confocal and transmission electron microscopy. P301S mice exhibited an intense reactive astrogliosis, increasing with aging in parallel to a strong phospho-tau pathology. ThyTau22 model showed a slighter astrocyte reactivity accompanied by a lesser accumulation of phospho-tau. Astrogliosis in P301S mice closely correlated with an acute DAM-like microglial activation, not observed in ThyTau22 hippocampus. Confocal and ultrastructural studies revealed that, in both models, astrocytic processes contained phospho-tau, especially those surrounding blood vessels. Our results support that astrocytes respond to tau pathology in the absence of Abeta. This reactivity highly correlates with phospho-tau pathology and markedly depends on microglial activation. Moreover, astrocytes may play a role in the elimination/spreading of phospho-tau species through the brain. Deciphering the mechanisms underlying these processes might help to develop therapies to slow down the progression of AD.
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CONTRIBUTION OF THE PRIMARY SOMATOSENSORY CORTEX TO REFLEX BLINK IN NAÏVE AND TEAR-DEFICIENT RATS

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Reflex blinking (RB) evoked by natural stimulation of the ocular surface (OS) is integrated in the brainstem. There is scarce evidence of its modulation by higher structures. The aim of the present work was to analyse the contribution to RB of the area of the primary somatosensory cortex (S1) where OS is represented, both in naïve and tear-deficient rats.

Multiunit activity of S1 neurons and electromyographic activity of the contralateral orbicularis oculi muscle (OO-EMG) were simultaneously recorded using high-impedance tungsten and silver electrodes, respectively, in 17 male Wistar anesthetized rats (8 naïve; 9 tear-deficient, previously subjected to lacrimal gland excision). RB was evoked by electrical stimulation of the OS at 0.1 Hz and different intensities, before and during application of 2% lidocaine to S1, and after drug washout. S1 activity was reduced or fully abolished by lidocaine.

In naïve animals, evoked OO-EMG activity preceded S1 activation. The area under the curve (AUC) of the evoked OO-EMG signals increased significantly during S1 anesthesia (0.007±0.009 vs. 0.013±0.013 V2, n=16 eyes, $p<0.01$). In tear-deficient rats, similar AUC values were obtained before (0.023±0.003 V2, n=18 eyes) and during S1 anesthesia (0.022±0.002 V2), although AUC was significantly larger than in naïve animals, both before ($p<0.001$) and during lidocaine application ($p<0.01$).

Results show that the activity of the somatosensory cortex modulates reflex blink characteristics in health conditions. In chronic tear deficiency there is an increased sensory input that drives orbicularis oculi activity, which appears not to be regulated by cortical activity.

CORTICAL AND HIPPOCAMPAL RHYTHMOPATHIES IN EXPERIMENTAL MODELS OF BRAIN METASTASIS

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Understanding the neurophysiology of brain tumours is critical to challenge the neurocognitive morbidity associated with the disease. Over recent years, several studies have shown alterations of neuronal network activity in peritumoral areas of primary brain tumours such as gliomas, with a bi-directional impact on cancer cells growth and on neural communication, which has been linked to hyperexcitability and seizures. However, the influence of brain metastasis on neural circuits remains relatively unexplored in spite of the high clinical prevalence of neurological symptoms. We evaluated neuronal activity from mice models of brain metastasis caused by local injection of melanoma, breast and lung cancer cells. By using multi-site silicon probes in peritumoral and contralateral areas of awake head-fixed mice, we measured the local field potential (LFP) in cortical and hippocampal areas. While control mice exhibited consistent laminar profiles of LFP signals, mice from the three models presented higher variability in the oscillatory power at different frequency bands. Animals with brain metastasis showed common impairments such as a generalized reduction of the oscillatory power at theta (4-12 Hz), alpha (9-14Hz), gamma (40-90 Hz) and high frequency oscillations (HFOs; >200 Hz) bands and reduced theta/delta (0.1-4Hz) ratio in cortical and hippocampal areas. Moreover, the hippocampal/cortical ratio for the HFOs band was significantly altered, suggesting an activity unbalance between both structures. Interestingly, we found several markers of hyperexcitability and seizures in some metastatic animals. Strikingly, we identified tumour-type specific alterations in the theta/delta ratio and the HFO frequency band, which did not depend on the size of the tumour mass. Our comprehensive analysis of these phenotypes includes not only neurons but multiple cell types of the metastasis-associated microenvironment, whose reactive state might indirectly contribute to interfere with brain homeostasis. Altogether, our data provide novel insights about the functional alterations associated with brain metastasis experimental models.

DECIPHERING THE MOLECULAR MECHANISM OF PLK1 CONTROL OF ADULT NEURAL STEM CELL ACTIVATION, SELF-RENEWAL AND DIFFERENTIATION

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Adult neural stem cells (NSCs) dwell in specialized microenvironments called niches, being the most studied the subependymal zone along the lateral ventricles, and the subgranular zone of the dentate gyrus. Despite being mainly quiescent, NSCs can be transiently activated by specific signals, so they can proliferate but also re-enter a state of inactivation to avoid exhaustion. The total pool of NSCs at a given time point is a consequence of NSC decision-making throughout their life-span transiting through ‘maintenance’, by the active regulation of quiescence and the self-renewing asymmetric divisions of activated NSCs; ‘reduction’, by terminal, symmetric or asymmetric differentiation; or ‘expansion’ due to self-renewing symmetric divisions. Whether the instructions that guide the making of these decisions are determined by intrinsic properties, by the microenvironment of the niche or a combination of signals is the subject of intense investigation. Data from our group indicate that Plk1 is a novel intrinsic regulator of adult neurogenesis. In an effort to unravel the molecular mechanism by which this control is attained, we have found a link between Plk1 and a master regulator of neurogenesis, through which we propose Plk1 controls activation, the mode of division and differentiation of NSC towards the neuronal lineage.

DETECTION OF BACTERIAL LIPOPOLYSACCHARIDE AND TRANSPORT MECHANISMS IN THE PREFRONTAL CORTEX OF ALCOHOL BINGE DRINKING-EXPOSED RATS

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Alcohol binge drinking (ABD) induces neuroinflammation in the prefrontal cortex of rats and damages the intestinal barrier allowing the entry of bacterial products, such as the endotoxin lipopolysaccharide (LPS), to the systemic circulation, which strongly activates the innate immune system. LPS is a big molecule and its being accepted it could not entry the brain. New evidences indicate that parts of the LPS such as Lipid A and core could reach the brain in physiological conditions by binding to specific apolipoproteins. However, the infiltration in the brain and the transport mechanisms have not been explored in ABD conditions. Here, we explored the presence of the endotoxic component of LPS, the Lipid A, in frontal cortex of rats exposed to ABD and study the mechanisms of transportation by binding to apolipoproteins ApoA1 and ApoB in male and female rats.

Animals were exposed to intragastric binge ethanol administrations (3 times/day x 4 days) with a maximum dose of 3 g/kg of ethanol in a solution of 30 percent (w/v). To verify intoxication, blood ethanol levels were determined in blood samples taken from tail. Female reproductive cycle was controlled by taking vaginal secretion once a day and microscopic analysis. Free ApoA1, ApoB and Lipid A levels and binding (Apo-Lipid A) aggregates were measured by western blot analysis and confirmed by Co-Immunoprecipitation.

Results indicate that neither free Lipid A nor ApoA1 and ApoB are increased in the frontal cortex of ABD male or female rats compared to controls. However, significant increases in the Lipid A / ApoA1 and Lipid A / ApoB ratios were observed in this structure, indicative of the binding of Lipid A with these apolipoproteins, as confirmed by Co-Immunoprecipitation.

Our results suggest that LPS infiltrates the brain in ABD conditions through a lipoprotein transport mechanism which does not show sexual differences.

DIAZEPAM ADMINISTRATION IN THE INTRAHIPPOCAMPAL KAINIC ACID ANIMAL MODEL OF EPILEPSY RESCUE BRAIN FDG-PET HYPOMETABOLISM IMAGING

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2-deoxy-2-(18F)fluoro-D-glucose in positron emission tomography (FDG-PET) is an efficient tool to characterize the changes in brain glucose metabolism, in which hypometabolism seems to be an early marker of epileptogenesis since it is associated with neuronal death and inflammatory processes. In animal epilepsy models, diazepam is usually administered after the status epilepticus in order to prevent high rate mortality. The aim of this study is to determine whether the administration of diazepam can modify the metabolic or neurohistochemical characteristics related to the intrahippocampal kainic acid (KA) animal model of epilepsy.

Intrahippocampal kainic acid surgery was carried out in 21-day-old male C57BL/6J mice, followed or not by diazepam (10 mg/kg, i.p.) administration. Static FDG-PET studies were performed before surgery (baseline measure), 24 hours and 7 days after surgery. Brains were extracted on day 8 to measure neurodegeneration (FluoroJadeC), neuroinflammation (GFAP, Iba1) and neuronal death (activated Caspase 3, Nissl) by neurohistochemistry.

FDG-PET neuroimaging showed differences in the dynamics of brain metabolism over time. The use of diazepam attenuates the hypermetabolism generated by KA 24 hours after surgery, reaching levels similar to the group without epilepsy. Furthermore, the epilepsy group without diazepam administration showed marked hypometabolism at 7 days, contrary to the group with diazepam. At the neurohistochemical level, differences in inflammation, neurodegeneration and neuronal death were observed even with the administration of diazepam.

In conclusion, diazepam in this model has an effect at the metabolic level, rescuing the differences that occur in the model without diazepam, so its use should not be recommended when the aim of the research is to use FDG-PET as a diagnostic tool in this model, although the neurohistochemical correlate associated with this disorder is maintained.

DOPAMINE D4R RESTORES MORPHINE-INDUCED IMPAIRMENT OF ADULT NEUROGENESIS IN THE SUBVENTRICULAR ZONE

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In the adult mammalian brain, neuroblasts from the subventricular zone (SVZ) migrate along the rostral migratory stream into the olfactory bulb, where they differentiate and synaptically integrate to contribute with the maintenance of the olfactory function. It has been established that endogenous as well as exogenous opioid signalling affects proliferation in adult brains. In fact, chronic administration of morphine reduces adult neurogenesis in SVZ although its implication in addiction has not yet been clarified. On other hand, dopamine has been also identified as a regulatory factor of adult neurogenesis as dopaminergic cells from the substantia nigra compacta project toward the dorsal SVZ whereas the ventral tegmental area innervates the ventral SVZ. Previous results demonstrated that morphine increases striatal dopamine signaling, which is restored by the specific stimulation of dopamine D4 receptor (D4R). The mechanisms by which D4R counteracts morphine effects is not completely understood, but the existence of a D4R-MOR heterodimer in the striosomes of the caudate putamen has been proposed . However, it is unknown how this interaction could affect both the adult neurogenesis and olfaction.

In the present work, we have studied the effects of a chronic treatment with morphine alone or in combination with a D4R agonist (PD168,077) on adult neurogenesis occurring in the SVZ. Furthermore, the impairment or improvement of odorants discrimination has also been analyzed.

Using immunohistochemical techniques, we found that chronic treatment with morphine increases dopamine signalling in the SVZ and promotes a depletion of cell proliferation, affecting both neural and glial precursors. These effects were counteracted by the coadministration of morphine with the D4R agonist. The present results support for a critical role of the D4R to prevent morphine effects in the SVZ.

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DYSFUNCTIONAL M2 CORTEX-SUPERIOR COLLICULUS-BASAL GANGLIA CIRCUIT IN HUNTINGTON'S DISEASE

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Alterations in cortico-Basal ganglia (BG) circuitry are deeply characterized and profoundly compromised in Huntington's Disease (HD). However, little is known about the implications of subcortical-BG circuits in HD. The superior colliculus (SC) is a primitive subcortical sensorimotor area that integrates visual and cortical afferents (particularly from M2-Cortex (M2) and the cingulate) and modulates BG circuitry. Particularly, the cortico-SC-BG circuit is implicated in sensorimotor transformations to direct gaze movements, including the saccadic movements known to be altered in HD patients. Also, we recently described that activating M2 terminals in the striatum using optogenetics is sufficient to restore motor deficits in symptomatic HD mice. Here, we aim to identify and characterize structural and functional alterations of the M2-SC circuits in HD, to finally develop circuit based therapeutic strategies.

Our data shows that symptomatic HD mice (20 week) has predominant approach vs defensive behaviours compared to WT mice, seen by a significant reduction in active escaping response in the looming task and an increase in active approaches in the beetle mania task, indicating alterations in SC function in HD. Moreover, we used in vivo MRI to study functional connectivity from the M2 with BG-related structures at symptomatic stages. Our data demonstrates that the SC is one of the nuclei with higher functional correlation with the M2 in WT mice, and strikingly, significantly diminishes in symptomatic HD mice. Then, we injected AAV-CamKII-GFP in M2 and observed reduced axon terminals arriving to the SC in HD compared to WT mice. We are currently analyzing neuronal response to M2 activation in the SC by combining optogenetics with multielectrode arrays.

Our results describe for the first time the structural and functional involvement of the M2-SC-BG circuits in HD pathophysiology. By using optogenetics, we hope to modulate circuit function and restore symptoms in HD.

DYSREGULATION OF THE AUTOPHAGIC-LYSOSOMAL PATHWAY IN PARKINSON'S DISEASE ASSOCIATED TO GBA

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Parkinson's disease (PD) is characterized by the death of dopaminergic neurons from the substantia nigra pars compacta and the presence in the affected brain regions of intracytoplasmic protein inclusions called Lewy bodies (LB). The main component of LB is α-synuclein, a protein prone to form neurotoxic oligomers and aggregates in a concentration-dependent process. Since increased levels of α-synuclein can promote PD progress, the turnover mechanisms of this protein play an important role in PD development. Particularly, the autophagic-lysosomal pathways have a key role in maintaining proper α-synuclein neuronal levels.

The first genetic risk factor to develop PD is the presence of mutations in the GBA gene, which encodes the lysosomal enzyme glucocerebrosidase (GCase) involved in sphingolipids metabolism. About 12% of PD patients present a mutation in GBA and more than 50% have pathogenic mutations in lysosomal enzymes, emphasizing the key role of the lysosomal function in PD. An inverse relationship between loss of GCase activity and α-synuclein accumulation has been shown in different PD models and in samples from PD patients carrying GBA mutations. This increase in the intracellular levels of α-synuclein is one of the main causes that contribute to neurodegeneration in PD.
We have characterized the role of the autophagic-lysosomal function and the presence of α-synuclein neurotoxic species in a new in vitro model of differentiated dopaminergic-like neurons expressing the two most prevalent GBA mutations, p.N370S and p.L444P, as well as GBA knock out. We observed that loss of GCase activity leads to the cellular and intralysosomal accumulation of GCase substrates, lysosomal dysfunction, macroautophagy and CMA impairment, mitochondrial dysfunction, ROS production, ER stress and ultimately increase of different α-synuclein species.
We are using this cellular model as a valuable in vitro system for the screening of a therapeutic approach based in the restoration of GCase activity in lysosomes through enzyme replacement therapy enhanced with nanotechnology. Restoration of the autophagic-lysosomal system may promote α-synuclein turnover and avoid the accumulation of α-synuclein PD-associated neurotoxic species.

E2F4DN-BASED GENE THERAPY RECOVERS LONG-TERM POTENTIATION AND HIPPOCAMPAL-DEPENDENT MEMORY IN HOMOZYGOUS 5xFAD MICE

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Compelling evidence demonstrates that neurons upregulate the expression of cell-cycle markers when subjected to stress. This is the case in Alzheimer's disease (AD), in which cell-cycle reentry, followed by DNA duplication (neuronal tetraploidization), precedes and recapitulates its neuropathological hallmarks, suggesting that this process participates in the etiology of AD. E2F4, a transcription factor involved in maintenance of quiescence, becomes phosphorylated in APP/PS1 mice and in Alzheimer's patients. We have demonstrated that the phosphorylation of two conserved Thr residues of E2F4 is necessary to induce neuronal tetraploidization and cognitive loss in AD. Therefore, it was developed a novel therapy consisting in neuronal expression of a dominant negative form of E2F4 (E2F4DN), not phosphorylatable. This therapy has been patented (US9567384B2, EP2783696B1, JP6100276B2), and is licensed by Tetraneuron, a Spanish spin-off biotech company.

Initial stages of AD are proposed to be linked to alterations in synapse function, responsible for cognitive deficits associated to the disease. Thus, we hypothesized that viral expression of E2F4DN could correct this synaptic dysfunction. First of all, basal membrane properties and spontaneous synaptic activity (sEPSC) was analyzed through whole-cell recordings in hippocampal primary cultures transduced with a viral vector containing E2F4DN. We demonstrated that it does not alter basal properties of hippocampal neurons. Afterwards we expressed E2F4DN through intravenous administration of AAV-E2F4DN in six-weeks old control and homozygous 5xFAD mice. Synaptic function was measured by electrophysiological recordings of field excitatory postsynaptic potential (fEPSPs) in hippocampal CA3-CA1 synapses. We found that E2F4DN reverts LTP inhibition. Furthermore, this LTP recovery leads to cognitive improvement. 5xFAD mice treated with our therapy present a notably improvement in two hippocampal-dependent memory tasks (novel-object location and contextual fear conditioning). Thus, we report here that E2F4DN-based gene therapy represents a promising approach for AD treatment with capacity to prevent cognitive decline associated to the disease.

EFFECT OF MACROPHAGES ON NEUROSPHERE FORMATION AND NEURONAL DIFFERENTIATION OF NEURAL STEM CELLS

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Among different damages that affect the CNS, traumatic brain injury, spinal cord injury disease and stroke are major worldwide causes of neurological impairment. These conditions exhibit common characteristics, including temporal break-down of the blood-brain barrier, which leads to the extravasation of molecules and blood cells, and the establishment of an inflammatory environment. The major three cell players to rise inflammation are microglia, astrocytes and infiltrated macrophages, which have shown polarization to the pro-inflammatory (type 1) or anti-inflammatory (type 2) phenotypes. Since inflammation can hinder or support neuronal regeneration, in this work we asked whether inflammatory environments mediated by monocyte-derived macrophages might have an influence on neurogenesis. We studied neurosphere formation and neuronal differentiation of NSCs cultured alone or in the presence of macrophages. Macrophages were derived from adult mouse bone marrow and expanded during seven days prior to the experiments. Thereafter, they were polarized to more cytotoxic (IFN-γ, 8 ng/mL, 24 h) or pro-survival (IL-4, 2 ng/mL, 24 h) phenotypes, or remained inactivated. NSCs were obtained from E11 mouse mesencephalon or adult SVZ, and cultured alone or onto a macrophage monolayer, in the presence of EGF and bFGF, to form neurospheres. To induce neuronal differentiation, growth factors were removed. We observed significantly less neurospheres formation in cocultures with macrophages, compared to controls, however, the proportion of neurons generated, by each neurosphere, was about 8x higher in the case of cocultures. Moreover, mesencephalon-derived neurons exhibited a higher proportion of cells immunoreactive for dopaminergic hallmarks (i.e. TH) and increased neurite outgrowth in the presence of macrophages. We did not find significant differences in neurosphere numbers or neuron ratio between conditions containing inactivated macrophages or pre-polarized to cytotoxic or pro-survival phenotypes. Our results suggest that macrophages modulate the neurogenic and differentiation potential of embryonic and adult NSCs. DMAV is supported by CONACYT(#770727).

EFFECT OF THE SRC INHIBITORY PEPTIDE TAT-Cx43$_{266-283}$ ON NEURAL STEM CELLS WITH EGFR OVEREXPRESSION OR EGFRVIII MUTATION

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Glioblastomas are one of the most malignant tumours worldwide. Among the causes of such malignancy is a subpopulation of tumour cells with stem cell properties known as Glioma Stem Cells (GSCs). These cells are resistant to standard treatments, such as temozolomide, which causes tumour recurrence. Several studies have proposed Neural Stem Cells (NSCs) from the subventricular zone (SVZ) as a possible origin for GSCs. The transition of NSCs to GSCs frequently concurs with epidermal growth factor receptor (EGFR) overexpression or mutations, such as EGFRvIII. Our group designed a cell penetrating peptide based on connexin43 (TAT-Cx43$_{266-283}$) that inhibits the activity of the oncoprotein c-Src and therefore targets GSCs, increasing survival rates in glioma-bearing mice. Because Src is involved in EGFR signaling, we aimed to explore the effect of TAT-Cx43$_{266-283}$ in the transition of NSCs to GSCs. For this purpose, we analysed the cell growth of SVZ NSCs (Control-NSCs), NSCs with EGFR amplification (EGFRwt-NSCs) and NSCs with the mutant EGFRvIII (EGFRvIII-NSCs). Our results show that TAT-Cx43$_{266-283}$ specifically inhibited the growth of EGFRwt-NSCs and EGFRvIII-NSCs, without significant effects in Control-NSCs. Importantly, we found that temozolomide and other control peptides did not affect the cell growth of any of these NSCs. To gain insight into the mechanism involved in the effect of TAT-Cx43$_{266-283}$, we analysed the EGFR signaling pathway by Western blot. Our preliminary results show that TAT-Cx43$_{266-283}$ decreased the activity of EGFR and EGFRvIII, as well as c-Src activity. So far, our results indicate that TAT-Cx43$_{266-283}$ impairs EGFR signaling pathway with the subsequent reduction in the proliferation and survival of NSCs that overexpress or exhibit mutations in EGFR. These results stress the relevance of TAT-Cx43$_{266-283}$ as a future therapy against glioblastoma, alone or in combination with temozolomide or other treatments that do not target stem cells with EGFR alterations.

EFFECT OF THE TRANSPLANT TYPE ON RGC NEUROPROTECTION AND AXONAL REGENERATION AFTER OPTIC NERVE CRUSH

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Bone marrow mesenchymal stem cell (BM-MSC) transplantation is widely studied in pre-clinical models because of their potential neuroprotective properties in central nervous system (CNS). However, microenvironmental differences between three transplants (Syn: syngeneic pigmented mouse in pigmented mouse, Allo: allogeneic pigmented mouse in albino mouse, Xeno: xenotransplant, human in pigmented mouse) are unclear and could determinate graft survival and action. Optic nerve crush (ONC) in mice is a well-studied model of neuronal degeneration in the retina, part of CNS, upon which retinal ganglion cells (RGC) die in a progressive way, so at 5 days, 50% of their population has died, and from a 1 month onwards <10% of them remain. We purposed to assess functional and histological differences between syngeneic, allogeneic, and xenotransplant of BM-MSCs after ONC.

Intravitreal injections of 20,000 cells of each transplant were performed after ONC in C57BL/6J and BALB/c mice. As controls matched groups of ONC+vehicle was done. Animals were perfused and flat mounted retinas analyzed at 3, 5 and 90 days after injury. In vivo functional retinal activity and anatomy were measured at 90 days by electroretinogram (ERG) and optic coherence tomography (OCT), respectively. To study axonal regeneration, choleric toxin tracer (CTB) assay was intravitreally injected at 85 days. Brn3a+RGCs were automated quantified and spatial distribution analyzed with isodensity maps.

Only syngeneic transplantation has significant neuroprotective properties from 5 days ($p<0.05$) to 90 days ($p<0.05$). In addition, compared ONC alone, axonal regeneration is significant in the syngeneic model, in contrast to allo- and xeno- transplantation. Finally, xenotransplant of human BMSC is the only that has negative effect in retinal function, decreasing most of ERG waves.

To evaluate graft outcome is not enough to clarify cellular properties but rather to study the microenvironment created after transplantation.

ENDO-LYSOSOMAL DISRUPTION DRIVES MICROGLIAL PHAGOCYTOSIS DYSFUNCTION IN STROKE

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Microglial phagocytosis of apoptotic cells is an essential process to maintain tissue homeostasis and avoid the spillover of the cytotoxic content that results from the cell death induced by excitotoxicity and/or inflammation. We have shown that microglial phagocytosis is chronically impaired in models of neurodegenerative diseases such as epilepsy and stroke, where microglial phagocytosis is blocked as early as 6h after transient Medial Cerebral Artery occlusion (tMCAo), a model of stroke. We hypothesize that microglial phagocytosis impairment in tMCAO was related to an energetic failure and used different in vitro systems to test the role of oxygen and nutrient deprivation (OND). To assess the effect of OND on phagocytosis we first used hippocampal organotypic slices and observed a similar defect in apoptotic cell phagocytosis, which was related to a reduced microglial surveillance and process motility by 2-photon microscopy, likely related to the generalized energetic failure in stroke. The OND-induced phagocytosis reduction rapidly recovered after reperfusion suggesting that, in addition to the acute energetic failure, a more complex mechanism is responsible for the long-term impairment of phagocytosis found in tMCAo mice. We then treated primary microglial cultures with OND and observed phagocytosis deficits, in particular, a reduced degradation of apoptotic cells. This reduction was related to an increased lysosomal pH, possibly as a consequence of alterations in energy-dependent proton pumps that lead to a deficient enzymatic activity. The energetic dysfunction not only affected phagocytosis but also autophagy, another endolysosomal pathway. We assessed autophagy using electron microscopy and found an increase in autophagy-like vesicles, presumably due to stalled autophagosomes related to a deficient lysosomal degradation. To revert the phagocytosis impairment, we tested the autophagy inductor rapamycin, both in vitro and in vivo, to restore the autophagy flux and the altered endolysosomal pathway, and hence, recover the phagocytic activity. Thus, the microglial phagocytic potential opens a novel approach to accelerate the recovery of the ischemic brain by harnessing microglial phagocytosis of apoptotic cells through the stimulation of autophagy.

EXPRESSION OF MICROGLIAL CX3CR1 IN ALZHEIMER'S DISEASE AND ITS REGULATION BY NORADRENALINE

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Noradrenaline (NA) is a major modulatory neurotransmitter of the central nervous system (CNS) and besides its actions as a neurotransmitter it presents a potent anti-inflammatory and neuroprotective effect. The major source of NA in the CNS is the Locus coeruleus (LC) which is one of the earliest brain region affected by Alzheimer's disease (AD). The loss of noradrenergic neurons in LC leads to a decrease in NA levels which facilitates the progression of AD. This seems to be due mainly to the ability of NA to reduce the activation of microglial cells. We previously observed that NA induces the production of the chemokine CX3CL1 in neurons. The activation of microglial CX3CR1, receptor for CX3CL1, reduces the activation of microglia, which is known to contribute to the neuronal damage characteristic of AD. Therefore, alterations of CX3CR1 production in microglia could translate into an alteration of CX3CL1 anti-inflammatory effects.

In order to determine if microglial CX3CR1 production is altered in AD and if NA can regulate it, CX3CR1 expression and synthesis were analyzed in 5xFAD mice and human AD brain samples. In addition, the effects of NA and its reuptake inhibitor reboxetine were analyzed in microglial cultures and 5xFAD mice respectively.

Our results indicate that the production of CX3CR1 is increased in the brain cortex of AD patients and 5xFAD mice. Also, in these mice reboxetine treatment further increases CX3CR1 and enhances microglial reactivity toward amyloid beta plaques. However, administration of NA to cells cultures of primary rat microglia or HMC3 cell line decrease CX3CR1 production, suggesting that microglia responses to NA may be altered in the absence of CX3CL1-producing neurons or other external factors.

FAIM KNOCKOUT LEADS TO GLIOSIS AND LATE-ONSET NEURODEGENERATION OF PHOTORECEPTORS IN THE MOUSE RETINA

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Fas Apoptotic Inhibitory Molecule protein (FAIM) is a death-receptor antagonist and an apoptosis regulator. It encodes two isoforms that have significant neuronal functions, FAIM-S (short) and FAIM-L (long). FAIM-S, which is ubiquitously expressed, is involved in neurite outgrowth. In contrast, FAIM-L is only expressed in neurons and it protects them from cell death. Interestingly, FAIM-L is downregulated in Alzheimer's disease patients and mouse models before the onset of neurodegeneration, and Faim transcript levels are decreased in retinal degeneration mouse models. Nonetheless, few studies have been directed to elucidate the role of FAIM in the central nervous system, yet alone the retina. The retina is a highly specialized tissue that has proved to precede pathological mechanisms of neurodegenerative diseases. Here we describe that Faim depletion in mice damages the retina unrelentingly and leads to late-onset photoreceptor cell death in older mice. Immunohistochemical analyses show that Faim knockout (Faim-/- mice present ubiquitinated aggregates throughout the retina from early ages. Moreover, retinal cells release stress signals that can signal to Müller cells, as shown by immunofluorescence and qRT-PCR. Müller cells monitor retinal homeostasis, and hereafter trigger a gliotic response in Faim-/- mice that becomes pathogenic when is sustained over time. In this regard, we found a pronounced vascular leakage at the latter ages, which can be caused by persistent inflammation. These results suggest that FAIM is an important player in the maintenance of retinal homeostasis and support the premise that FAIM could be a plausible early marker for late photoreceptor and neuronal degeneration.

FAIM-L AS A MODULATOR OF TAU-PATHOLOGY IN ALZHEIMER'S DISEASE AND OTHER TAUOPATHIES

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Alzheimer's disease (AD) is characterized by two main biological hallmarks: beta-amyloid aggregates and the formation of intracellular neurofibrillary tangles (NFTs). NFT formation is linked to neuronal death and cognitive dysfunction. NFTs are composed of abnormally hyperphosphorylated and aggregated Tau protein. Tau, a MAPT protein, is essential to maintain molecular dynamics in mature neurons. Aberrant modifications of Tau, such as phosphorylation, are associated with a reduction in Tau functionality, the formation of the aforementioned NFTs, and the consequent neuronal death. Our laboratory has been focused in the study of the Fas apoptotic inhibitory molecule (FAIM-L). FAIM-L is an anti-apoptotic protein only expressed in neurons. It has also been involved in non-apoptotic functions such as neuronal pruning and axonal degeneration. We have previously reported that FAIM-L is reduced in AD patients and in the APPxPS1 mice model.

In the present study, we show that FAIM-L is only reduced in mice models that show Tau pathology, such as P301S and VLW mice. Other AD models, which only present beta-amyloid pathology, do not present FAIM-L downregulation. In P301S mice, FAIM-L reduction is observed previously to synaptic deficits (3 months) or extensive neurodegeneration (9 months).The mechanism by which aberrant Tau could be reducing FAIM-L levels needs further investigation. Here, we demonstrate an interaction between Tau and FAIM-L and propose that this reduction could be dependent of Tau phosphorylation status. We hypothesize that FAIM-L reduction may enhance the Tau-associated pathology. Using AAVs we aim to overexpress FAIM-L in hippocampus of P301S mice and determine the effect of restoration of FAIM-L levels in the progression of the disease. With this work would like to establish FAIM-L as a possible new therapeutic target in AD and other Tau-related neurodegenerative diseases.

FAMILIAL ALZHEIMER'S DISEASE GENE MUTATIONS REGULATE MITOCHONDRIAL DNA REPLICATION, TRANSCRIPTION AND RELEASE.

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Mitochondrial DNA (mtDNA) encodes proteins that are necessary for the production of cellular energy by the mitochondria. In neurons, shortage of this energy due to mitochondrial dysfunction triggers neurodegeneration. In our previous studies, we reported that subjects with pathogenic mutations that cause familial Alzheimer's disease exhibit low content of cell-free mtDNA (cf-mtDNA) in cerebrospinal fluid (CSF) before the appearance of clinical signs, suggesting that a decrease in the CSF content of cf-mtDNA precedes the clinical signs of dementia. However, the source and the mechanisms of cf-mtDNA release are unclear. Moreover, the molecular mechanisms involved in regulation of mtDNA copy number and release by different genes that cause familiar Alzheimer's disease are unknown. To explore these questions, we have now investigated the effect of APP-Swe/Ind and PSEN1dE9 mutations on mtDNA replication, transcription and release in SH-SY5Y cell clones that permanently express these mutations. We found that either APP-Swe/Ind or PSEN1d9 gene mutations both reduce mtDNA copy number, the amount of L-strand and H-strand mtDNA transcripts per cell, and the release of cf-mtDNA. In addition, we found that pharmacological inhibition of mitophagy enhances the release of cf-mtDNA in control cells, but not in cell clones expressing APP-Swe/Ind and PSEN1dE9 mutations. These results indicate the presence of an mtDNA quality control system independent of mitophagy that is impaired by APP-Swe/Ind and PSEN1dE9 mutations. Moreover, the present results show that alteration of mtDNA dynamics is a common factor of different pathogenic mutations that cause Alzheimer's disease. Supported by SAF2017-89791-R, CIBERNED PI2020/09-04 and CIBERNED CB06/05/0050 grants.

FROM ENGRAMS TO MEMORY PATHOLOGY IN DOWN SYNDROME

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Recently, it has been proposed that memories are stored in a sparse subpopulation of neurons called engram cells, that become functionally connected during learning. The strengthening of their connections upon learning enable these cells to reactivate synchronically during memory recall. Thus, engram cells activated at the same time for a particular event would become the physical representation of the memory trace in the brain. Activity-dependent genetic tagging of engrams allows artificial reactivation or suppression of memories. We studied whether engram alterations can account for memory deficits in cognitive disorders. We propose that aberrant engram cells contribute to memory deficits in Down syndrome (DS), the most common form of intellectual disability. We found sparser neuronal activation during the acquisition of a contextual-fear memory in the hippocampus in a trisomic mouse model for DS (Ts65Dn), suggesting that less cells would be supporting a given memory. However, artificial reactivation of trisomic engram cells did not rescue the Ts65Dn memory deficits. We also found that astrocytes could contribute to memory deficits in Ts65Dn mice. Specifically, astrocyte activation using DREADDs depressed hippocampal synaptic transmission. Our results suggest that memory deficits in DS might be contributed by impaired “engram allocation”, in which new players, such as astrocytes might also participate.

FUNCTIONAL AND MORPHOLOGICAL STUDY OF TRANSIENT ISCHEMIA-REPERFUSION IN ADULT PIGMENTED MICE

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Purpose: Transient ischemia-reperfusion (I/R) induced by acute elevation of the intraocular pressure (IOP) is a commonly employed method to study acute angle-closure glaucoma. Here, we assess various ischemic and survival intervals with functional and histological techniques.

Methods: I/R was induced in C57BL/6J mice by inserting a 30G needle in the anterior chamber of the left eye connected to a 500mL bottle of 0.9%NaCl at a high of 150cm, to increase the IOP from 8 to ≈90 mmHg. Four intervals of ischemia were studied (45', 60', 75' or 90') (n=4 each group) and 3 days later mice were perfused, their retinas flat-mounted and immunolabelled against Brn3a to identify retinal ganglion cells (RGCs). We also studied the effects of 60' I/R: in vivo retinal function by electroretinograms recordings at 3, 7, 14, and 21 days after injury (n=12) and ex vivo the survival of several retinal populations at 3, 7, 14 or 21 days, including RGCs, horizontal cells and S-cone and L/M cone photoreceptors; identified with Brn3a, calbindin, S- and L/M-Opsin antibodies, respectively.

Results: At 3 days of 45' of I/R the Brn3a+RGC population remained intact, however after 60', 75' or 90' the Brn3a+RGC population it was reduced to 75, 37.5 and 12.5 percent, respectively. At 7 days after 60' of I/R, Brn3a+RGCs decreased to 50%, and remained unchanged at 21 days. Horizontal cells and S-cones remained unchanged at every time interval examined. L/M cones diminished significantly at 7 days (≈70%) and further decreased to 58% at 14 days without further changes at 21 days. Functional analysis shows a permanent decrease in all waves studied, from 3 to 21 days after I/R.

Conclusions: Pigmented mice retina shows a damage threshold between 45' and 60' minutes of I/R for RGC survival. 60' of I/R leads to a rapid loss of innermost retina that from 3 to 7 days, the horizontal cells are not affected, while in the outer retina, L/M cones are affected but S cones are not.

FUNCTIONAL EPI-GENOMICS UNVEILS NEW RISK GENES AND TREATMENTS FOR ALZHEIMER´S DISEASE

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Alzheimer's disease (AD) is a complex disorder caused by a combination of genetic and non-genetic factors, which are investigated by genome- (GWAS) and epigenome- (EWAS) wide association studies, respectively. Combining the strengths of both type of studies, we have recently identified a new genetic-epigenetic interaction on Peptidase M20 Domain Containing 1 (PM20D1) associated with AD. We showed that PM20D1 expression depends on a haplotype-dependent chromatin loop between PM20D1 enhancer and promoter regions, that PM20D1 expression is increased by AD-like stressors, upregulated in AD mice and non-risk human haplotype carriers, and that its overexpression improves cognitive performance and reduces AD pathologies.

However, the precise mechanism by which PM20D1 exerts its protective role in AD remains largely unknown. PM20D1 facilitates the condensation of fatty acids and amino acids generating a series of compounds named N-acyl amino acids (NAAs). NAAs are present in all tissues, including brain, notwithstanding, little is yet known about their function and regulation. To investigate their role in AD, we NAA treated AD primary cultures, worms and mouse models, and measured AD-related pathologies and cognitive performance, and to unveil the underlying mechanisms, we applied scRNA-seq approaches and cell-type specific manipulations. Following this approach, we demonstrated that NAAs are effective in multiple cell-types and AD models, improving cognitive performance and amyloid pathologies, and activating neuronal pro-survival and microglia amyloid-clearance mechanisms.

Our approach and results not only supports the use of NAAs as a therapeutic approach for AD, but also broaden current experimental and therapeutic strategies, which is sorely needed to alleviate the burden of this devastating disease.

FUNCTIONAL SELECTIVITY OF SEROTONIN 5-HT2A RECEPTOR DRUGS ON GAI1-PROTEINS IN POSTMORTEM HUMAN BRAIN CORTEX

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Cell culture and animal studies have demonstrated that both hallucinogenic and non-hallucinogenic 5-HT2A receptor (5-HT2AR) agonists promote Gαq/11-protein activation. In contrast, only hallucinogenic drugs acting at 5-HT2ARs activate inhibitory Gαi1-proteins. Whether these mechanisms operate in brain tissue remains unknown.

The activation of 5-HT2AR coupling to Gαi1- and Gαq/11-proteins induced by hallucinogenic ((±)DOI and LSD) and non-hallucinogenic (lisuride and pergolide) 5-HT2AR drugs was evaluated at 10 μM concentration in post-mortem human brain cortex. The effects of different 5-HT2AR antagonist/inverse agonists (pimavanserin, altanserin, nelotanserin, eplivanserin, volinanserin and ketanserin) on both pathways were also analysed. Modulation of the [35S]GTPγS binding coupled to antibody immunoprecipitation by using the Scincillation Proximity Assay (SPA) technology was chosen as methodological approach.

(±)DOI and LSD increased the [35S]GTPγS binding to both Gαi1- and Gαq/11-proteins by a mechanism sensitive to selective 5-HT2AR antagonists (MDL-11,939, ketanserin and altanserin). Other inhibitory Gαi/o-protein were also stimulated but less sensitive to 5-HT2AR antagonists. Moreover, equivalent stimulatory effects on both pathways were observed in brain of wild-type mice and disappeared in 5-HT2AR knock-out animals. The non-hallucinogenic drugs lisuride and pergolide behaved as 5-HT2AR agonists exclusively on the Gαq/11-protein pathway. On the other hand, pimavanserin, altanserin, and volinanserin exerted an inhibitory response on the [35S]GTPγS basal binding to Gαi1-proteins that was sensitive to MDL-11,939 and absent in 5-HT2AR knock-out mice, confirming their inverse agonist properties on this 5-HT2ARs pathway. Regarding 5-HT2AR coupling to Gαq/11-proteins, only volinaserin displayed inverse agonism.

These results demonstrate a differential profile of G-protein signalling between hallucinogenic and non-hallucinogenic 5-HT2AR agonists in human brain cortex. The functional coupling to Gαi1-proteins may be involved in the hallucinogenic liability of (±)DOI, LSD and other 5-HT2AR agonists. These results also suggest that selective inverse agonism contributing to uncoupling of 5-HT2ARs from Gαi1-proteins could be a new pharmacological approach in the development of new antipsychotic drugs.

FUSΔ14 MUTATION CAUSES CHANGES IN LIPID METABOLISM IN MICE WITH MOTOR AND COGNITIVE ALTERATIONS

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Amyotrophic Lateral Sclerosis (ALS) is a lethal neurodegenerative disease characterised primarily by the progressive loss of motor neurons in the spinal cord and cerebral cortex. There are alterations in the regulation of lipid metabolism in ALS but the origin of those remains unknown. Mutations in FUS (Fused in sarcoma) are causative of ALS and forms of cognitive impairment and dementia. The FUSDelta14 mutation causes aggressive juvenile cases of ALS. The mouse model of physiological expression of mutant FUSDelta14 in heterozygosity causes very mild and late onset motor neuron degeneration with no other major phenotypes. The homozygous mice die at birth. We aim to generate homozygous mice and evaluate the effect of the mutation in the regulation of lipid metabolism and its impact in motor and cognitive phenotypes.

We generated FUSDelta14 homozygous mice in a defined F1 hybrid background of C57Bl/6JxDBA/2J. We use both males and females for this study and conduct a comprehensive set of metabolic, motor and cognitive tests at early age. We also performed RNA-sequencing of frontal brain, spinal cord, white and brown fat, muscle and liver from wild type and homozygous FUSDelta14 mice.

Homozygous FUSDelta14 mice are smaller than the heterozygous and wildtype littermates. These mice show systemic metabolic dysregulation with altered glucose tolerance, increased fat depots, higher blood cholesterol levels and lower resting energy expenditure. Their brains are also thinner and have reduced marble burying capacity and indifference in the recognition of noble objects. Homozygous mice develop mild motor alterations evidenced by altered electromyogram. A systemic transcriptomic analysis of several tissues confirmed the lipid metabolic alterations phenotype observed.

We show that FUSDelta14 mutation causes early central lipid metabolic alterations that affect cognitive and motor phenotypes, placing lipid metabolism dysregulation as one of the main pathological mechanisms in the ALS-FTD spectrum of disorders.

GABAB-RECEPTOR ACTIVATION PARTIALLY RESTORES NETWORK DYSFUNCTION ASSOCIATED WITH NMDA-RECEPTOR HYPOFUNCTION

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Cognitive deficits and impaired sensory processing are hallmarks of neurodevelopmental and neuropsychiatric disorders, such as schizophrenia and autism. N-Methyl-d-aspartate receptor (NMDAR) hypofunction is considered to be involved in causing these deficits by disrupting excitation-to-inhibition balance in neuronal circuits. Activation of GABAB-receptor is hypothesized to restore this imbalance, but its effect on translational electrophysiological biomarkers is poorly understood.

Here we studied the physiology of limbic-auditory circuits under pharmacological NMDAR blockade (using MK-801) and GABABR activation (using Baclofen) in freely-moving rats, implanted with electrodes in key brain areas. The pharmacological effects were assed on several translational readouts from resting-state EEG, auditory-evoked oscillations and mismatch-negativity paradigms, evaluated through power spectral, coherence and event-related estimates.

Across brain areas, MK-801 increased the power of both spontaneous and auditory-evoked brain oscillations, mainly in the gamma and higher frequency range. Baclofen partially normalized this aberrant oscillatory activity. Furthermore, coherence analysis indicated that functional coupling in auditory-limbic circuits is substantially altered by MK-801, such as increased coupling of the frontal cortex with the auditory cortex and the amygdala in the theta band. For higher frequency bands (e.g. high gamma), coherence was increased in a network comprising frontal cortical regions and thalamic nuclei. Baclofen normalized coherence between frontal cortex and amygdala and augmented the MK-801 induced increase of gamma coherence in thalamic and thalamocortical circuits. Additionally, we found clear deficits in auditory mismatch responses under MK-801, involving both prediction error and adaptation components across limbic-auditory brain regions. Baclofen did not restore these deficits but even impaired mismatch responses when applied alone.

GABABR activation partially restores oscillatory changes associated with NMDAR-hypofunction, but had a detrimental effect on functional coupling and context-dependent auditory processing. Our results indicate that GABABR agonists may be of limited therapeutic value for neurodevelopmental disorders associated with NMDAR hypofunction.

GENERATION AND CHARACTERISATION OF NEW MOUSE MODELS OF TDP-43 PROTEINOPATHIES INCLUDING A NEW GENOMICALLY HUMANISED KNOCK-IN STRAIN

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TDP-43 (encoded by the TARDBP gene) is the main component of the pathogenic inclusions that define the great majority of cases of amyotrophic lateral sclerosis (ALS) and ~50% of frontotemporal dementia (FTD) cases. Moreover, mutations in TARDBP are causative of ALS and/or FTD. Despite its critical role in these neurodegenerative diseases, it is not yet clear how TDP-43 dysfunction leads to neurodegeneration.

To try to recapitulate as faithfully as possible human ALS disease pathogenesis, we have a long-term interest in creating Knock-in (KI) mouse models of ALS. Here, we present the characterization of a novel TDP43 mutant Knock-in mouse line carrying a pathogenic point mutation within the C-terminal domain of TDP43 (Q331K). We include behavioral analysis of this strain on a B6 and B6/DBA background as well as cellular and molecular analysis of derived primary cell lines. As other C-terminal mutations, the Q331K mutation leads to a splicing gain of function, as well as stress response abnormalities and dysfunction in pathways previously associated with ALS pathogenesis, such as the PI3K pathway.

In addition, we present the generation and initial molecular characterisation of the first genomically humanized TDP-43 (hTARDBP) mouse model, on which we have replaced the mouse Tardbp gene for its human TARDBP counterpart, from the start to the stop codon, including all introns in between, producing the first mouse model expressing a human TDP-43 protein under mouse endogenous physiological expression control.

GENERATION AND CHARACTERIZATION OF HUMAN PLURIPOTENT STEM CELL (hPSC**)-DERIVED ASTROCYTES TO MODEL ALZHEIMER´S DISEASE**

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Alzheimer disease (AD) is characterized clinically by memory loss and pathologically by amyloid-β (Aβ) accumulation, neurofibrillary tangle formation, extensive neuroinflammation, synaptic toxicity and neurodegeneration. Recent studies highlight the importance of glial cells on the pathogenesis and progression of AD. Among glial cells, astrocytes are fundamental for maintaining homeostasis and protecting neurons but, under different pathological conditions, when stimulated by specific factors, acquire different activation states that can be protective or harmful. While it is well established that astrocytes undergo profound alterations in gene expression, morphology and function during the course of AD, such changes are still poorly defined and mostly unknown in the case of human astrocytes.

To analyze human astrocyte reactive states in the context of AD, we are using the stem-cell technology to generate astrocytes derived from human pluripotent stem cells (hPSCs) and in vitro models of AD in which astrocytes are exposed to various Aβ challenges. Human astrocyte identity as well as reactivity after Aβ stimulation are being characterized at molecular and functional levels with various assays.

hPSC-derived astrocytes at day in vitro 90 express main markers of astrocytes (GFAP, S100, EAAT1, EAAT2, Vimentin and AQP4) without expression of neuron (MAP2) and oligodendrocyte (O4) markers. After stimulation with oligomeric Aβ, hPSC-derived astrocytes show a reactive profile that we are characterizing.

In sum, our approach allows exploration of the human astrocyte reactivity on an AD context and will provide insights into the contribution of astrocytes to the pathophysiology of AD.

GENERATION OF GRIN-RELATED DISORDERS ZEBRAFISH MODELS LIBRARY FOR ENDOPHENOTYPIC CHARACTERIZATION AND PHARMACOLOGICAL SCREENING

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NMDA ionotropic glutamate receptors play pivotal roles in synaptic development, plasticity, neural survival, and cognition. Recent advances on Next-Generation Sequencing revealed the association of de novo mutations affecting GRIN genes (encoding GluN subunits of the NMDAR) with neurodevelopmental disorders, so-called GRIN-related disorders (GRD) or Grinpathies. GRD is a rare condition with a clinical spectrum dictated by both the affected GRIN gene and the functional outcomes of the mutated residue/s, primarily affecting glutamatergic neurotransmission and causing synaptopathies. Accordingly, generation of an in vivo library is required, in order to cover the genetic aetiologies underlying GRD phenotypes, to delineate the neurological alterations and ultimately to identify personalized therapeutic approaches for GRDs. In the context of GRD, zebrafish appears as an optimal animal model, since it provides several advantages from biomedical and industrial points of view (e.g. fast generation of genetic models, multidimensional phenotyping and reliable in vivo pharmacological screening).

To address this objective, CRISPR-Cas9-based genome editing technology has been applied for the obtention of knockout models of Zebrafish paralogous GRIN1, GRIN2A and GRIN2B genes. Single mutant larvae showed no effect on survival rate, and allowed to define the spatio-temporal expression pattern of Grin genes in Zebrafish larvae. Interestingly, in addition to a prominent expression in the brain, NMDAR subunits were also detected in peripheric organs (e.g. heart, intestine). Currently, phenotypic assessment is being performed in pharmacological acute GRD models that revealed the presence of both behavioural and motor endophenotypes. In the short term, the comprehensive phenotyping of Zebra-GRIN models will allow to define GRD-like alterations and, importantly, to evaluate the therapeutic efficacy of repurposed and EMA-approved putative NMDAR allosteric modulators, to ultimately allow personalized therapies for GRD patients.

GENE-REGULATORY DYNAMICS OF MICROGLIA STATES DURING NEUROINFLAMMATION

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Gene expression and genetic variation studies suggest an important contribution of microglia cells to the onset and progression of most prevalent neurodegenerative conditions, such as Alzheimer's disease (AD), where a putative diversity of microglia states with divergent homeostatic or pathophysiological roles has been proposed. However, very little is known on the mechanisms regulating the ability of these highly plastic cells of the brain to adopt specialized roles when exposed to different conditions. Here, we combine high-throughput genomics and in situ RNA expression analysis at the single-cell level with functional assays to reveal the molecular underpinnings of the transitions and maintenance of the distinct phenotypic and functional states of brain's innate immune cells through the initiation, activation and resolution of the neuroinflammatory response. These data deepen our understanding of microglia heterogeneity which is critical to devise new therapeutics for most prevalent neurodegenerative diseases.

HIGH-FAT FEEDING SHIFTS THE GUT MICROBIOME AND ACCELERATES RETINAL DEGENERATION IN RETINITIS PIGMENTOSA MICE

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High-fat diets (HFD) can lead to shifts in the gut microbiota and metabolic alterations and have been associated with increased risk of cognitive dysfunction. Here, we analyze the effects of a HFD on the gut microbiome and the neurodegenerative process in an animal model of retinitis pigmentosa (RP). Wild-type C57BL/6J and rd10 mutant (RP) mice were fed either with normal chow (5.5% fat kcal) or with a HFD (61.6% fat kcal) for two weeks after weaning (P19). At the endpoint, retinal function was evaluated by optomotor test and electroretinography. The structure and integrity of the retina were studied by immunohistochemistry. The gut microbiome was analyzed by 16S rRNA gene sequencing. Rd10 mice showed diminished retinal responsiveness and visual acuity, loss of photoreceptor rows, and morphologic anomalies in the remaining photoreceptor cells, compared to C57BL/6J mice. Photoreceptor degeneration was associated to proliferation of microglial cells and reactive gliosis of Müller cells. The gut microbiome analysis revealed differences between healthy and dystrophic mice in alpha and beta diversity at the genera, species and amplicon sequence variants levels. HFD consumption produced significant dysbiosis in the gut microbiome of both wild-type and mutant mice, increasing potentially pro-inflammatory bacteria. In wild-type mice, HFD consumption did not affect retinal structure and function. But HFD intake by rd10 mice decreased retinal responsiveness and visual acuity, increased photoreceptor degeneration, and exacerbated microglial cell activation and Müller cell gliosis. Also, HFD consumption enhanced the expression of inflammatory and cell death markers in rd10 retinas. In conclusion, retinal degeneration in retinitis pigmentosa is linked to significant changes in the gut microbiome, which can be altered by the diet, leading to deterioration of the disease. The results suggest that increased consumption of fat could aggravate the progression of the disease in patients with retinal degenerative disorders. MINECOFEDER-BFU2015-67139-R, MICIINN-FEDER PID2019-106230RB-I00, RETICS-FEDER-RD16/0008/0016, IDIFEDER/2017/064

HUMAN AMYGDALA INVOLVEMENT IN ALZHEIMER'S DISEASE REVEALED BY MALDI IMAGING AND SWATH ANALYSIS.

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Alzheimer's disease is characterized by executive dysfunction and memory impairment, underlying accumulation of extracellular amyloid-β and intracellular hyperphosphorylated tau. Amygdala atrophy have been shown to be pronounced in early stages of Alzheimer's disease, with correlation between the degree of atrophy and disease severity. Due to the early damage to the amygdala, personality changes often precede other clinical symptoms of the disease, such as cognitive impairment. Moreover, the amygdala constitutes a key that may contribute to the spreading of pathologic molecules due to its vast connectivity with other brain regions. These findings hypothesizing the amygdala as a central participant in Alzheimer's disease pathology. State-of-the-art -omic approaches would allow multilevel analysis but are currently underdeveloped. Proteomic studies on human tissue would be especially suitable to identify peptide fingerprint changes in human amygdala during pathology.

Post-mortem tissue was provided by IDIBAPS, BTCIEN, BIOBANC-MUR, BPA and NAVARRABIOMED Spanish National Biobanks. Experimental procedures were approved by Ethical Committee of Clinical Research at Ciudad Real University Hospital (SAF2016-75768-R and PID2019-108659RB-I00). A total of 16 cases were used for MALDI Imaging and SWATH analysis. Proteomic analyses consisted in PCA, heatmap, volcano plot and detection of activated/deactivated pathways. MALDI Imaging reflected differences in distribution of up/downregulated proteins identified by SWATH Analysis.

The study was sponsored by the UCLM/ERDF (2020-GRIN-29145 to NPND), Spanish Ministries of Economy and Competitiveness/ERDF (grant no. SAF2016-75768-R) and Science and Innovation (grant no. PID2019-108659RB-I00) to AMM and Autonomous Government of Castilla- La Mancha/ERDF (grant no. SBPLY/17/180501/000430) to AMM and DSS). MGR and SVC held a predoctoral fellowship granted by UCLM/ESF and VAL held an assistant professorship granted by UCLM/ERDF. Authors thank Dr. Pilar Alberdi (technical specialist UCLM/ERDF supported) her proteomic expertise.

IMMATURE OLIGODENDROCYTES WITH R-RAS1 AND R-RAS2 DEFICIENCY PRODUCE AXONAL DEGENERATION

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Fast synaptic transmission in vertebrates is critically dependent on myelin for insulation and metabolic support. Myelin is produced by oligodendrocytes that maintain multilayered membrane compartments that wrap around axonal fibers, and alterations in myelination can therefore lead to severe pathologies such as multiple sclerosis. Given that hypomyelination disorders have complex etiologies, reproducing clinical symptoms of myelin diseases from a neurological perspective in animal models has been difficult. We recently reported that R-Ras1-/- and/or R-Ras2-/- mice, which lack GTPases essential for oligodendrocyte survival and differentiation processes, present different degrees of hypomyelination in the Central Nervous System with a compounded hypomyelination in double knockout (DKO) mice. Here, we discovered that R-Ras1 and/or R-Ras2 loss of function is associated with aberrantly myelinated axons with increased numbers of mitochondria and a disrupted mitochondrial respiration that leads to increased ROS levels. As a consequence, aberrantly myelinated axons are thinner with cytoskeletal phosphorylation patterns typical of axonal degeneration processes characteristic of myelin diseases. Although we observed different levels of hypomyelination in single mutant mice, the combined loss of function in DKO mice lead to a compromised axonal integrity triggering a loss in visual function. Our findings demonstrate that R-Ras loss of function reproduces several characteristics of myelin diseases, and we therefore propose that R-Ras1-/- and R-Ras2-/- neurological models are valuable approaches for the study of myelin pathologies.

IMMUNODENSITY OF DOPAMINE D2, CANNABINOID CB1, METABOTROPIC GLUTAMATE mGlu2 AND mGlu3 RECEPTORS IN SCHIZOPHRENIA SUBJECTS

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Background: Dysregulation of dopamine D2 (D2R), cannabinoid CB1 (CB1R) and/or metabotropic glutamate 2/3 (mGlu2/3R) receptors may contribute to the pathophysiology of schizophrenia. The aim of this study was to quantify the immunodensities of D2R, CB1R, mGlu2R and mGlu3R in post-mortem brains of schizophrenia subjects.

Material and methods: Brain samples from the dorsolateral prefrontal cortex (DLPFC; Brodmann's area 9) of subjects with schizophrenia (n=48) and sex-, age-, and post-mortem interval-(PMI) matched controls (n=48) were obtained at autopsies performed in the Basque Institute of Legal Medicine. Schizophrenia cases were divided into antipsychotic-treated (AP+; n=29) and antipsychotic-free (AP–; n=19) groups, according to blood toxicological screening at death. Receptor cortical amounts were estimated by Western blot (WB). Antibody selectivity was validated in knockout mice lacking target receptors. Case-control datasets were compared by paired t-tests.

Results: The immunodensities of both mGlu2R (–31%; $p<0.001$) and CB1R (–17%; $p<0.01$) were significantly lower in schizophrenia, compared to those in control brains. Regarding the antipsychotic treatment, CB1R downregulation was only observed in AP+ cases (–18%; $p<0.05$), whereas mGlu2R expression was reduced both in AP– cases (–27%; $p<0.05$) and more robustly in AP+ subjects (–34%; $p<0.001$). In contrast, D2R and mGlu3R cortical amounts did not differ significantly between cases and controls.

Conclusions: The present results indicate that low mGlu2R protein expression might be a core feature of the schizophrenia brain, which could be further aggravated following antipsychotic treatment. On the other hand, downregulated CB1R levels might be attributed to antipsychotic medication. Surprisingly, D2R and mGlu3R protein expression did not seem to be altered in the DLPFC of schizophrenia subjects. However, this does not imply that the functional signalling of these receptors is not altered. Future studies will determine the implications of mGlu2R dysregulations in schizophrenia brain functioning.

IMPACT OF TAUOPATHIES ON THE FUNCTIONAL ORGANIZATION OF NEURONAL CULTURES

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Tau protein aggregates are a key element in the development of certain neurodegenerative diseases, defined as tauopathies. This type of diseases includes relevant pathologies such as Alzheimer's Disease (AD) which affects more than 47 million people worldwide [1]. When Tau becomes pathological it might induce neurodegeneration through different mechanisms [2], causing a disruption of synaptic communication and, therefore, changing the functional organization of the neuronal circuit. Due to sheer size of the brain, and the difficulty to monitor tauopathies at a cellular scale, an interesting approach is to use in vitro neuronal circuits derived from mouse brain. Here we use such idea to understand tau-induced functional alterations in vitro and to develop strategies to block tau action and protect the neuronal circuits.

To prepare the neuronal cultures, cortical mouse embryos tissue is mechanically dissociated and planted in glass covers. Cells are infected with an adeno-associated virus, GCamp-6, a calcium marker, at day in vitro 1 (DIV 1) and treated with Tau and different control conditions every time the medium is changed. Spontaneous activity is registered every from DIV 7 to DIV 16 with the help of a fluorescence microscope attached to a hight-speed camera. Data then is analysed applying MATLAB routines developed in Soriano's Lab.

We found that the tau-treated, pathological neuronal cultures exhibit a tendency towards an excessive synchronization, which we associate to the damage or loss of activity-regulatory mechanisms in the neuronal networks.

The project leading to these results has received funding from "la Caixa" Foundation (ID 100010434) under the agreement LCF/PR/HR19/52160007. Its main objective is to determine the molecular mechanism involved in different tauopathies, characterise the spread of Tau in neuronal circuits and develop possible selective treatments that slow or prevent the spread of Tau molecular aggregates.

IMPACT OF WHITE ADIPOSE TISSUE IN AD PATHOLOGY

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-Alzheimer's disease (AD) is a complex disorder and multiple cellular and molecular mechanisms are involved in AD onset and progression. Recent evidences has suggested that metabolic alterations are an important pathological feature in disease progression in AD. Likewise, diabetes and obesity, two mayor metabolic illnesses, are risk factors for AD. In addition, novel studies has suggested that AD induces peripheral metabolic alterations, facilitating the development of diabetes. Overall, these studies suggest that there is an important two-way crosstalk between AD and peripheral metabolic disorders. Here, we seek to understand the mechanisms underlying this association and we hypothesize that the white adipose tissue may serve as a key communicator organ between the brain and peripheral metabolic illnesses, and alterations in this organ may affect both types of disorders.
-Here, we used histological stains, immunohistochemistry and biochemical means to determine changes in the white adipose tissue from WT and 3xTg-AD mice. Moreover, similar techniques were used in the brain of 3xTg-AD mice that received white fat pads from WT and 3xTg-AD donors to determine any changes in amyloid and tau pathology.
-Our study shows that 3xTg-AD mice develop significant peripheral metabolic alterations which in turn affected the white adipose tissue biology. Moreover, adipose tissue transplanted from donor 3xTg-AD and WT mice into recipient 3xTg-AD mice indicate that AD associated white fat tissue induced profound AD pathology changes in recipient 3xTg-AD mice.
-Overall, our study demonstrate a novel important crosstalk between AD and peripheral metabolic disorders thought white adipose cells. A more profound understanding in these processes may turn in novel and promising therapeutic strategies for AD and metabolic illnesses.

INCREASED SEROTONIN 5-HT2A RECEPTOR CONSTITUTIVE ACTIVITY ON GAI1-PROTEIN IN POST-MORTEM FRONTAL CORTEX OF SUBJECTS WITH SCHIZOPHRENIA

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In animal and cellular models, activation of Gαi1-proteins by serotonin 5-HT2A receptor (5-HT2AR) agonist drugs represents a molecular fingerprint of hallucinogenic properties. On the other hand, supersensitive 5-HT2AR signalling through Gαi1-, but not Gαq/11–proteins, has been observed in schizophrenia. If this supersensitivity is consequence of altered constitutive activity of 5-HT2ARs in schizophrenia, is still unknown. Currently, inverse agonists of 5-HT2ARs are available, which allows the analysis of constitutive activity conditions. In this context, pimavanserin has been described as selective 5-HT2AR inverse agonist on the Gαi1-protein-mediated pro-hallucinogenic pathway and neutral antagonist on the canonical Gαq/11-protein-mediated pathway, in human prefrontal cortex.
[35S]GTPγS binding assays associated to immunoprecipitation with specific antibodies for Gαi1- and Gαq/11-proteins were carried out with pimavanserin (10-10-10-6 M). The selectivity of pimavanserin on 5-HT2ARs was measured by using the antagonist MDL-11,939 (10 µM). Moreover, the selectivity was confirmed by using brain samples of wild-type (5-HT2AR(+/+)) and knock-out (5-HT2AR(-/-)) mice. Post-mortem frontal cortex samples from 23 subjects with schizophrenia and their matched controls were analysed.
Pimavanserin induced a higher inhibitory effect on 5-HT2AR coupling to Gαi1-proteins in schizophrenia subjects (Imax=20±2%) than in controls (Imax=14±1%) (p=0.0004). Pimavanserin did not induce effects on 5-HT2AR coupling to Gαq/11-proteins (Imax=0±1% and Imax=-1±1%, respectively). Pimavanserin activity on Gαi1-proteins was sensitive to MDL-11,939 confirming the involvement of 5-HT2ARs. Moreover, pimavanserin exerted a significant inhibition of the coupling to Gαi1-proteins (Imax=13±2%, n=6) in 5-HT2AR(+/+) mice, while no effect was found in 5-HT2AR(-/-) mice. No effect of pimavanserin on Gαq/11-proteins in mice brain tissue was observed.
These findings demonstrate an enhanced constitutive activity of 5-HT2ARs through the pro-hallucinogenic pathway in prefrontal cortex of subjects with schizophrenia. The constitutive 5-HT2AR hyperactivity could underlay the vulnerability to psychotic symptoms and suggest the relevance of the inverse agonism on 5-HT2ARs as new anti-psychotic pharmacological strategy.

INFLAMMATION AND AUTOPHAGY IN GLYCOGEN-INDUCED NEURODEGENERATION

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Lafora disease (LD) is a fatal childhood-onset dementia characterized by the extensive accumulation of glycogen aggregates—the so-called Lafora Bodies (LBs)—in several organs. The accumulation of LBs in the brain underlies the neurological phenotype of the disease. LBs are composed of abnormal glycogen and various associated proteins, including p62, an autophagy adaptor that participates in the aggregation and clearance of misfolded proteins. Our results demonstrate that p62 participates in the formation of LBs and suggest that the sequestration of abnormal glycogen into LBs is a protective mechanism through which to reduce the deleterious consequences of its accumulation in the brain (1). Until recently, it was widely believed that brain LBs were present exclusively in neurons and thus that LD pathology derived from their accumulation in this cell population. However, we have demonstrated that LBs are also present in astrocytes. Strikingly, impeding LB accumulation in astrocytes prevents the increase in neurodegeneration markers, autophagy impairment, and metabolic changes characteristic of LD in a mouse model. Conversely, mice that overaccumulate glycogen in astrocytes show an increase in these markers. These results unveil the deleterious consequences of the deregulation of glycogen metabolism in astrocytes and change the perspective that LD is caused solely by alterations in neurons (2).

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INHIBITION OF RECEPTOR PROTEIN TYROSINE PHOSPHATASE B/Z PREVENTS DECREASES ON HIPPOCAMPAL NEUROGENESIS INDUCED BY ACUTE ETHANOL IN MALE AND FEMALE ADOLESCENT MICE BUT ONLY DECREASES ETHANOL INTAKE IN MALE MICE

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Pleiotrophin (PTN) is a cytokine that has been shown to modulate ethanol drinking and reward. PTN modulates neuroinflammation in different contexts. PTN is an endogenous inhibitor of Receptor Protein Tyrosine Phosphatase (RPTP) β/ζ. Pharmacological inhibition of RPTPβ/ζ reduces binge-like drinking in mice. We hypothesize that RPTPβ/ζ also plays a role in chronic ethanol consumption and in the neural alterations associated with ethanol.

Male and female adolescent C57BL/6J mice were used in an intermittent access to ethanol (IAE) model using a 2-bottle choice protocol. Before each drinking session, mice received an administration of MY10 (60 mg/kg, i.g.), a small-molecule inhibitor of RPTPβ/ζ, or vehicle as control, and ethanol consumption was measured. At the end of the 4-weeks IAE protocol, brains were removed for immunohistochemistry analysis of Iba-1, GFAP and doublecortin (DCX). In the acute ethanol experiments, mice were treated with MY10 one hour before the administration of 6 g/kg ethanol (i.p.). Eighteen hours after ethanol administration, brains were dissected and subjected to the same immunohistochemistry analysis.

Male mice treated with MY10 drank less ethanol than controls and showed a reduced preference for the ethanol solution in the IAE model. In contrast, MY10 did not seem to have relevant effects on ethanol intake in female mice. This effect of MY10 was not accompanied by significant alterations in glial responses. In acute ethanol experiments, we observed an overall increased activation of microglia in female adolescent mice, especially in those treated with MY10 and ethanol. Acute ethanol induced a significant decrease on hippocampal neurogenesis in both male and female adolescent mice, which was completely prevented by pre-treatment with MY10 in both sexes.

In summary, inhibition of RPTPβ/ζ significantly reduced ethanol consumption in the IAE model only in male mice. RPTPβ/ζ critically modulates ethanol-induced decreases on hippocampal neurogenesis in both male and female adolescent mice.

INTERMEDIATE ALLELES IN HTT GENE MAY PLAY A ROLE IN SPORADIC TAUOPATHIES

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In Huntington´s disease (HD), the pathological condition occurs when the number of repeats exceeds a critical threshold (>35 CAG). However, sometimes these expansions remain within an intermediate range (27-35 CAG), denominated intermediate alleles (iaHTT), normally associated with normal phenotype. We previously analysed the association between iaHTT and neurodegenerative diseases other than HD. Interestingly, we found that the frequency of iaHTT is significantly higher among Alzheimer´s disease patients (AD; n=1126) regarding control subjects (n=509) (6.03% vs. 2,9%, respectively), suggesting that iaHTT might have a role in the pathogenesis of AD. Additionally, in a cohort of frontotemporal lobar degeneration patients (FTLD; n=225) there was an increased frequency of iaHTT regarding controls (5.3% vs. 2.9%), although the association was not statistically significant. Thus, the first aim of this study was to increase the number of the FTLD cohort. Therefore, we analysed the presence of iaHTT, by blood cells genotyping, in 440 FTLD patients. Although we found a nonsignificantly increased frequency of iaHTT in FTLD patients, results organization by clinical subgroups revealed that only progressive nonfluent aphasia subjects (PNFA) showed a significant difference of iaHTT regarding controls (13.6% vs. 2.9%, respectively). These data suggest that the presence of iaHTT may play a role in the development of tauopathies, such as AD and PNFA, since no association was found in Parkinson's disease patients (n= 610). The second aim was to determine whether the presence of iaHTT implies the existence of HTT protein aggregates in patients with tauopathy. To this end, we performed sequential biochemical fractionation of protein aggregates in post-mortem amygdala samples from both iaHTT AD and FTD patients, and their corresponding controls. Immunoelectron microscopy studies suggest that, in iaHTT patients, small HTT aggregates may exist in the same fractions where positive phosphorylated Tau protein filaments are present. Further studies are needed to determine whether the presence of iaHTT alters the physiology of Tau or other toxic proteins, which could explain its role as a pathogenic risk factor.

INVOLVEMENT OF THE NEUROPEPTIDE CORTISTATIN IN NEUROINFLAMMATION AND BLOOD-BRAIN BARRIER DYSFUNCTION IN ISCHEMIC STROKE

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Ischemic strokes are the result of a permanent or transient occlusion of a major brain artery. The energy/oxygen deprivation that follows leads to irreversible tissue injury and long-term sequelae. Despite continuous improvements, therapeutic failure is still notorious and strokes remain the second cause of death worldwide. Therefore, many studies advocate that better stroke management requires focusing on endogenous neuroprotective mediators that globally modulate the immune response, rather than approaching pathogenic mechanisms independently. In this context, we study cortistatin (CST), a neuropeptide widely distributed in the Central Nervous System and the Immune System. This molecule displays anti-inflammatory, immunomodulatory and neuroprotective properties, characteristics that potentially make it an attractive endogenous target and a novel therapeutic agent for strokes. Hence, we are currently investigating its involvement in the immune dysregulation and neuroinflammation processes associated with strokes, as well as its possible therapeutic application. For this purpose, we use the MCAO (middle cerebral artery occlusion) model by temporarily (30') occluding the middle cerebral artery in WT and CST-deficient mice (CST-/-). Our preliminary results suggest more susceptibility to stroke development and worse prognosis in CST-/- mice 24h post-MCAO, i.e. larger neuronal lesions, higher neurological score and a striking acute glial activation. Moreover, blood-brain barrier (BBB) dysfunction in CST-/- mice may be also contributing to this great susceptibility, as shown in a BBB in vitro model, where the CST-/- endothelial cells barrier showed increased permeability and reduced and altered tight-junctions/adherens-junctions expression. Altogether, our results suggest the relevance of endogenous CST modulating neurodegeneration and neuroinflammation events during ischemic damage and its key role in the BBB structural and functional viability. Moreover, our findings add evidence supporting that recovering CST levels by its exogenous administration may be a novel multifunctional therapeutic agent for the treatment of ischemic strokes.

KINETIC AND DISTRIBUTION OF NANOEMULSIFIED ALPHA-TOCOPHEROL IN AGEING MOUSE MODELS

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Vitamins, especially the alpha-tocopherol (αT) isoform of vitamin E (VitE), have been widely studied as an anti-ageing agent due to its well-known antioxidant properties. αT is highly sensitive to the action of external agents and its action is reduced by the presence of biological barriers, moreover it shows an erratic and irregular oral absorption due to its lipophilic characteristic. In this work, we had tested a αT nanoemulsion developed in our group after a single oral dose administration in C57BL/6J, SAMR1 and SAMP8 mice. After administration, mice were sacrificed and blood, liver, brain and lung samples were collected. Blood profile and tissue distribution of αT were analyzed by means of an optimized and validated LC-ESI-MS/MS method. The results revealed that the nanoemulsion increase αT levels in plasma, serum and liver compared to free αT and the senescence mice model (SAMP8 and SAMR1) present higher concentration of αT compared to C57BL/6J mice. However, as expected the results obtained in brain samples were completely different. In all groups of mice αT levels remained constant, none of the formulations administrated managed to increase αT concentration after a single dose administration. Considering that the development of nanostructures has made possible to increase the bioavailability of these compounds in blood and liver, further studies are needed to increase αT concentration in the brain in order to improve the neuroprotection through chronic administration.

LACTOPEROXIDASE MIGHT BE A PATHOGENIC FACTOR IN PARKINSON´S DISEASE

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Introduction. Hemeperoxidases have been proposed to play a pathogenic role in neurodegenerative diseases. Lactoperoxidase (LPO), an antimicrobial hemeperoxidase, has been reported by our group to be enhanced in the cerebrospinal fluid (CSF) in patients with Parkinson disease (PD). The objective was to look at the relationship of LPO in the CSF and serum with clinical features of idiopathic PD. Material and methods. LPO concentration was analyzed through ELISA techniques. Correlation of CSF or serum LPO and MDS-UPDRS, dopaminergic medication, and other clinical parameters and scales was examined. Results. The findings revealed that LPO concentration in the CSF, not serum, was found to be elevated in patients with PD relative to controls ($p<0.001$). CSF LPO concentration negatively correlated with MDS-UPDRS part-IV score ($p<.0001$), a rating scale that allows evaluating motor complications; and with the dose intensity of the dopaminergic medication regimen, as evaluated with levodopa equivalent dose or LED (mg/day; $p<.0001$). Conclusions. CSF LPO is found to be elevated in the CSF of PD patients, and CSF LPO holds promise as potential biomarker for diagnosis of PD. Increasing the dose intensity of dopaminergic medication regimen attenuates the elevation in LPO levels in the CSF. The pathophysiological mechanisms that seem to be responsible for LPO increase would include dopamine deficiency, oxidative stress, and less likely, microbial infection.

LIPID METABOLISM DYSREGULATION IS AN EARLY AND PROGRESSIVE PATHOLOGICAL MECHANISM IN THE SPINAL CORD OF TRANSGENIC SOD1 MICE

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Amyotrophic lateral sclerosis (ALS) is a multifactorial and multistep fatal degenerative disorder. There are several pathological mechanisms leading to motor neuron death, although there are many unknowns in the disease aetiology of ALS. Alterations in lipid metabolism are well documented in the progression of ALS. Both patients and animal models have significant metabolic dysregulation such as hypermetabolism, dyslipidemia and progressive weight loss. In the spinal cord of mouse models and ALS patients there are alterations of lipid metabolites, including oxysterols, ceramides and sphingolipids. The origin of these lipid metabolic alterations remains unclear. We aim to identify early lipid metabolic pathways altered before disease symptoms in the spinal cord of mouse model of ALS. We performed a transcriptomic analysis of the spinal cord of SOD1-G93A and wild type littermate mice at pre-symptomatic disease stage and identified several altered genes involved in the regulation of lipid metabolism. We expanded our study conducting a transcriptomic meta-analysis combining RNA-seq studies from the spinal cord of SOD1 mice at different stages of disease: three pre-symptomatic at 90 days, and three symptomatic at 120-150 days. The meta-analysis identified several lipid metabolic pathways dysregulated from pre-symptomatic that were progressively worsening at symptomatic disease stage. The cholesterol biosynthesis and transport, ceramide catabolism, and eicosanoid synthesis were the main lipid metabolic pathways altered from early stages. Here, we present an insight into the pathological mechanisms in ALS, supporting that lipid metabolic alterations are central to ALS aetiology, which opens new options for the treatment of these devastating conditions.

LOCAL PROTEIN SYNTHESIS IN HEALTH AND DISEASE

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Neurons are able to respond to changes in their environment by producing proteins locally. Lopcal protein synthesis plays relevant roles in growth cone behavior and synapse formation during embryonic development, and in axon maintenance by regulating mitochondrial function in adulthood. Importantly, a role for local translation in mediating amyloid-induced neurodegeneration, a hallmark for Alzheimer´s disease, has recently been described. Although most localized mRNAs are believed to be transported to distal neurites (dendrites and axons) from the somatodendritic compartment, recent evidence suggests that some neuritic transcripts might be delivered to neurons by glial cells. Among the mechanisms proposed for this horizontal transfer, is the secretion extracellular vesicles (EVs) by glia and subsequent internalization into axons. These extracellular vesicles contain RNAs and proteins which might be involved in translation regulation.

One of our aims is to determine the contribution of glial EVs in the ability of neurites (axons in particular) to translate proteins locally in models of amyloid-induced pathology. Ultimately we wish to understand if EVs play a role in neurological disorders such Alzheimer´s disease, by regulating intra-axonal signaling events that require local translation.

Experiments performed in primary cultures thus far indicate that EVs from different origins (neuronal, glial, mixed.) elicit distinct effects on local translation depending on whether they are secreted by healthy cells or in the context of amyloid-induced pathology.

While analyzing the effect of glia on local translation in neurons we realized that glial cells (microglia in particular) was able to produce proteins locally. This event has only been recently reported in this cell type and its role in brain pathology has not been addressed. Thus, we established a second line of research in which we aim at deciphering the role of local protein synthesis in glia itself in nervous system physiology and pathology.

Here we summarize the results gathered so far in the context of the maion research lines of our lab.

MALDI IMAGING IN MICE BRAINS REVEALS NOVEL PEPTIDES SIGNATURES ASSOCIATED TO THE PROGRESSION OF ALZHEIMER`S DISEASE THAT CAN BE REVERSED BY UBIQUINOL

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Oxidative stress (OS) is a prodromal signature associated to the debut and progression of Alzheimer's disease (AD). OS is often described as a self-propagating phenomenon caused by an imbalance between oxidant and antioxidant systems, namely an overproduction of reactive oxygen species (ROS), which overwhelms the intrinsic antioxidant defenses. In AD, there is a growing evidence supporting the link between ROS-mediated damage and mitochondrial dysfunction, with endoplasmic reticulum (ER) stress and proteostasis imbalance. Our previous work demonstrated the protective role of ubiquinol (Ubi), the reduced form of coenzyme Q10 (CoQ10), in the 3xTg-AD mice model of AD, preventing hypoxia and amyloid-β (Aβ) peptide deposition in hippocampal and neocortical areas at onset (6 months) and advanced (12 months) stages of disease in animals fed with Ubi-supplemented diets from premorbid (2 months) ages. Using a similar approach, 2-month-old wild-type and 3xTg-AD mice were fed with standard or Ubi-supplemented diet, up to 6- and 12-month. After performing behavioural tests, animals were perfused, brains were included in paraffin and sections were analyzed and visualized by MALDI Imaging. Proteins inferred from significantly altered m/z peaks were analyzed and clustered with MEV4 and Metaboanalyst 5.0, followed by FunRich and STRING. Results were validated by immunofluorescence and confocal microscopy in hippocampal and cortical areas. Behavioral tests showed an Ubi-dependent rescue in the AD-associated cognitive decline. MALDI-Imaging analysis revealed age-dependent differential peptide signatures in the 3xTg-AD model vs. wt mice in hippocampal and cortical areas, being altered those related to protein translation, ROS production and ER stress, among others. Our results indicate that Ubi-supplemented diets could delay AD progression in the 3xTg-AD model by reducing OS and maintaining the proteostasis balance.

MALE SEX BIAS IN PARKINSON'S DISEASE IS LINKED TO AN ACCELERATED AGE-DEPENDENT NEUROMELANIN ACCUMULATION

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Men have a higher incidence and prevalence of Parkinson's disease (PD), earlier disease onset, more severe motor symptoms and progression, and more frequent cognitive decline compared to women. However, most PD studies do not consider the influence of sex, thus the molecular mechanisms underlying sex differences in PD remain unknown. Sex steroids modulate dopaminergic pathways, in both normal and pathological states, and estrogens improve PD symptoms in both men and women. Estrogens are also able to modulate melanin production in the skin and we have recently reported, in both humans and experimental animals, that excessive age-dependent intracellular neuromelanin accumulation above a pathogenic threshold triggers PD pathology. Here we assessed whether differences in neuromelanin production/accumulation could underlie the differential effect of sex on PD. First, using postmortem human brain tissue, we found that intracellular neuromelanin levels within nigral dopaminergic neurons from age-matched control subjects are significantly higher in men than in women and that men reach earlier the pathogenic threshold of neuromelanin accumulation, even in absence of overt PD. We then assessed the effect of sex on the only rodent model currently available of age-dependent neuromelanin production within PD-vulnerable neurons, based on the viral vector-mediated expression of melanin-producing enzyme tyrosinase (AAV-hTyr) in the substantia nigra of rats. This model, developed by our group, exhibits major PD features in parallel to progressive neuromelanin accumulation. We observed that AAV-hTyr-injected male rats exhibit an earlier and greater accumulation of neuromelanin compared to female animals, reaching earlier the pathogenic threshold of intracellular neuromelanin accumulation. Remarkably, ovariectomized (OVX) female rats injected with AAV-hTyr accumulated neuromelanin more rapidly than non-OVX female animals and ultimately reached pathological neuromelanin levels similar to their male counterparts. These results suggest that an increased/accelerated accumulation of neuromelanin in men across life may underlie their higher risk to develop PD, compared to women.

MATERNAL SEPARATION DECREASES THE EXPRESSION OF DOUBLECORTIN IN THE OLFACTORY SYSTEM OF BOTH MEPC2-HETEROZYGOUS FEMALE MICE AND THEIR HEALTHY CONTROLS

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Mutations in the X-linked gene MECP2 are the main cause of Rett syndrome, a rare disease affecting females, and other neurodevelopmental disorders. This gene codes for methyl CpG binding protein 2, a transcriptional regulator expressed in neurons. Our previous data show that the density of immature neurons, expressing doublecortin, is significantly higher in the piriform cortex, but not in the olfactory bulbs or the hippocampus, of symptomatic Mecp2-mutant mice, as compared to their age-matched wild-type controls. Since previous pieces of evidence suggest an effect of early life stress in neuronal maturation, here we sought to analyze the impact of maternal separation, and its interaction with Mecp2 deficiency, in the expression of doublecortin in the postnatal brain. To do so, we performed a double immunofluorescent detection of doublecortin and NeuN, a marker of mature neurons, in adolescent Mecp2-heterozygous and wild type female mice that were either subjected to 3 hours of maternal separation from postnatal day 3 until weaning, or left undisturbed with the dam. The effects of genotype and type maternal care were analyzed using the classical frequentist ANOVA and Bayesian inference. Both statistical analyses revealed a significant increase in doublecortin expression in the olfactory tubercle in Mecp2-heterozygous females irrespective of maternal care. By contrast, doublecortin expression was decreased in the piriform cortex and granular cell layer of the olfactory bulbs of females subjected to maternal separation, irrespective of their genotype. Similarly, the percentage of doublecortin/NeuN cells in the periglomerular layer of the olfactory bulbs was decreased in both groups of maternally-deprived females. Finally, we found no effect of either genotype or maternal deprivation in doublecortin expression in the dentate gyrus of the hippocampus. Our results suggest that early environmental intervention could help rescuing region-specific deficits in neuronal maturation in Mecp2-mutant mice. Funded by Ministerio de Ciencia e Innovación (PID2019-107322GB-C22).

MICROGLIA ARE KEY REGULATORS OF THE INNATE ANTI-TUMOURAL RESPONSE IN LATE ADULTHOOD

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Glioblastoma and metastatic brain tumours are incurable diseases with short life expectancy. Microglia are the resident macrophages of the Central Nervous System, accounting for up to the 30% of the total tumour mass. They show an immune suppressive response supporting cancer growth. In general, microglia can adopt different states of polarisation, although the reasons of the phenotypic switch are not fully understood. However, it is known that cancer cells drive the phenotype of surrounding immune cells into a pro-tumoural state.
In turn, during aging, microglia evolve into a 'priming' state with clear pro-inflammatory features. This inflammatory phenotype has been widely associated with the onset of different neurodegenerative diseases. Nevertheless, in the context of brain tumours, we hypothesise that the incidence of brain tumours at the old age is significantly reduced mainly because of the pro-inflammatory microenvironment generated by aged-microglia.
To prove that, we have used in vitro mouse models of microglia (BV-2), glioblastoma (GL261) and breast cancer brain metastasis (EO771). We have also performed in vivo studies to quantify the tumour burden in aged mice (18 and 24 months) compared with young mice (3 months).
We show by qPCR, western blot and immunohistochemistry how senescent microglial cells develop a strong pro-inflammatory response once exposed to tumour-conditioned media. In the same vein, we have seen a significantly reduction of the tumour size in aged mice. Key pro-inflammatory biomarkers (e.g. iNOS and TNFα) were significantly up-regulated not just in the tumour microenvironment but also in the tumour-associated aged microglia.
To the best of our knowledge, this is the first empirical demonstration of how senescent microglial cells are associated with the reduction of brain tumours growth. Unraveling the molecular mechanisms behind the pro-inflammatory activation of senescent microglial cells could lead us to potential new treatments to modulate the immune response against brain tumours.

MiR-138 AS A RESTORATIVE THERAPY FOR SPINAL CORD INJURY

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Injury of the spinal cord triggers a set of damaging events that spread cell death to unaffected tissue. Apoptosis among spinal neurons begins few hours after injury and proceeds during weeks. On the other hand, injury to the spinal cord (SCI) alters the local expression of multiple microRNAs. These are short RNA sequences that inhibit the expression of hundreds of genes to regulate cell state and fate. MicroRNA-based therapies for SCI are currently under study because the available evidence indicates that microRNA dysregulation contributes to the onset of processes such as astrogliosis. Previous in silico analysis showed that miR-138-5p, a microRNA highly enriched in the central nervous system, targets components of apoptotic cell death pathways, such as caspase-3, caspase-7 and Bak-1.

We hypothesize that miR-138-5p downregulation after SCI contributes to the overexpression of apoptotic genes, therefore sensitizing neurons to death, and that a therapy restoring physiologic levels of miR-138-5p will reduce neuronal apoptosis after SCI. The aims of this work are to evaluate the changes in expression of miR-138-5p and its apoptotic targets and to test the neuroprotective effect of miR138-5p overexpression. To accomplish these objectives we used histological, cellular and molecular methods to measure miR-138-5p and protein expression in neurons after damage.

Gene expression data reveals that downregulation of miR-138-5p during the first week after injury coincides with the upregulation of apoptotic proteins. In situ hybridizations of spinal cord samples reveal that miR-138-5p is highly expressed in neurons and it is downregulated after SCI, particularly evident among neurons in the perilesional regions. Finally, the transfection of miR-138-5p mimic in primary neuronal cultures reduces the effector caspases activity and is neuroprotective against apoptosis induced by L-glutamic acid.

Our results suggest that a miR-138-5p-based therapy may be a potential candidate as treatment of SCI.

MiR-182-5p AND miR-138-5p REGULATE NOGO-A/NOGO RECEPTOR EXPRESSION, PROMOTING NEURITE OUTGROWTH IN NEURAL CELLS

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During postnatal development, central nervous system (CNS) neurons lose their ability to regenerate in part due to the presence of myelin-derived inhibitors of neurite outgrowth and neuroregeneration. Nogo-A is the most important myelin-associated inhibitor in CNS. This protein is expressed on the surface of oligodendrocytes and mediate the inhibition of axonal outgrowth through binding to its receptor, Nogo receptor (NgR), located in CNS neurons. The activation of this pathway at lesion sites may explain the lack of axonal regeneration in the CNS after trauma in adult mammals. Previous studies have shown that blockage either Nogo-A or NgR leads to growth cone formation and promotes neurite outgrowth.

MicroRNAs (miRNAs) regulate important processes in CNS such as neuronal differentiation, neuritogenesis, excitation, synaptogenesis, and plasticity. Two of these miRNAs are miR-182-5p (miR-182) and miR-138-5p (miR-138). Studies demonstrated that miR-182 promotes axonal growth and regulates neurite outgrowth via the PTEN/AKT pathway in cortical neurons, whereas miR-138 regulates axon growth during development and regeneration by targeting SIRT1.

In this study, we used luciferase reporter assays to demonstrate that miR-182 and miR-138 target Nogo-A and NgR mRNAs, respectively. Accordingly, both miRNAs downregulate the protein expression of their targets in different neural cell lines, such as Neuro-2a, C6 and PC12. Furthermore, we demonstrate that both miRNAs promote neurite outgrowth of neural cells in vitro. Specifically, the co-culture of miR-182 transfected C6 cells with rat primary hippocampal neurons increased neurite length of neurons in comparison with its negative control miRNA. Moreover, NgR downregulation by miR-138 in PC12 cells also increased neurite lengths. In conclusion, these miRNAs could be a potential miRNA-based therapy for the treatment of CNS traumatic injuries such as spinal cord injury.

MITOCHONDRIAL DYSFUNCTION AND NEUROTOXICITY INDUCED BY FRATAXIN DEFICIENCY IN ASTROCYTES ARE ATTENUATED WITH THE SONIC HEDGEHOG AGONIST SAG

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Friedreich´s ataxia (FRDA) is predominantly a neurodegenerative disease caused by a deficiency in frataxin (FXN). FXN is a protein with a major mitochondrial localization enriched in tissues with a high-energy demand, like the brain. These low FXN levels lead to a progressive degeneration of neurons of the spinal cord, brainstem and the deep cerebellar nuclei, responsible for the loss of movement coordination and equilibrium, main symptoms observed in FRDA patients.
As for other neurodegenerative diseases, increasing evidence supports the idea that other non-neuronal cells like astrocytes are actively involved in the FRDA neurodegenerative process. Depending on the stimuli they respond to, astrocytes acquire different activation states in a process called astrogliosis, where neuroinflammatory stimuli induce the formation of A1 reactive astrocytes, which upregulate proinflammatory genes, being harmful for neurons. Recent studies have demonstrated positive effects of Sonic Hedgehog (SHH) agonists in astrocyte viability and proliferation, astrocyte-mediated neuroprotection, and also positive effects in mitochondrial activity and dynamics. Thus, we have thoroughly characterized astrocyte reactivity phenotype and mitochondrial status of FXN-deficient astrocytes, evaluating as well the effect of SHH agonists on astrocyte reactivity, viability, and function.
We have observed that FXN-deficient astrocytes have reduced cell viability and higher expression of several A1 reactive astrocyte markers. Moreover, FXN-deficient astrocytes also showed defects in mitochondrial function and dynamics. All these alterations were prevented by a chronic treatment with the smoothened agonist (SAG), a SHH signaling agonist. Regarding the possible neuroprotective effects of SHH agonists, previous results showed that FXN-deficient astrocytes are able to induce neurodegeneration, and we have observed that the chronic treatment with SAG attenuated the neurotoxicity triggered by the treatment of mouse cortical neurons with conditioned medium of FXN-deficient astrocytes.
Overall, our results indicate that astrocytes might be considered as key players in the neurodegenerative process associated with FRDA, suggesting as well that the treatment of FXN-deficient astrocytes with a SHH agonist like SAG, could be used as a possible target to reduce FRDA-associated neurodegeneration.

MODELING PARKINSON'S DISEASE WITH THE ALPHA-SYNUCLEIN PROTEIN

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The aggregation of alpha-synuclein (α-Syn) in form of Lewy bodies and neurites is the main neuropathological hallmark of Parkinson's disease (PD). PD is characterized by the loss of dopaminergic neurons in the substantia nigra. Precisely, the dysfunctionality and degeneration of these neurons is associated with α-Syn aggregation. Moreover, mutations in the SNCA gene, which encodes α-Syn, cause familial forms of PD and are the basis of sporadic PD risk. Animal models that reflect the dopaminergic neuronal loss and the widespread and progressive α-Syn aggregation constitute a valuable tool for studying the molecular mechanisms of the disease and might contribute to the development and validation of new therapies. Here, we summarize the main features of the α-Syn protofibrils (PFFs) models and recombinant adeno associated viral vector (rAAV) mediated α-Syn overexpression models, providing a detailed comparative analysis of both models. To characterize these PD models, we use SNCA-OVX mice injected with human PFFs of α-Syn and wild type mice injected with viral particles containing human mutated E46K α-Syn. Our results show that injection of α-Syn PFFs and overexpression of α-Syn mediated by rAAV lead to a different pattern of PD pathology in rodents. First, α-Syn PFFs model lead to the formation of Lewy body-like inclusions in brain regions directly interconnected with the injection site, suggesting that there is an inter-neuronal transmission of the α-Syn pathology. In contrast, rAAV-mediated α-Syn overexpression in the brain limits the α-Syn aggregates within the transduced neurons. Second, phosphorylated α-Syn inclusions obtained with rAAV are predominantly nuclear with a punctate appearance that becomes diffuse along the neuronal fibers, whereas α-Syn PFFs model lead to the formation of cytoplasmic aggregates of phosphorylated α-Syn reminiscent of Lewy bodies and neurites.

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MORPHOLOGICAL AND STEREOLOGICAL STUDY OF NEURONS AND INTERNEURONS IN THE NON-HUMAN PRIMATE STRIATUM

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The striatum is composed of projection neurons, the medium spiny neurons (MSN), and of a small population of interneurons which modulate and control the striatal output. Most interneurons are GABAergic; they are divided into several subtypes based on their immunostaining for various proteins such as parvalbumin (PV), calretinin (CR), neuropeptide Y, somatostatin, nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH) and tyrosine hydroxylase (TH). There is an additional non-GABAergic interneuron subtype, the cholinergic interneurons which expressed choline acetyltransferase (ChAT). Most information on the striatal neuronal populations has been gathered in rodents (3-5%), whereas the evidence in the primate striatum is scarce and fragmented (6-26%). Thus, up to date there is no accurate stereological data on the absolute number of each neuronal subtypes in primates. This raises a question: is this figure accurate for the primate brain?

Performing histological staining for each molecular marker, we provide the morphological description and the distribution pattern in the control non-human primate for each striatal neuronal population caudate, putamen and ventral striatum. We also have used unbiased stereological methods on consecutive sections where densities of projection neurons and subpopulations of interneurons were calculated to obtain a general landscape of the proportion of striatal neurons and interneurons.

We report a gradient of striatal neuronal subtypes, being the most abundant the MSN projection neurons, followed by CR+, PV+, NADPH+ and ChAT+ and TH+ interneurons. We have estimated the percentage of interneurons in the whole macaque striatum as a 14%, resembling the relative proportion of striatal interneurons in humans reported previously and significantly higher than in the rodent striatum. The presented data is important for the understanding of striatal circuits and reinforce the importance of considering the relative presence of the different neuronal populations to draw functional conclusions.

MORPHOMETRIC CLUSTER ANALYSES OF SIBLING NG2-CELLS IN RESPONSE TO MULTIPLE SCLEROSIS LESION MODELS

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NG2-cells, also known as oligodendrocyte precursor cells (OPC), are a heterogeneous glial cell population. This heterogeneity is still under debate and whether it can be driven by intrinsic factors, such as their ontogenic origin, remains unclear. Nevertheless, the NG2-glia display different functions during the development and after brain damage. At this respect, we recently revealed the heterogenic response of NG2-glia clones to experimental autoimmune encephalomyelitis (EAE) brain damage. Now, we sought to analyze the relationship between both their morphology and ontogeny in the adult brain and their changes in two different Multiple Sclerosis (MS) models' scenarios. To address this question, we combined the StarTrack, a multicolor genetic lineage-tracing tool that enables the long-term in vivo tracing of progenitor lineages, along with a single-cell morphometric cluster analysis, in the two MS murine models induced by EAE and Cuprizone, to compare with control brains. We correlated the reactivity, with different morphometric parameters measured in the derived NG2-cell progeny of StarTrack targeted single progenitors. Data from the morphometric analysis allowed us to unravel different NG2-cell clusters sorted by morphological parameters related, not just by their ontogenic origin, but also related to the different MS lesions. In summary, a better understanding of NG2-glia heterogeneity is relevant to decipher the physiological role of these cells both in healthy brain and in response to MS.

NATIVE AND NITRATED A-SYNUCLEIN, AND PATTERNS OF NITRO-A-SYNUCLEIN-POSITIVE INCLUSIONS IN SALIVA AND SUBMANDIBULARY GLAND IN IDIOPATHIC PARKINSON'S DISEASE

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Background. Salivary α-synuclein (aSyn) and its nitrated form or 3-nitrotyrosine-α-synuclein (3-NT-αSyn) hold promise as biomarkers for idiopathic Parkinson's disease (IPD). The objectives are to study the levels and clinical correlations of native and nitrated αSyn in saliva and submandibulary glands in patients with IPD and controls. Material and methods. Salivary and serum αSyn, and 3-NT-αSyn level is evaluated with ELISA and immunoblots. Correlations of native αSyn and 3-NT-αSyn, and clinical features of the disease are examined. Submandibulary gland sections are analyzed. Results. A) Salivary concentration and saliva/serum ratios of native and nitrated αSyn are found to be similar in patients and controls; b) salivary αSyn and 3-NT-αSyn do not correlate with any clinical feature; c) three patterns of 3-NT-αSyn-positive inclusions are observed in histological sections: rounded "Lewy-type" aggregates of 10-25 µm in diameter, and coarse aggregates with varied morphology that are all located within the interlobular connective tissue of the gland, and spheroid bodies of 3-5 µm in diameter in the cytoplasm of duct cells. Lewy-type inclusions are only observed in IPD patients, and the remainder aggregates are observed in the patients and controls. Conclusions. The patients´ saliva presents similar concentration of native αSyn and nitrated αSyn than controls, and no clinical correlations with clinical features are found. These findings preclude the utility of native and nitrated αSyn as biomarkers. "Lewy-type" inclusions expressing 3-NT-αSyn are observed only in patients, a novel finding which suggests that biopsy of submandibulary glands, if proven safe, could be a useful technique for diagnosing IPD. Finally, to our knowledge, this is the first description of 3-NT-αSyn-immunoreactive spheroid bodies within the cytoplasm of duct cells. These bodies are present in the submandibulary gland sections from all subjects regardless their pathology, and they could be related to aging.

NEURAL MIGRATION IS IMPAIRED IN THE APP/PS1 ALZHEIMER'S MICE MODEL DUE TO INCREASE SENESCENCE IN MIGRATING PRECURSORS CELLS

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Adult neurogenesis occurs in two neurogenic niches: the ventricular-subventricular zone (V-SVZ) and subgranular zone (SGZ). The V-SVZ is composed by a heterogeneous cellular population: neural stem cells (B cells), intermediate progenitors (C cells), young migrating neurons (A cells), ependymal cells and mature astrocytes. V-SVZ zone can generate new neurons, oligodendrocytes, and astrocytes, which migrate to an adequate position. New neurons mainly migrate to olfactory bulbs (OB) throughout the rostral migratory stream (RMS). In Alzheimer's disease, both olfactory loss and neurogenesis impairment have been described at a very early state.

We aimed to investigate neurogenesis and neuronal migration in the V-SVZ in a murine model of Alzheimer's disease, the APP/PS1 mice model. We measured the levels of proteins involved in senescence (□-gal), apoptosis (Smac-DIABLO), neural progenitors (DCX and PSA-NCAM) and mature neurons (NeuN) and in some cases, we determine their co-localization by immunofluorescence technique and we obtained microscopy images from the V-SVZ, RMS and OB; also we use flow cytometry for cell cycle characterization.

Our results showed that migrating precursors neurons were accumulated in the V-SVZ in the APP/PS1 mice model. Furthermore, we found an increase of cells in the G1 phase and a decrease in the S phase in V-SVZ from APP/PS1 mice. We observed that the accumulated migrating cells were in a senescence state, while an important proportion of astrocytes suffered apoptosis in the V-SVZ of the APP/PS1 mice. Finally, we determined that in the RMS from APP/PS1 mice there were fewer migrating neurons than in WT; and in the olfactory bulb of APP/PS1 mature neurons decreased. These results indicate a failure in the neural migration.

To sum up, we conclude that neural migration is impaired in a mice model of Alzheimer's disease probably contributing to the early symptoms of the disease.

NEUROCLUEDO: WHICH, WHEN AND WHERE NEURONS DIE AFTER SPINAL CORD INJURY?

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Neuronal death is a central event of spinal cord injury (SCI) pathophysiology and a major determinant of the resulting functional deficits. Neuronal loss depends on features of the injury (type, severity, location), on the temporal and spatial context relative to insult, and on specific features of the neurons. Multiple articles have quantified neurons in the damaged spinal cord using different approaches but there is neither access to the original images nor a reference method for neuron detection after SCI. Here we present NeuroCLUEDO, an Open Science Framework repository aimed to determine which, where and when do neurons die after SCI. NeuroCLUEDO comprises a documented image repository of spinal cord sections stained with neuronal markers, test benchs for comparing methods in the naïve and injured spinal cords, and repositories of the obtained results. In the first analysis at NeuroCLUEDO we have uploaded raw images from a study of our laboratory testing the effects of a neuroprotective drug (ucf-101) in a mice model of traumatic SCI and use them to compare the effects of employing manual-, semiautomatic- and AI-based methods of cell detection on the number and location of identified neurons. Results from the analysis of 20 full sections of naïve, contused and treated mice spinal cords reveal that the number of identified neurons broadly agrees among the compared methods but that agreement is very poor concerning their position, that is, methods are not counting the same neurons despite the total number is similar. Interestingly, the comparison of manual identification data from different researchers revealed limited repeatability and reproducibility in both the number and position of the neurons. Both the repository and the test bench are open to anyone and contributions either as manual identifications or as new identifications methods are welcome.

NEURONAL EXPRESSION OF E2F4DN ATTENUATES THE IMMUNE RESPONSE OBSERVED IN THE CEREBRAL CORTEX AND HIPPOCAMPUS OF 5xFAD MICE

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Alzheimer's disease (AD) is a neurodegenerative disorder in which altered immune response is an important etiological factor. The transcription factor E2F4 participates in tissue homeostasis and regulates gene networks affected in AD, thus constituting a potential target for intervention. We have studied whether neuronal expression of a dominant negative form of E2F4 (E2F4DN), unable to become phosphorylated in a Thr motif that controls its activity, can modulate the immune response observed in AD. To this aim, we generated Mapt:E2F4DN knock-in mice (E2F4DN mice) that, together with control Mapt:EGFP knock-in mice (EGFP mice), were crossed with 5xFAD mice, a known murine model of AD. Neuronal expression of E2F4DN in 5xFAD mice led to reduced astrogliosis (i.e. area occupied by GFAP) at 3 months of age, both in cortex and hippocampus. In addition, Iba1-positive cells exhibited reduced size in the cortex and hippocampus of 5xFAD/E2F4DN mice at 3 and 6 months, suggesting that microglia activation is attenuated by the presence of neuronal E2F4DN. To analyze the crosstalk between E2F4DN expressing neurons and microglia, we cultured microglial cells with conditioned media from E2F4DN- or EGFP-expressing neurons. The differences in morphology observed suggest that neuron-microglia communication occurs via soluble factor. In vivo, most Iba1-positive cells of 5xFAD/E2F4DN mice were associated to amyloid beta (Aβ) deposits, which were increased in size, but not in number at 3 months of age. Moreover, neuronal expression of E2F4DN slowed down the accumulation of Aβ at 6 months of age. We speculate that the crosstalk between E2F4DN-expressing neurons and microglia favors the aggregation of oligomeric Aβ at early stages of AD, thus reducing its toxicity, and attenuates Aβ deposition at later stages. Altogether, our data are consistent with a beneficial immune response in 5xFAD mice expressing neuronal E2F4DN, which we propose as a therapeutic agent against AD.

NEUROPROTECTIVE EFFECT OF REMOTE ISCHEMIC PERCONDITIONING AND POSTCONDITIONING IN A PRECLINICAL MOUSE MODEL OF ACUTE ISCHEMIC STROKE

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Remote ischemic conditioning (RIC) is an endogenous procedure that reduces ischaemic injury by repeated transient mechanical obstruction of vessels at a remote limb from the injury site. It represents a new paradigm in neuroprotection with unknown mechanism of action. This study aimed to evaluate the neuroprotective effect of per-RIC (during ischemia) and post-RIC (after ischemia) in a preclinical mouse model of acute ischemic stroke.

A mouse model of transient focal cerebral ischemia by compressing the distal middle cerebral artery (tMCAO) for 60 min was used. Animals were classified into three groups: MCAO group, per-RIC group (during MCAO) and post-RIC group (10 minutes after MCAO). RIC consisted of 3 x 5 min cycles of right hind limb ischemia. Infarct volume, functional neurological score and histological examination were evaluated 72h after reperfusion. Multiple inflammatory cytokines in the peripheral blood were measured using a Multiplex Assay. Light-sheet fluorescence microscopy technique was used to determine the effects of RIC on the microvascular network.

Per-RIC (n=10) and post-RIC (n=10) significantly reduced the infarction size 3 days after reperfusion compared to the group that did not receive RIC (n=10). In addition, RIC treatments significantly improve the neurological outcome and shown specific cellular pattern and morphological profile in the peri-infarct region. MCAO had a specific cytokine profile with a peak of inflammatory expression at 6 hours post stroke. RIC treatments shown a specific time-dependent cytokine profile. Per-RIC significantly increased the expression of both proinflammatory (GM-CSF) and anti-inflammatory cytokines (IL-10 and IL-13) at 6h, while Post-RIC significantly decreased the levels of proinflammatory cytokines (IL-6 and IL-12p70) and chemokine (KC). In addition, RIC strategies had different effect on the brain vasculature determined by a quantitative analysis of estimated density and vessel diameter.

Our results suggest that per-RIC and post-RIC may be used as a novel neuroprotective strategy against ischemia injury. Both strategies showed neuroprotective role but distinct signalling pathways.

NEW EXPERIMENTAL MODELS FOR THE STUDY OF FRIEDREICH'S ATAXIA

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Friedreich's ataxia (FRDA) is a rare autosomal recessive neurodegenerative disease. Although is a systemic disease, spinal cord and cerebellum are among the most severely affected tissues. Gait instability, loss of coordination in arms and legs or dysarthria are some of the hallmarks of the disease with most of the cases detected during the childhood. At a molecular level, FRDA is mainly caused by a GAA triplet repeat expansion in the first intron of the gene codifying for frataxin (FXN), leading to a decreased expression of this mitochondrial protein. One of the main challenges for the study of FRDA is the absence of good experimental models that mimic the human disease, likely due to the difficulty to recreate the pathological expansion. New models are currently emerging in order to better understand the physiopathology of the disease and test for therapeutic approaches. Thus we have characterized two experimental models which bear the human frataxin gene with a pathological expansion. As a cellular model, we have generated induced FRDA neurons by direct reprogramming from adult human fibroblasts. Furthermore we have characterized a new humanized mouse model harbouring a human frataxin gene with a >800 GAA repeat expansion (www.jax.org/strain/030395), herein referred to as YG8JR, and some of their neuronal populations in culture. In comparison to control conditions, induced FRDA neurons and neuronal cells cultured from the YG8JR mouse show low levels in FXN leading to modifications in the mitochondrial network pattern as well as in the complexes of the mitochondrial electron transport chain. Moreover, the YG8JR mouse model show an ataxic phenotype, with a decline in motor tests, such as rotarod, severe loss of weight, atrophy of the cerebellum, loss of neuronal cells and synaptic alterations. Overall, we think these new models will help to gain a better knowledge of the disease and may be helpful for drug screening to treat the disease.

NON-MOTOR SYMPTOMS AND NEURONAL ALTERATIONS IN A COMORBIDITY MICE MODEL OF DEPRESSION AND PARKINSON'S DISEASE

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Parkinson´s disease (PD) is the second more prevalent neurodegenerative disease. Besides motor impairments, increasing evidences demonstrate the presence of non-motor symptoms co-morbid with PD. Some of these symptoms can appear at early stages of the disease and may worsen the pathology, resulting in a poorer quality of life. One of the most common and disabling co-morbidities are the emotional alterations, such as anxiety and depressive symptoms, present in more than 50% of PD patients. The neuronal alterations that cause motor symptoms in PD are well characterized, thanks to the availability of several models that successfully reproduce them. In contrast, comorbid PD models that recapitulate the non-motor symptoms to study the neuronal alterations involved are not available. Beyond dopamine decline, there are other monoaminergic systems altered in PD, such as serotonin or noradrenaline. The dorsal raphe nucleus and the locus coeruleus, respectively the main serotonin and noradrenalin source of the brain, are affected at early stages of PD, when the emotional symptoms appear. The altered activity in these nuclei and in their projection fields may be involved in the pathophysiology of these mental comorbidity. In this work, we used the aphakia mice, a genetic PD model induced by the down-regulation of the Pitx3 transcription factor, that triggers a failure in the nigro-striatal dopaminergic pathway and a moderate parkinsonian phenotype, mimicking mild stages of PD, when emotional symptoms appear. We aim to, first, stablish the aphakia mice as a parkinsonian model with mental comorbidities and, second, define the neuronal circuits involved in the appearance of anxiety and depression. We found that the aphakia mice show anxiety and depressive signs associated with a decreased number of dopamine neurons in the DRN and decreased catecholaminergic transmission in the projection fields.

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Nrg1 HAPLOINSUFFICIENCY ALTERS INHIBITORY CORTICAL CIRCUITS

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Neuregulin 1 (NRG1) and its receptor ERBB4 are schizophrenia (SZ) risk genes that control the development of both excitatory and inhibitory cortical circuits. Most studies focused on the characterization ErbB4 deficient mice. However, ErbB4 deletion concurrently perturbs the signaling of Nrg1 and Neuregulin 3 (Nrg3), another ligand expressed in the cortex. In addition, NRG1 polymorphisms linked to SZ locate mainly in non-coding regions and they may partially reduce Nrg1 expression.

Here, to study the relevance of Nrg1 partial loss-of-function in cortical circuits we characterized a recently developed haploinsufficient mouse model of Nrg1 (Nrg1tm1Lex). These mice display SZ-like behavioral deficits. The cellular and molecular underpinnings of the behavioral deficits in Nrg1tm1Lex mice remain to be established.

With multiple approaches including Magnetic Resonance Spectroscopy (MRS), electrophysiology, quantitative imaging and molecular analysis we found that Nrg1 haploinsufficiency impairs the inhibitory cortical circuits. We observed changes in the expression of molecules involved in GABAergic neurotransmission, decreased density of Vglut1 excitatory buttons onto Parvalbumin interneurons and decreased frequency of spontaneous inhibitory postsynaptic currents. Moreover, we found a decreased number of Parvalbumin positive interneurons in the cortex and altered expression of Calretinin. Interestingly, we failed to detect other alterations in excitatory neurons that were previously reported in ErbbB4 null mice suggesting that the Nrg1 haploinsufficiency does not entirely phenocopies ErbB4 deletions.

Altogether, this study suggests that Nrg1 haploinsufficiency primarily affects the cortical inhibitory circuits in the cortex and provides new insights into the structural and molecular synaptic impairment caused by NRG1 hypofunction in a preclinical model of SZ.

OLEOYLETHANOLAMIDE TREATMENT MODULATES NEUROINFLAMMATION AND MICROGLIOSIS IN A MOUSE MODEL OF CEREBELLAR NEURODEGENERATION

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The endocannabinoid oleoylethanolamide (OEA) has proven to exert anti-inflammatory and neuroprotective effects in different animal models with brain injury of varied etiology. Indeed, a previous study of our laboratory demonstrated that the exogenous administration of OEA -prior to the onset of the neurodegenerative process- resulted in neuronal protection of Purkinje cells and in an improvement of the behavioral defects observed in Purkinje Cell Degeneration (PCD) mutant mice.

In this study, we tested whether OEA treatment (10 mg/kg, i.p. at postnatal day 12) modulates neuroinflammation and counteracts microglial activation in the model of severe cerebellar degeneration PCD. First, changes in mRNA levels of proinflammatory, neurotrophic and neuroprotective factors were measured by quantitative PCR in both the short- (3- and 24-hours post-treatment) and the long-term (P30). Secondly, modulation of microglial activation and its phenotype was analyzed in parasagittal sections of cerebellar vermis by immunohistochemistry at P30.

Our results showed that OEA treatment significantly reduced mRNA levels of the proinflammatory factors IFNγ, IL1β and TNFα, and increased the neurotrophic/neuroprotective factors BDNF, GAP43 and MAP2 in cerebellum 3 and 24 hours after administration. Additionally, OEA decreased mRNA levels of IL1β and IL6 in the long term, which were upregulated in PCD mice at the age of P30. Finally, results from immunohistological analysis of cerebellar microglial cells showed that OEA administration reduced the microglial density in the cerebellum of PCD mice, which was exacerbated as a consequence of neurodegeneration. These findings demonstrate the neuroimmunomodulatory properties of OEA in the PCD mouse and shed light on the possible mechanisms of action of OEA as a therapeutic agent which could be a co-occurrence among down-regulation of neuroinflammation, modulation of microglial activation and neuronal protection.

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OLIGODENDROCYTE MATURATION AND MYELINATION: IMPLICATIONS OF DEFICIENT THYROID HORMONE TRANSPORT TO THE BRAIN

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Oligodendrocytes are glial cells that play a crucial role in the CNS. Their maturation and myelination are finely regulated processes that require key trophic signals important for growth and metabolism. Thyroid hormone (TH) is a potent signal that regulates oligodendrocyte maturation, oligodendroglial-synaptic interactions and myelination. TH transport across the blood-brain barrier and cellular membranes is mediated by a specific transmembrane transporter, the monocarboxylate transporter 8 (MCT8). Dysfunction of MCT8 leads to inherited hypomyelination and psychomotor disabilities in the X-linked Allan-Herndon-Dudley syndrome (AHDS) or MCT8-deficiency. Although impairments in myelination in AHDS patients represent one of the main hallmarks of the disease, there is no consensus on whether there is a permanent hypomyelination or a delay on myelination that is restored later in life.

To address this point, we made use of multiple techniques to study myelination processes in Mct8/Dio2 knockout mice (KO), an already validated model for AHDS, from postnatal to adult stages, to gain new insight into the pathophysiological mechanisms of AHDS and the effects of TH on myelination.

Myelination was studied histologically by assessing the content of myelin proteins and lipids. These studies revealed persistent myelination defects in the brain of Mct8/Dio2 KO mice, consistent with observations at the ultrastructural level showing severely decreased percentage of myelinated axons in the Mct8/Dio2 KO mice brain using transmission electron microscopy analyses. Myelination was also assessed by Magnetic Resonance Imaging, showing microstructural alterations in the white matter. These data obtained on myelination led to the study on oligodendroglial dynamics, showing altered proliferation and differentiation patterns from oligodendrocyte precursor cell stages.

Myelination and oligodendroglial dynamics in Mct8/Dio2 KO mice are altered from early developmental stages and these alterations persist throughout later stages. Altogether, these data provide new understanding on the pathophysiological mechanisms underlying MCT8 deficiency to design and evaluate possible future treatments.

OXIDATIVE DAMAGE IN MIDDLE-AGED APOLIPOPROTEIN E4 CARRIERS

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Apolipoprotein E4 (APOE4) is the main genetic risk factor for many diseases, including Alzheimer's disease (AD). However, while oxidative stress is a characteristic of AD pathology, our previous study found that young healthy APOE4 carriers present reductive stress. As AD pathology starts years before the onset of clinical symptoms, it is possible that the oxidative status of APOE4 carriers might change as they reach middle age. Therefore, we conducted a prospective study with the objective of analyzing the oxidative status of the same APOE4 carriers, after a 10-year interval. We recruited 39 cognitively healthy adults that participated in the previous study, 24 APOE4 carriers (14 heterozygous and 10 homozygous) and 15 non-carriers. Subjects had a mean age of 52 years (range 35-64 years) and 61% were women. Blood samples were collected from all subjects and levels of plasma malondialdehyde (MDA) and whole blood glutathione were measured by high performance liquid chromatography and spectrophotometry, respectively. Levels of oxidized (GSSG) and reduced glutathione were used to calculate oxidized/reduced ratio. Current results showed that, although there were no differences in glutathione levels, APOE4 carriers presented significantly higher levels of MDA when compared to non-carriers. Furthermore, APOE4 carriers presented a significant increase in GSSG, oxidized/reduced ratio, and MDA levels through time, which did not occur in non-carriers. Although age affected MDA levels in APOE4 carriers, with a higher increment in those subjects older than 50 years, gender and genotype had no effect on either blood parameter. We concluded that the oxidative status of the cognitively healthy, middle-aged APOE4 carriers in our study changed through time, with a reversal of the previous reductive stress and current higher markers of oxidative damage.

P53 DEPLETION PROMOTES NEOVASCULARIZATION AND BRAIN REPAIR AFTER INTRACEREBRAL HEMORRHAGE

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Brain neovascularization has been associated with good prognosis of intracerebral hemorrhage (ICH) patients. We previously showed that the human Tp53 Arg72Pro SNP modulates brain endothelial cells survival after ICH, which is essential for the secretion of growth factors and cytokines (i.e. VEGF) that mediate the mobilization of endothelial progenitor cells (EPCs) from the bone marrow to the peripheral blood. EPCs promote brain vascular repair after ICH. Then, pro-apoptotic p53 would be a negative regulator of EPC mobilization, thus affecting the functional outcome after ICH. Since p53 is accumulated in the brain after ICH, we speculate that p53 destabilization not only will promote cell survival, but also vascular recovery and brain repair.

p53 KO mice were subjected to an experimental model of ICH in vivo by injecting bacterial collagenase into the basal ganglia. Proliferative markers (BrdU, Ki67) and perfusion status of newly-formed blood vessels in the brain was also analyzed after Evans blue injection.

We observed that p53 loss reduced lesion volume and significantly boost levels of circulating EPCs in mice from 72 hours after ICH. Knockdown of p53 also increased proliferative events in SVZ and lesion areas, as evidenced by BrdU incorporation and Ki67 staining. Consequently, an improved vascular repair response was achieved in p53 KO mice, in comparison with those expressing an active p53 protein.

Our results point out the impact of the p53 signaling pathway in the balance between brain damage and repair, which might condition functional recovery after ICH.

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PARVALBUMIN INTERNEURONS AND PERINEURONAL NETS IN THE HIPPOCAMPUS AND RETROSPLENIAL CORTEX OF A MURINE DOUBLE HIT MODEL FOR SCHIZOPHRENIA

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Schizophrenia (SCZ) is a multifactorial disease resulting in cognitive and emotional dysfunctions, usually appearing from late adolescence to early adulthood. Although its etiology is not fully understood, early life aversive experiences and alterations in neurodevelopment are considered predisposing factors. Certain brain regions such as the prefrontal cortex and the hippocampus known to be affected by early life stress are also altered in SCZ. Different reports have also described alterations in the retrosplenial cortex (RSC). Studies in patients and animal models of SCZ have found alterations in the parvalbumin (PV) expressing interneurons, making them good candidates to study the mechanisms underlying this psychiatric disorder. Some of the alterations observed in PV+ interneurons may be mediated by perineuronal nets (PNNs), specialized regions of the extracellular matrix, which frequently surrounding these inhibitory neurons. In the present study, we have used a murine double hit model combining a single perinatal injection of an NMDAR antagonist (MK801) to slightly disturb early postnatal development and post-weaning social isolation as an early life aversive experience. We have investigated the effect of the model and each of its factors on the subpopulation of PV expressing interneurons and PNNs in the hippocampus and RSC of adult male mice, using unbiased stereology. In the CA1, but not in the CA3 region of the hippocampus, the number of PNNs and PV+PNN+ cells was affected by the treatment with MK-801. In the RSC, we observed a significant impact of isolation, treatment with MK-801, and the interaction of both interventions on the number of PV expressing interneurons, PNNs, and PV+PNN+ cells. The present double-hit model constitutes a useful tool to investigate the effects of early life aversive experiences and the biological basis of schizophrenia. Our results may constitute the basis for further studies on PV expressing interneurons and the PNNs in this disorder.

PATHOLOGICAL AND THERAPEUTIC IMPLICATIONS OF MYELIN ALTERATIONS IN THE ACID SPHINGOMYELINASE DEFICIENCY

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The myelin sheath is a modified plasma membrane that surrounds axons, providing support, protection and controlling the nervous conduction. Myelin is produced by oligodendrocytes (OLs) in the central nervous system, but myelination homeostasis depends not only on OLs but also on their active interplay with neurons, astrocytes and microglia. Myelin alterations occur in many lysosomal storage disorders (LSD). However, these deficits have been traditionally seen as a consequence and assigned to play a secondary pathological role. Acid Sphingomyelinase Deficiency (ASMD) is a prototypical LSD characterized, in its infantile neurovisceral form, by cellular accumulation of sphingomyelin and a rapid neurodegeneration that leads to death in early childhood. A recent study from our laboratory pointed to demyelination as the triggering factor for microglia dysfunction and neuronal death in ASMD. We have tested this hypothesis in the mouse model of ASMD, which lacks the acid sphingomyelinase (ASMko), by characterizing the myelin anomalies and their underlying molecular mechanisms.
Our results show that demyelination is a very early event in the cerebellum of ASMko mice that is accompanied by structural alterations in the myelin sheath. We also observed defects in the OL lineage resulting in an increased number of immature OLs. Experiments performed in primary cultures showed a defect in the radial and longitudinal growth of ASMko OLs, which show less and shorter processes than wt OLs due to sphingomyelin accumulation. Altogether, these results suggest that sphingomyelin-induced alterations in the OL differentiation may be at the basis of demyelination in ASMD. This information will enable us to preclinically validate strategies rescuing ASMko OL differentiation, which could open therapeutic perspectives for ASMD and other LSDs that share sphingomyelin storage, myelin alterations and neurodegeneration.

PROGRESSIVE BEHAVIOURAL, BIOCHEMICAL AND MICROBIAL CHANGES DURING DIFFERENT STAGES OF STRESS

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Major depression (MD) is a common, relapsing mental illness that affects millions of people worldwide. One of the most studied risk factors associated with MD is chronic stress. Current treatment is ineffective in 30% of patients so there is a need to fully understand the pathophysiology of MD. The role of the microbiota-gut-brain axis in regulating stress-response is emerging as a particular area of interest.

Here, we want to evaluate if the changes induced by stress are accumulative and time dependent. To do so, we divide our mice into control non-stressed (NS), short-term stress mice (STS) and long-term stress mice (LTS). STS mice received only two days of stress whereas LTS mice underwent the chronic unpredictable mild stress protocol (CUMS) for 28 days. After the stress protocol, we analyse the biochemical, behavioural, and microbial changes induced by stress.

Results indicate that LTS induces much more severe depressive-like symptoms than STS as demonstrated in the body weight, the fur appearance, and in the anxiety levels in the open field. Moreover, biochemical analysis reveals huge differences depending on the duration of the stress. The study of the microbiome in faeces also shows that the changes in the microbiota are mostly observed in chronically stressed mice.

In summary, our work suggests that there is a progression in the biochemical, behavioural and microbial changes induced by stress. Future work trying to restore the microbiome alterations will allow us to better understand the importance of the gut-brain axis in response to chronic stress.

PROTEOMIC AND STEREOLOGICAL STUDY OF HUMAN AMYGDALA IN PARKINSON'S DISEASE

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Emotional impairments such as anhedonia are common non-motor symptom in Parkinson's disease (PD). Amygdala involvement by α-synuclein (Braak stage 3) could constitute neural substrates underlaying this emotional deterioration.

Differential α-synucleinopathy among amygdaloid nuclei has been described. MRI and VBM studies have revealed volumetric changes in the amygdala with conflicting results. To our knowledge, only one stereological study has studied the neuronal population in the amygdala, indicating neurodegeneration in the cortical and basolateral nucleus. However, glial populations have been neglected. Likewise, proteomic analysis focused on the amygdala in PD are lacking. This work, therefore, aims at analyzing volumetric and glial changes as well as to reveal the profile of proteomic changes of the amygdala in PD.

Brains from PD (Braak 5-6) and non-PD age-matched subjects were obtained from Spanish Biobank network. All procedures were approved by the Ethical Committee for Clinical Research at the University Hospital of Ciudad Real (SAF2016-75768-R and PID2019-108659RB-I00). Volume changes were estimated by Cavalieri's method. Optical Fractionator method was used to analyze microglia (Iba-1) and astroglia (GFAP). SWATH-MS and MALDI on tissue approaches were used for the proteomic study.

Results reveal differential glial involvement among amygdaloid nuclei as well as identify important proteomic alterations that could constitute potential biomarkers.

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PURIFICATION AND CHARACTERIZATION ofhPSC-DERIVED STRIATAL PROGENITOR SUBPOPULATIONS FOR TRANSPLANTATION IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a currently incurable neurodegenerative disease primarily characterized by the loss of medium spiny neurons in the striatum. Cell replacement therapy is the only approach currently focused on structural and functional restoration of atrophied tissue in HD by replenishing the degenerating MSN population.

Human pluripotent stem cell (hPSC)-derived neural progenitors can relieve motor deficits in animal models of HD; however, clinical translation of protocols is still limited by the heterogeneity of cell products. Cell sorting is considered instrumental to ensure reproducible generation of defined cell products. Here, we describe that marker X is a cell surface marker suitable for enrichment of hPSC-derived striatal neuroblasts.

Based on this, we have successfully set up and optimized an immunomagnetic sorting pipeline which allows for high-yield enrichment of striatal neuroblasts in heterogenous cell populations resulting from in vitro differentiation. We have demonstrated that the implementation of this approach leads to a reduction of not only the heterogeneity of the final cell product, but also of batch-to-batch variation in both control and HD cell lines. Furthermore, we have proved the versatility of our strategy showing that different neuroblast subtypes can be enriched under different conditions.

Selected neuroblasts from control and HD cell populations have been characterized in vitro in terms of their identity and potential to generate different neuron subtypes after striatal differentiation. Furthermore, we have transplanted these neuroblasts into the striatum of adult mice and observed evidence of their in vivo survival and integration into the striatum up to one-week post transplantation.

In conclusion, we anticipate that X-based cell sorting prior to transplantation has the potential to enable the development of safer and more reproducible cell products to be used for clinical cell replacement strategies in HD.

RIFAXIMIN PREVENTS MOTOR INCOORDINATION IN RATS WITH MILD LIVER DAMAGE BY PREVENTING IMMUNE CELL INFILTRATION AND NEUROINFLAMMATION IN THE CEREBELLUM

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Patients with liver cirrhosis may show minimal hepatic encephalopathy (MHE), with mild cognitive impairment, psychomotor slowing and motor incoordination, which reduce life quality and span. MHE onset is associated with a shift in peripheral inflammation that would promote infiltration of lymphocytes into the brain. Cerebellum of patients with steatohepatitis show T-lymphocytes infiltration and neuroinflammation suggesting that the changes triggering MHE may already occur at early stages of liver disease. Moreover, patients with steatohepatitis may show cognitive and motor impairment. The mechanisms leading to MHE and how to prevent or reverse it remain unclear. Rifaximin improves neurological function in MHE but the underlying mechanisms remain unclear. The aims of this work were to study the mechanisms triggering immune cells infiltration into the cerebellum, neuroinflammation and motor incoordination at early stages of liver disease in rats and advance in the understanding of the mechanisms of action of rifaximin by analyzing its effects on the above alterations.

Mild liver damage was induced in rats by CCl_4 injection during 4 weeks. Rifaximin was administered daily orally starting at 2 weeks of CCl_4. Motor coordination was assessed in the Rotarod. Immune cells infiltration, chemokines, TNFα expression and neuroinflammation in cerebellum were analyzed by immunohistochemistry. The content of extracellular GABA and glutamate and membrane expression of their transporters were analyzed.

TNFα, CCL20, CCL2 and CX3CL1 increased in cerebellum promoting T-lymphocytes and macrophages infiltration which induce neuroinflammation. Mild liver damage altered neurotransmission in cerebellum inducing motor incoordination. Rifaximin prevents immune cells infiltration and neuroinflammation and restores neurotransmission in cerebellum, improving motor incoordination.

This report shows that the induction of motor incoordination occurs at early stages of liver damage, provides clues on the mechanisms of the beneficial effects of rifaximin and suggests that early treatment with rifaximin could improve cerebellar neuroinflammation and motor alterations in patients with steatohepatitis.

ROLE OF DOPAMINE D4 RECEPTOR IN THE DEVELOPMENT OF MORPHINE-INDUCED ANALGESIC TOLERANCE

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Morphine is one of the most effective analgesic used in the clinical management of pain. However, long-term use of morphine can cause many side effects including respiratory depression, constipation, analgesic tolerance, hyperalgesia and addiction. The mechanisms underlying morphine tolerance are complex and nowadays it is not yet completely understood. As a primary mediator of morphine analgesia, the mu opioid receptor (MOR) contributes to morphine tolerance through downregulating the expression of MOR and its uncoupling from G-proteins in the dorsal horn of the spinal cord. It has been reported that the colocalization of the dopamine D4 receptor with MOR in the dorsal striatum counteracts the addictive effects induced by morphine through a putative D4R-MOR heteroreceptor that modulates dopamine signaling from nigral dopamine nerve cells. As D4R is also expressed in both the dorsal root ganglia (DRG) and dorsal horn neurons, we hypothesize that D4R could interfere the development of morphine-induced tolerance to its analgesic effects at dorsal horn level.
Using a chronic treatment paradigm of morphine with the D4R agonist PD168,077, we have first investigated the nociceptive response to noxious thermal stimulation (tail flick), mechanical stimulation (von Frey) and to persistent noxious chemical stimulation (formalin). Furthermore, using immunohistochemical techniques, we have studied primary afferent fibers (peptidergic and non-peptidergic C fibers), spinal interneurons and NK1 spinal projection neurons, and the balance between glutamate and GABA in the dorsal horn.
Results from the evaluation of analgesic activity showed that D4R activation prevents the development of morphine-induced analgesic tolerance. In addition, D4R preserves the appropriate balance between glutamate and GABA for a proper analgesic effect by modulating the spinal circuit. The present results give support for the existence of antagonistic functional D4R-MOR receptor-receptor interaction in the dorsal horn that could help to the development of a new pharmacology strategy in the treatment of pain.

ROLE OF mGLUR5 IN THE PSYCHIATRIC ALTERATIONS OF NIEMANN PICK DISEASE TYPE C

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Niemann Pick Disease type C (NPC) is a lysosomal storage disorder with severe neurological implications. Although early-onset forms of the disease are associated with more severe visceral symptoms, patients most commonly develop a progressive neurological disorder resulting in ataxia, seizures, cognitive impairment and psychiatric alterations in later onset forms. Mutations in the gene encoding NPC1, an endolysosomal protein that mediates intracellular cholesterol trafficking, lead to the accumulation of cholesterol and other lipids in the endolysosomal compartments and cause NPC. Preliminary evidence obtained in our laboratory has unveiled mood and synaptic anomalies in NPC1nmf164 mice, which bear mutant NPC1 and mimic NPC. Here we propose that these anomalies can be partly explained by alterations in the metabotropic glutamate receptor 5 (mGluR5). mGluR5 is a G-protein coupled receptor involved in the fine tuning of synaptic activity, mediating synaptic plasticity events such as Long Term Depression (LTD). Alterations of this receptor have been associated with different psychiatric conditions.
Western blot, immunofluorescence and electron microscopy analysis in the brain of NPC1nmf164 mice showed an increment of intracellular mGluR5 levels in neurons. This increment can be reproduced in cultured neurons from wild type mice by blocking cholesterol trafficking and rescued in NPC neurons by cholesterol extraction with (2-Hydroxypropyl)-β-cyclodextrin. Electrophysiological analysis showed increased mGluR-LTD in NPC1nmf164 mice, which is accompanied by enhanced downstream signaling pathway. These anomalies can be ameliorated by pharmacological inhibition of mGluR5 through oral treatment with its allosteric modulator CTEP. This drug also prevents psychiatric alterations in the NPC1nmf164 mice. Our results hence involve mGluR5 alterations in the synaptic plasticity and mood anomalies typical of NPC and provide a new therapeutic strategy that might help patients suffering from this fatal disease.

ROLE OF THE IMPRINTED GENE CDKN1C IN THE DIFFERENTIATION PROCESS OF NEURAL STEM CELLS

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Neurogenesis throughout adult life is supported by multipotent neural stem cells (NSCs), characterized by their abilities of self-renewal and differentiation into the three different neural lineages: neurons, astrocytes and oligodendrocytes. Both capabilities are maintained by specific intracellular mechanisms that are activated by extracellular signaling from the microenvironment or niche in which they reside in vivo, the subventricular zone (SVZ) and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus. Genomic imprinting is an epigenetic process that causes genes to be expressed depending on their parental origin, causing a monoallelic expression of a subset of genes called imprinted genes. This process is implicated in the control of gene dosage in the neurogenic niches. Cyclin-dependent kinase inhibitor 1C (Cdkn1c) is an imprinted gene expressed by the maternal allele and encodes the P57 protein that plays a crucial role during development of cerebral cortex and also in the maintenance of quiescence in NSCs from the SGZ. Alterations in Cdkn1c expression have implications in neurological syndromes such as Beckwith-Wiedemann or Prader-Willi. However, it is not known the role of P57 in the neurogenesis process. This work characterizes the expression of the imprinted gene Cdkn1c and its coding protein P57 in the adult SVZ, showing that P57 is present in GFAP+ cells located close to the lateral ventricles and in differentiated cells, suggesting an important role of P57 at differentiation process. We also evaluate the role of P57 in adult SVZ NSCs quiescence and differentiation by in vivo electroporation of NSCs in a Cdkn1c-deficient murine model, showing that P57 is involved in terminal differentiation of NSCs into glial linages.

ROLE OF THE NMDAR-NR2B SUBUNITS IN THE FUNCTION OF SUPRAMOLECULAR NMDAR-BK COMPLEXES.

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Large conductance calcium- and voltage-activated potassium channels (BK) are widely expressed across many tissues, contributing to many physiological functions. Membrane depolarization and relatively high (micromolar) intracellular concentrations of Ca2+ are required for their activation. Such concentrations are reached in the vicinity of Ca2+-permeant ion channels, to which BK proteins are closely located in various tissues, including the brain. N-methyl-D-aspartate receptors (NMDAR) are sodium (Na+)- and Ca2+-conducting glutamate-activated ion channels that are functionally coupled to BK channels in the olfactory bulb and the dentate gyrus (Isaacson et al., 2001; Zhang et al., 2018), contributing to decreased neuronal intrinsic excitability. Recently, we have shown that postsynaptic NMDAR-BK complexes at basal dendrites of somatosensory cortex layer 5 pyramidal neurons regulate synaptic transmission and long-term plasticity. However, the specific contribution of different NMDAR subunits (NR1 and NR2A/NR2B) to the NMDAR-BK interaction remains elusive. Defects in this association may be of pathological relevance in the context of syndromes MRD6 (mental retardation, autosomal dominant 6) and EIEE27 (epileptic encephalopathy, early infantile, 27), which have been related to mutations in the NR2B-encoding gene (GRIN2B).
In this work we aimed to understand the role of NR2B subunits in the function of NMDAR-BK complexes and, more specifically, if NR2B-related human mutations modify NMDAR-BK coupling. Function of NMDAR-BK complexes containing different MRD6- and EIEE27-related NR2B mutations was tested using electrophysiology in heterologous expression systems. Proximity between channel proteins within complexes was assessed using in situ proximity ligation assays (PLA). Our results reveal some disease-related NR2B mutations that reduce the NMDAR-BK interaction, either by altered interactions with the BK channels and/or by functional uncoupling between the channels within the NMDAR-BK complexes.

ROS-INDUCED SP1 REGULATES WRAP53 LEVELS AND NUCLEAR ACCUMULATION LEADING TO NEUROPROTECTION AFTER ISCHEMIA

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Ischemia-induced oxidative stress compromises genome integrity, which results in DNA damage and neuronal loss after stroke. We described that reactive oxygen species (ROS) generated during ischemia upregulate WRAP53 (WD40 encoding RNA antisense to p53) and trigger its translocation to the nucleus, where it promotes DNA repair [1]. However, the molecular mechanism remains unknown. Transcription factor Sp1 acts as a pleiotropic oxidative stress response protein in neurons. Particularly, ROS-induced Sp1 expression promotes neuroprotection against ischemia [2]. Interestingly, Wrap53 promoter contains putative consensus sequences (GC boxes) for Sp1. We analyze the role of Sp1 as a modulating factor of WRAP53-mediated neuronal survival and its impact on brain repair after stroke.

Primary cortical neurons were subjected to in vitro ischemia (oxygen and glucose deprivation, OGD). Levels of WRAP53 and Sp1 were modulated by lipofection with plasmids and siRNA. Protein location and 53BP1 foci formation (DNA repair marker) were analyzed by immunofluorescence. For in vivo experiments, mice were subjected to middle cerebral artery occlusion (MCAO), a validated model of cerebral ischemia.

We found that ischemia rapidly induced Sp1 expression in neurons, which preceded WRAP53 accumulation. Sp1 and WRAP53 accumulated in the nuclei after OGD, which was confirmed in vivo. By ChIP assay on SH-SY5Y cells, we found that the GC boxes-containing Wrap53 promoter region co-immunoprecipitated with anti-Sp1, suggesting Sp1 as a modulator of WRAP53 expression. Interestingly, Sp1 downregulation by siRNA prevented WRAP53 upregulation and nuclear translocation induced by OGD in neurons, leading to inactivation of DNA repair response and neuronal death.

Sp1 modulates WRAP53 expression and nuclear accumulation after ischemia through a ROS-dependent pathway. This new ROS-Sp1-WRAP53 signaling pathway poses Sp1 and WRAP53 as attractive targets for new neuroprotective strategies in ischemic stroke.

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SEX DIFFERENCES IN THE KYNURENINE PATHWAY IN A MOUSE MODEL OF NEUROPATHIC PAIN AND DEPRESSION COMORBIDITY

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Pain and depression are frequently comorbid disorders and both are more prevalent in women than men. The neurobiological mechanisms underlying this comorbid situation are still unknown and poorly investigated according to the sex. Kynurenine metabolism is hypothesized to be a pathway linking pain and depression, in part, by the role of the kynurenine in the central nervous system.
Hence, we propose that neuropathic pain could alter the kynurenine pathways promoting the onset of anxiety and/or depressive-like behaviors. Thus, sensorial and depressive-like behaviors, as well as plasma and central levels of tryptophan and kynurenine in a mice model of neuropathic pain (chronic constriction injury, CCI) were evaluated by using ELISA technique at short (ST) and long-term (LT) after nerve injury in both sexes.
Basal kynurenine plasma levels were significantly higher in females than in males. Nerve injured animals, CCI-ST and CCI-LT, showed higher levels of kynurenine in the prefrontal cortex (PFC). This increase was specially relevant in the CCI-LT group and it temporally coincide with the onset of the depressive-like phenotype showed by an increase of the immobility time in the forced swimming test. Interestingly, kynurenine enhancement was significantly higher in the PFC of females in comparison with male mice.
These data suggest that kynurenine is an important mediator in the comorbid chronic pain-depression situation and could be involved in the different sex-vulnerability observed in both pathologies.

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SMALL RNAS DERIVED FROM DIFFERENTIALLY AFFECTED BRAIN REGIONS OF HUNTINGTON'S DISEASE PATIENTS RECAPITULATE DIVERSE NEUROPATHOLOGICAL OUTCOMES IN WILD-TYPE MICE

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Progressive motor alterations and selective death of medium-sized spiny neurons in the caudate and putamen are key pathological hallmarks of Huntington's disease (HD), a neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in the coding region of the huntingtin (HTT) gene. Most research has focused on the pathogenic effects of the resultant protein product(s); however, growing evidence indicates that expanded CAG repeats within mutant HTT mRNA and derived small CAG repeat RNAs (sCAG) participate in HD pathophysiology. The individual contribution of protein versus RNA toxicity to HD pathophysiology remains largely uncharacterized and the role of other classes of small RNAs (sRNA) that are strongly perturbed in HD is uncertain.

Here, we show that sRNA produced in the putamen of HD patients (HD-sRNA-PT) are sufficient to induce HD pathology in vivo. Moreover, sRNA obtained from the motor cortex (as an affected region) or from the cerebellum (as a less-affected region) are able to differently compromise motor function in wild-type mice. This observation prompted us to identify which sRNA species are enriched in HD putamen and present neurotoxic potential. We detected high levels of tRNA fragments (tRFs) in HD putamen, and we validated the neurotoxic potential of an Alanine derived tRF in vitro. These results highlight that HD-sRNA-PT are neurotoxic, and suggest that multiple sRNA species contribute to striatal neuropathology, favouring therapeutic strategies based on the blockage of sRNA-mediated toxicity.

SPERM CYTOSKELETON ODFS GENES AS A POTENTIAL MECHANISM OF GLIOBLASTOMA PROGRESSION

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Glioblastoma (GB) is the most common and aggressive malignant primary brain tumor of the central nervous system in humans, it is originated from neoplastic glial cells. GB affects 1/100.000 persons and the median survival is 14,6 months. Neoplastic glial cells are characterized by a high degree of proliferation, diffuse invasion and resistance to conventional therapies. Patients suffer from synapse loss, an early symptom of a neurodegenerative processes. Our group has demonstrated that Drosophila GB cells extend a network of tumor microtubes (TMs) that enwrap the surrounding neurons, and promote glia–neuron signaling communication that favors glial proliferation and invasion, leading to a reduction of synapse number in the neighboring neurons. All these results suggests that GB is a neurodegenerative disease.

We use Drosophila melanogaster as a model of GB based on the constitutive activation of PI3K and EGFR in glial cells, this GB model faithfully reproduces the progression of the tumor, and associated-neurodegeneration. Our group is focused in GB growth mechanisms through TMs and I study the role of ODFs as potential network modulators. I study the role of dODF3B and dODF3L2, that have human orthologues upregulated in glioma, and correlate with poor prognosis.

We have found that dODFs act as sperm actin cytoskeleton modulators and are upregulated in GB brains, correlating with tumor progression. More specifically, the downregulation of dODF3B and dODF3L2 in GB cells prevents GB proliferation, infiltration and neurodegeneration. In addition, preliminary results suggest that signaling pathways described in GB as WNT/Wg modulate dODF3B levels of expression in glial cells. Consequently, we suggest that dODFs emerge as potential targets involved in glia-neuron communication, and GB progression.

SPHINGOMYELIN 16:0 IS A SPECIFIC TARGET FOR BRAIN PATHOLOGY IN THE ACID SPHINGOMYELINASE DEFICIENCY

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Acid Sphingomyelinase Deficiency (ASMD) is a fatal lysosomal storage disorder caused by mutations in the acid sphingomyelinase (ASM) gene leading to neurodegeneration. ASM loss of function in neurons increases total levels of sphingomyelin (SM) resulting in lysosomal alterations, oxidative stress and cell death. Lipidomic analysis have indicated that certain SM species, which differ in the fatty acid chain length and unsaturation degree, increase much more than others in brain extracts from mice lacking ASM (ASMko) that mimic ASMD.

To test the pathological effects of different SM species we added them individually to cultured cortical neurons from wild type (WT) and ASMko mice. SM 16:0, which shows the highest relative increase in the ASMko mouse brain, is also the SM species accumulating the most in neuronal cultures leading to lysosomal permeabilization and exocytosis, oxidative stress and cell death. In contrast SM 24:1 had no deleterious effects.

Gene expression analysis by qPCR in brain extracts of ASMko mice indicated the upregulation of Ceramide Synthase 5 (CerS5), which is involved in SM16:0 synthesis. We therefore proposed CerS5 inhibition as a suitable strategy to prevent SM16:0 associated toxicity. Infection of cultured ASMko neurons with adenovirus encoding shRNA-CerS5 reduced lysosomal alterations, oxidative stress and death. Moreover, intracerebellar injection of AAV9-shRNA-CerS5 improved motor abilities and Purkinje cell survival in the ASMko mice.

These results demonstrate the different toxicity of SM species in neurons and unveil SM16:0 accumulation as a specific target to address brain pathology in ASMD.

STAT3 INHIBITION PREVENTS THE TRANSFORMATION OF NSCS INTO REACTIVE-NSCS IN EPILEPSY

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Adult neurogenesis persists throughout adulthood in the hippocampus of most mammals because of a population of neural stem cells (NSCs) that remains in the dentate gyrus. The capability of NSCs to generate neurons is promoted by neuronal activity. However, hyperexcitation at the level of epileptic seizures induce NSCs to transform into reactive NSCs (React-NSCs), that become multibranched and hypertrophic and abandon neurogenesis to enter massively in mitosis and transform into reactive astrocytes that contribute to gliosis. We are now exploring signaling mechanisms that control the transformation of NSCs into React-NSCs.

One of the candidates is STAT3 (signal transducer and activator of transcription 3) which plays a critical role in astrogliogenesis and NSCs proliferation and differentiation. We have confirmed by quantitative rtPCR (Q-rtPCR) that STAT3 is overexpressed and by confocal microscopy that the phosphorylated form (P-STAT3) is increased in React-NSCs in a mouse model of mesial temporal lobe epilepsy (MTLE). Further we have stablished a model of React-NSCs in culture, which allows an easier manipulation of the STAT3 activity. We have confirmed also by Q-rtPCR and by confocal microscopy that these cultured React-NSCs also overexpress STAT3 and have more P-STAT3 when compared to control NSCs.

We hypothesize that the inhibition of STAT3 activity will prevent the induction of React-NSCs. To test this hypothesis, we are using two strong inhibitors of STAT3 activity: pharmacological agent WP1066 and silibinin, the main component of silimarin which is isolated from the seeds of milk thistle (Sylibum marianum). Our preliminary results suggest that indeed the inhibition of STAT3 reduces the transformation of NSCs into React-NSCs, as it decreases their overproliferation as well as their morphological transformation.

STEREOLOGICAL ANALYSIS OF NEURONS AND GLIA IN THE SUBICULAR COMPLEX IN ALZHEIMER'S DISEASE

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Alzheimer´s disease (AD), the most prevalent neurodegenerative disorder worldwide, is clinically characterized by cognitive deficits. Neuropathologically, deposits of amyloid-β and tau proteins accumulate in the brain in a six-stages predictable pattern. These misfolded proteins can propagate cell-to-cell in a prion-like manner and induce native proteins to become pathological.

Neuronal loss and volume reduction in entorhinal cortex (EC) and hippocampus (HP) have been largely reported, key regions in both the onset of the disease and the cognitive deficits observed in AD patients. In this sense, the subicular complex (SC) is a region of special interest because it represents the connecting bridge between the EC and the HP. However, the role of the SC in AD remains to be elucidated. Therefore, the aim of this work has been to quantify the volume and stereologically analyze neuronal and glial changes within the human SC including subiculum, presubiculum and parasubiculum.

Experimental procedures were approved by the Ethical Committee of Clinical Research at Ciudad Real University Hospital (SAF2016-75768-R). Post-mortem human brain samples were provided by the Spanish Biobank Network. Two experimental groups were used: n=9 AD cases (stages V/VI) and n=9 age-matched non-AD cases. Volumetric quantification of SC was performed using Cavalieri method. Neurons (Neu-N), microglia (Iba-1) and astroglia (GFAP) were also quantified by optical fractionator method using immunohistochemical staining.

Volumetric changes and differential involvement of neural and glial populations by proteinopathies in the SC could help to understand how cortical circuitry is involved in the spreading throughout the medial temporal lobe.

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STIMULATION OF MICROVESICLE/EXOSOME SECRETION BY POLYPHENOLS FOR THE TREATMENT OF NIEMANN PICK DISEASES

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Niemann Pick diseases types A (NPDA) and C (NPDC) lead to cognitive impairment, neurodegeneration and early death. NPDA and NPDC have different genetic origin being caused by mutations in the acid sphingomyelinase or the cholesterol transport protein NPC1, respectively. However, they share as pathological hallmark the accumulation in the endolysosomal compartment of lipids such as sphingomyelin and cholesterol. Recently, secretion of microvesicles/exosomes (ECV) has been described as a mechanism to eliminate toxic material from cells. Polyphenols, which are organic molecules present in fruits and vegetables, have been shown to stimulate ECV secretion preventing endolysosomal lipid accumulation in different cellular models. The objective of this work was to analyze the efficacy and safety of different polyphenols to stimulate ECV release and reduce lipid overload in NPD cells, both in vitro and in vivo. To this aim we have used mice deficient in the acid sphingomyelinase (ASMko) or mutant for NPC1 (NPC nmf164), which mimic NPDA and NPDC respectively. We have found that, among the different polyphenols tested, urolithins, are the safest and most efficient in increasing ECV secretion, reducing sphingomyelin and cholesterol levels and lysosomal size in cultured bone marrow derived macrophages and neurons derived from ASMko and NPC nmf164 mice. Moreover, oral treatment with ellagic acid, the precursor of urolithins, reduced lipid levels, improved neuronal survival and diminished inflammation in the brain of the NPD mouse models. These results support the therapeutic value of ECV secretion and polyphenols opening treatment perspectives not only for NPD patients but also for other storage disorders in which intracellular lipid overload occurs.

STRIATUM-ENRICHED TRANSCRIPTION FACTOR FOXP2 AMELIORATES EARLY PSYCHIATRIC-LIKE DISTURBANCES AND MOLECULAR ALTERATIONS IN HUNTINGTON´S DISEASE

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Patients with Huntington's disease (HD) may have difficulty controlling impulses and emotions, resulting in outbursts, yelling, or aggression. Mild to severe depression have been also widely reported. All these symptoms together are the ones of the primary complaints of HD. These disturbances have an early onset and could be associated to a very early dysfunction of the striatal circuitry. To understand how these psychiatric alterations take place, its underlying molecular mechanisms must be delineated.

Previous studies showed that the striatal-enriched gene Foxp2 is among the earliest genes dysregulated in the striatum of mouse models of HD. Foxp2 is a crucial gene for the formation and maturation of the cortico-striatal pathway. Coinciding with the HD pre-symptomatic psychiatric symptoms, it has been described in genomic wide association studies (GWAS) that Foxp2 is a major risk factor to develop depressive symptoms, irritability and sleep disturbances among others.

We performed a battery of behavioral tests in the juvenile R6/1 mouse model of HD to evaluate early psychiatric-like disturbances as impulsivity, aggressive behavior or hyperactivity.

We show that the detected dysregulation of Foxp2 in the R6/1 mouse is strongly correlated with alterations in impulsivity, decreased aggressive behavior, hyper-activity and several early biochemical changes. Moreover, overexpression of Foxp2 in the striatum of juvenile R6/1 striatum ameliorates impulsive-behavior displayed by R6/1 mice respect to control WT littermates and rescue their spine loss detected in medium spiny neurons.

Our data suggest that FoxP2 is one of the major and early targets for the mutant huntingtin which leads to an altered maturation striatal neuronal populations in HD that finally culminate in the appearance of pre-symptomatic psychiatric symptoms. Understanding the contribution of Foxp2 in HD could lead us to propose a comprehensive molecular mechanism with its associated circuit underlying major psychiatric symptoms in pre-symptomatic HD patients.

STUDY OF BONE MARROW-DERIVED MICROGLIAL CELLS IN A MODEL OF SELECTIVE NEURODEGENERATION

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The Purkinje Cell Degeneration (PCD) mouse presents a mutation in the Ccp1 gene that produces the selective post-natal death of Purkinje cells. Along with this neuronal loss, a strong microgliosis takes place in the cerebellum, but it is not fully understood whether it plays a beneficial or a harmful role in the development of the pathology. In this sense, it is also unknown if this gliosis is a direct consequence of the neurodegeneration, or if, by contrast, the pcd mutation itself causes an aberrant microglial behavior. Therefore, the direct effect of the pcd mutation on the functioning of the microglia was studied using cell cultures, without the influence of a neurodegenerative environment.

For this purpose, hematopoietic cells were isolated from the bone marrow of both wild-type and PCD mice and differentiated into microglia. Subsequently, immunofluorescence techniques and qPCR analyses were performed to characterize microglia by studying different markers and gene expression. Likewise, the viability of these cells was studied by means of a proliferation essay with Alamar Blue.

The preliminary results obtained suggest that the hematopoietic cells of PCD mice differentiated into microglia have a predominant polarization towards an anti-inflammatory phenotype. Besides, a differential gene expression has been observed for all the analyzed genes. Finally, the Alamar Blue essay demonstrated that PCD cells show a higher proliferation than the wild-type cells.

Therefore, it can be concluded that the mutation of the Ccp1 gene affects some microglial features related to cell morphology and neurochemical, gene expression and proliferation.

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STUDYING THE RELEVANCE OF HUMAN APOE POLYMORPHISM IN ALZHEIMER'S DISEASE THROUGH THE APPLICATION OF iPSCs

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Alzheimer´s disease (AD), which is characterized by progressive neurodegeneration, is the most common form of dementia. The ε4 allele of gene encoding apolipoprotein E (APOE gene) is the strongest genetic risk factor for AD among the three polymorphic alleles (APOE- ε2, APOE- ε3 and APOE- ε4). Increasing evidences have shown that APOE4 is associated with diverse aspects of AD pathogenesis, but the impact of different alleles on human astrocyte and neuronal differentiation, maturation and function remains to be fully elucidated. In order to clarify these questions, we obtained induced pluripotent stem cells (iPSCs) from fibroblasts of AD patients carrying the ε3 and ε4 alleles (in homozygosis) and from healthy patients. These iPSCs were fully characterised and their undifferentiated and pluripotent nature was confirmed. We also used gene-edited iPSC lines homozygous for the main APOE variants and an APOE knock-out line. Astrocytes and neurons were generated from human iPSCs by establishing differentiation protocols through the sequential addition of different small molecules and growth factors. iPSCs-derived astrocytes expressed typical markers (GFAP, GLT1, AQP4 and S100ß) as well as APOE and they exhibited functional features such as glutamate uptake capacity, calcium waves production and inflammatory stimuli response. iPSCs-derived neurons expressed neuronal markers (MAP2, TBR1, vGLUT, Calbindin, GABA) and showed a functional profile. APOE4 neurons displayed signs of degeneration confirmed by the release of amyloid-beta and increased levels of phospho-Tau. These findings support the use of iPSCs-derived astrocytes and neurons as cellular models to investigate mechanisms of degeneration in AD and the connection between APOE and AD pathology.

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SYNERGISTIC EFFECTS OF APPLYING STATIC MAGNETIC FIELDS AND DIAZEPAM TO CONTROL EEG ABNORMALITIES IN AN EPILEPTIC RAT MODEL

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In previous work, we have found that transcranial static magnetic fields (tSMS) application was able of delaying and reducing EEG seizure signs in the lithium-pilocarpine rat model of epilepsy1. Here we explore the putative synergistic effect of combining tSMS with low doses of diazepam, a drug commonly used to treat status epilepticus.

Experiments were carried out on 8 Sprague-Dawley rats, 2-3 months old. LiCl (127 mg/kg, i.p.) was administered 24 hours before pilocarpine injections. Pilocarpine was given in two doses injected (i.p.) 30 min apart. The first one was preceded (30 minutes) by Scopolamine, 1 mg/kg. Animals were randomly classified into two groups: “Magnet” (a neodymium nickel-plated cylinder, 45mm diameter and 30mm height, magnetic field of 0.5T was placed over the skull just before the first pilocarpine injection and for a total period of one hour), or “Control” (a stainless steel replica without magnetic properties was used). 1.25 mg/kg of diazepam (i.p.) was injected sixty minutes after of the second dose of pilocarpine.

Thirty minutes after diazepam injection, there was a clear reduction in the number of EEG spikes in both groups, being more pronounced in the magnet group (T-test, $p<0.05$). The Root Mean Square - an estimation of the EEG amplitude- showed a greater reduction in the Magnet group compared with those animals that only received the diazepam dose (T-test, $p < 0{,}001$). Furthermore, the power spectrum analysis showed a stronger reduction in theta, alpha and beta bands, on the diazepam+magnet animals compared to the diazepam+sham group (T-test, $p < 0.05$ beta band; T-test, $p< 0.0001$ theta and alfa bands).

The results show synergistic actions between magnetic fields and diazepam in controlling EEG abnormalities in the epilepsy model used here, and pave the way for the combination of tSMS with pharmacological treatments for epilepsy, improving seizures control and, perhaps, allowing dose reduction.

TARGETING MTOR/4E-BP1 AXIS BY THE ANTIDEPRESSANT SERTRALINE AMELIORATES MOTOR DEFICITS IN THE R6/1 MOUSE MODEL OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a dominant inherited neurodegenerative disorder caused by an unstable expansion of a CAG repeat within the exon 1 of the huntingtin (HTT) gene. Early symptoms include psychiatric alterations as depression or irritability that progressively add up to cognitive and motor alterations in later stages, both related to dysfunction in hippocampal and corticostriatal pathways. Previous results from our group suggest that an aberrant increase in translation in the striatum of HD contributes to the pathophysiology. This hypothesis is well-supported by the amelioration of the characteristic motor deficits in the R6/1 HD mouse model as a consequence of the normalization of translation by intracerebral injection of 4EGI-1, an inhibitor of a protein synthesis initiation complex. Here, we show that striatal neuronal cultures from R6/1 mouse recapitulate translational alterations present in the adult striatum providing a model for drug screening. We took advantage of this to understand the molecular mechanism underlying aberrant translational control and to assess the ability of Sertraline, an antidepressant known to act as an inhibitor of the mTOR/4E-BP1 axis, to restore protein synthesis levels in the HD molecular context. In the same line, we used intraperitoneal administration of Sertraline to normalize the translation rate in the striatum of R6/1 mice, leading to the amelioration of motor learning and coordination deficits. Accordingly, these results suggest a potential new use of the antidepressant Sertraline for the treatment of motor symptoms in HD.

TAUROURSODEOXYCHOLIC ACID SUPPORTS EARLY FUNCTIONAL RECOVERY OF RATS WITH SPINAL CORD INJURY BUT DOES NOT IMPROVE EFFECTS OF TRANSPLANTED BONE MARROW-DERIVED STROMAL CELLS

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Background: Tauroursodeoxycholic acid (TUDCA) is a bile acid with anti-inflammatory effects on microglia and macrophages. Implants of bone marrow-derived stromal cells (bmSC) are currently under investigation in clinical trials of spinal cord injury (SCI). We studied the therapeutic effect of TUDCA and a combinatorial treatment with human bmSC in a rat model of SCI.

Methods: Spinal cord contusion injury was induced at thoracic level T9. Treatment consisted of two injections of 100 mg/kg TUDCA, immediately after lesion and at 1 dpo, combined with one sub-occipital injection of human bmSC into the cisterna magna. Control groups received injections of saline or TUDCA treatment only. The recovery of motor functions was assessed during a surveillance period of six weeks. Rats were sacrificed after 4 days for biochemical and histological investigation or after 6 weeks for histology of the tissue.

Outcome: Treatment with TUDCA improved the recovery of autonomic bladder control and had a positive effect on motor functions in the subacute phase. Biochemical analysis of spinal cord tissue confirmed its anti-inflammatory activity. Effects on motor function were only transient, however, such that no significant differences between vehicle and TUDCA-treated animals were observed 1-6 weeks after lesion. Combinatorial treatment with TUDCA and bmSC failed to have an additional effect compared to treatment with bmSC only.

Keywords: bile acid, spinal cord injury, stromal cells, rat

THALAMUS RETICULAR NUCLEUS ALTERATIONS IN RESPONSE TO PERIPUBERTAL STRESS IN FEMALE AND MALE MICE

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Early exposure to stressful events is known to be a cause for alterations in neural development. These changes can be maintained during adult life, leading to stress-related psychiatric disorders. The thalamus reticular nucleus (TRN) is a GABAergic nucleus mostly formed by parvalbumin positive interneurons. This nucleus receives collateral glutamatergic projections form both corticothalamic and thalamocortical neurons and exerts a strong inhibition towards the thalamus. Although it is mainly known for regulating the informational flow between the cortex and the thalamus, it has also been related with the pathology of some psychiatric disorders, including some in which stress acts as a precipitating or predisposing factor. In this study we used a peripubertal unpredictable chronic stress model in mice, based on psychogenic stressors, to assess whether aversive events during peripubertal development lead to changes in the structure and connectivity of the TRN. This model has been previously used in our laboratory to recreate the effects of early stress in the prefrontal cortex, showing interesting and differential results between female and male mice. In the present study, we analyzed some molecules expressed in the TRN and related to interneuronal plasticity and the closure of critical periods, such as components of perineuronal nets and PSA-NCAM. In addition, we are analyzing other parameters related to the connectivity of the TRN with the cortex and the rest of thalamic nuclei. We intend to find some insight in the effects that adverse experiences during early life have on the development and connectivity of the TRN. This may lead to an advance on the knowledge of the neurobiological basis of some psychiatric disorders in which early-life stress acts as a predisposing factor.

THE EFFECT OF BONE MARROW DERIVED- MESENCHYMAL STEM CELLS ON A NOVEL IN VITRO MODEL OF X-LINKED ADRENOLEUKODYSTROPHY

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X-linked adrenoleukodystrophy (X-ALD) is an inborn error of metabolism caused by a mutation in ABCD1 gene, which encodes a peroxisomal transmembrane protein called ALDP. This protein is involved in the transportation of very-long chain fatty acid (VLCFA) to the peroxisome for degradation. X-ALD patients present an accumulation of VLCFA in tissue and blood due to alterations in ALDP function. The outcome observed in patients with cerebral form of X-ALD is an acute inflammatory demyelination.

The use of bone marrow derived- mesenchymal stem cells (BMSCs) has been studied for many years. BMSCs have been proposed as a therapeutical approach in many diseases.This study will address if BMSCs has a beneficious effect in X-ALD.

Here, we are investigating a novel in vitro model to study X-ALD from dental pulp stem cells (DPSCs), an accessible source of mesenchymal stem cells. DPSCs from a X-ALD patient showed an alteration in ALDP expression and lipid accumulation located in the cytoplasm .

To identify the effect of BMSCs in this in vitro model, we differentiated DPSCs from healthy and X-ALD individuals into neural-like cells. Immunocytochemistry experiments showed the expression of neuronal markers in the differentiated cells.

Electrophysiological assays were carried out to further characterized the neural-like cells. Analysis of the sodium and potassium currents from neural-like cells showed a lower peak amplitude and slower kinetics in X-ALD-derived cells compared to healthy cells.Interestingly, when X-ALD neural-like cells were directly co-cultured with BMSCs, the sodium currents had a larger peak amplitude and faster kinetics, rescuing a healthy phenotype.

Further investigation of the effect of BMSCs on X-ALD cells may provide relevant insights to develop a possible therapy for X-ALD.

THE EFFECT OF NEUROTROPHIC FACTORS ON THE CEREBELLAR DESTRUCTURATION ASSOCIATED WITH AUTISM SPECTRUM DISORDERS

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Autism spectrum disorder (ASD) is an abnormal neurodevelopmental process characterized by a central symptomatology. The cerebellum is a key structure in the study of ASD for both its motor function and its involvement in cognitive, affective and social behaviour. Patients with ASD have a specific loss of Purkinje cells, together with an affectation of neurotrophic factors (NF), which also causes neuronal alterations.

This work aims at studying the social conditions present in ASD and related with cerebellum, employing the PCD mutant mouse, an animal model that presents a specific loss of Purkinje cells. For the analysis of the relationship between NF and cerebellar destructuration, both gene and protein expression of NF, such insulin growth factor 1 (Igf-1), vascular endothelial growth factor A and B (Vegf-A and Vegf-B) and brain-derived neurotrophic factor (Bdnf) were analysed at different ages: P10, P15, P20, P25, P30 and P40 (i.e. before and during the Purkinje cell loss).

Our preliminary results demonstrated a statistically significant increase in both gene and protein expressions of Igf-1 at P25 and P40, and Vegf-B at P15 and P20 in the cerebellum of PCD mice, while the other NF remained similar to control mice. In addition, the pattern of expression of Igf-1 and Vegf-B resulted radically different amongst genotypes: in wild type mice we observed oscillations in their expression, being higher at P20 and P30, while in PCD mice we did not observe that, but a constant increase of these NF. These fluctuations seem to be important for proper cerebellar function and development, whereas the increase may be related to an attempt to neuroprotection in front neuronal death.

Finally, these data have been used to carry out a pharmacological treatment with neuroprotective factors, which is currently underway.

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THE GENETIC LOAD DETERMINES BEHAVIOURAL PHENOTYPE AND GUT MICROBIOTA COMPOSITION IN THE 5XFAD MOUSE MODEL OF ALZHEIMER'S DISEASE

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There are many hypotheses about the neuropathological origin of Alzheimer's disease (AD). The amyloid cascade hypothesis remains the most widely accepted by the scientific community, although the role of beta-amyloid as the sole cause of neuropathological hallmarks of the non-inherited form of AD (accounts for 95% of all cases) is questionable. The 5xFAD mice are commonly used as AD animal models that co-overexpress human APP and PSEN1 transgenes with a total of five AD-linked mutations, thus accelerating amyloid plaques formation. Many reports have described that 5xFAD mice presented cognitive alteration. However, no attention is paid to the effect of genetic load, heterozygous versus homozygous condition, on the histology, physiology, and gut microbiota composition. Hence, in this study, heterozygous and homozygous 5xFAD mice were used to test whether additional factors beyond hippocampal amyloid burden contributes to cognition impairment at 11 months old. Our results suggest that accumulation of Aβ1-40 and Aβ1-42 is present in both heterozygous and homozygous hippocampus of 5xFAD mice. However, only homozygous mice had cognitive impairment flexibility in the Morris water maze and impairment of working spatial memory in the Y-maze test. This strikes differences between behavioral and immunopathological phenotypes extend to microbiota, where the bacterial population is different in the three studied genotypes. In homozygous 5xFAD mice, a lower bacterial diversity is observed compared to the wild-type and heterozygous 5xFAD genotypes, decreasing the abundance of bacteria of the Firmicutes phylum and increasing bacteria of the Bacteroidetes phylum. Therefore, we suggest that the accumulation of beta-amyloid may not be the only cause of the worsening of cognitive impairment, but additional factors including the microbiota-brain axis might be altering the function of the immune system, favouring inflammation, and nervous system through bacterial product influencing the development of AD.

Keywords: 5xFAD, hippocampus, cognition impairment, neuroinflammation, microbiota
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THE GUT-BRAIN AXIS IN A NOVEL HUMANIZED TRANSGENIC MOUSE MODEL FOR PARKINSON´S DISEASE AND BRAIN AGING

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Accumulating genetic, epidemiological, neuropathological and clinical data indicate that alterations in the gastrointestinal (GI) function and the gut microbiota represent a risk factor for Parkinson's disease (PD). Changes in the bidirectional communication between the gut and the brain can affect both the enteric nervous system and the central nervous system, which might have important implications in understanding disease pathophysiology and for the development of disease modifying therapeutic strategies. However, the precise molecular mechanisms underlying this bidirectional communication in the context of PD are not fully understood.

In order to clarify how GI dysfunction is involved in disease pathogenesis and/or in modulating the manifestation of PD symptoms, we have characterized the GI function and the key mechanisms involved in the gut-brain axis of a new humanized transgenic PD mouse model (Tg-Th-hTyr). This model is based in the progressive accumulation of neuromelanin in all catecholaminergic nuclei of the brain, including the dorsal motor nucleus of the vagus nerve innervating the GI system. We have performed a battery of motor and non-motor behavioral tests to assess the phenotype of these animals, including the GI function. In addition, we have evaluated gut dysbiosis in fecal samples by 16S RNA gene sequencing and intestinal inflammation using cytokine profiling and histological examination of Tg and wild-type (wt) littermates.

Our results show impaired motor activity in Tg mice compared to wt littermates at 6 months of age. We also detected increased fecal output in Tg mice placed in a novel environment, suggesting alterations in the hypothalamic-pituitary-adrenal (HPA) axis. In the same line, we observed a significant increase in body weight and water/food intake in Tg mice. Our results indicate that the gut-brain axis is altered in neuromelanin-producing transgenic mice and that this novel PD model can contribute to clarify the role of gut dysfunction in PD pathogenesis.

THE INCREASE IN DOUBLECORTIN-IMMUNOREACTIVE IMMATURE NEURONS IN THE OLFACTORY CORTEX IS LINKED TO SYMPTOM ONSET IN A MOUSE MODEL OF RETT SYNDROME

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Rett syndrome (RTT) is a rare neurodevelopmental disorder that affects mainly females, predominantly caused by mutations in the X-linked gene coding for methyl CpG-binding protein 2 (MeCP2). This protein is involved in the epigenetic regulation of gene expression, and is broadly expressed in mature neurons. Patients with RTT suffer from intellectual disability, loss of speech and motor abilities, and seizures. We have previously shown that lack of MeCP2 leads to a region-specific increase in immature neurons, expressing doublecortin (DCX), in the olfactory cortex of young adult, symptomatic, Mecp2-null male mice, but not in age-matched, asymptomatic, Mecp2-heterozygous females. Here, we sought to investigate whether the impairment in neuronal maturation would be overt in Mecp2-heterozygous symptomatic females. To do so, we performed an immunohistochemical detection of DCX in 2 and 6 months old Mecp2-heterozygous female mice and their wild-type littermates. We found that DCX-immunoreactivity was increased in the piriform cortex, but not the olfactory bulbs or the hippocampus, of 6 months old mutant females, as compared to their wild-type controls. This increase was mainly due to an excess of DCX-tangled cells, the most immature ones, in the piriform cortex of Mecp2-heterozygous females. A slight increase in the proportion of DCX-tangled cells was already apparent in 2 months old Mecp2-heterozygous females, but became significant in older mice. These results confirm and extend our previous data, supporting that MeCP2 is involved in neuronal maturation in a region-dependent manner, and showing that this effect is linked to the onset of overt pathology. Funded by Ministerio de Ciencia e Innovación (PID2019-107322GB-C22).

THE LOSS OF STARBURST AMACRINE CELLS AND THEIR SYNAPTIC CONTACTS WITH DOPAMINERGIC AMACRINE CELLS MAY EXPLAIN THE VISUAL MOTION PERCEPTION DISTURBANCE IN PARKINSON'S DISEASE

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The main clinical characteristic symptoms of Parkinson's diseases (PD) are bradykinesia, tremor, and other motor deficits. However, there are other non-motor symptoms that can be identified at early stages of the disease, such as visual disturbances. One of these symptoms is the impairment of the motion perception. Therefore, the purpose of this study was to determine if the starburst amacrine cells (direction-selective amacrine cells), which is the main cellular type involved in motion perception, are degenerated in PD and if the dopaminergic system is related to this degeneration.

Human eyes from control and PD donors were available for this study. Dopaminergic amacrine cell density (tyrosine hydroxylase positive cells), starburst amacrine cell density (choline acetyltransferase positive cells), and their synaptic contacts (vesicular monoamine transporter-2 positive presynapses) were evaluated by immunohistochemistry and confocal microscopy in whole-mount retinas.

We observe that the number of dopaminergic amacrine cells was significantly decreased in PD retinas. Also, there is a degeneration of starburst amacrine cells observed by a decrease in the density of these cells in the two plexuses where they are located. Importantly, here we describe for the first time that dopaminergic amacrine cells contact with choline acetyltransferase positive cells in healthy control retinas and that these connections decrease in PD.

This work indicates that dopamine could modulate starburst amacrine cells and that the decrease of this input in PD may explain the degeneration of starburst amacrine cells that we describe and thus, the motion perception disturbance in this pathology.

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THE MATRICELLULAR PROTEIN HEVIN´S EXPRESSION IN NUCLEUS ACCUMBENS IS ALTERED BY ALCOHOL CHRONIC TREATMENT AND ADMINISTRATION AFTER WITHDRAWAL

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The matricellular protein hevin is highly expressed in adults, in both neurons and astrocytes, and enhances functional synapses formation. In a previous study, we showed a higher expression of hevin in postmortem human brain of alcoholic subjects compared to controls, which raised the question whether this alteration was the result of acute and/or chronic alcohol exposure or it was a consequence of alcohol withdrawal. Therefore, our aim was to determine the possible alterations in hevin expression due to acute or chronic alcohol intraperitoneal administration and alcohol relapse in C57BL/6J mice. We performed four different treatments: 12 days of chronic treatment with saline or ethanol (1.75 g ethanol/kg) followed by 3 days of withdrawal, and finished with a challenge dose of saline or ethanol. Groups: 1) saline – saline, 2) saline – ethanol, 3) ethanol-saline and 4) ethanol-ethanol. We used western blot technique to measure the expression levels of hevin in bilateral punches of five brain areas: frontal cortex (FC), amygdala (AMY), hippocampus (HIP), dorsal striatum (CPu) and nucleus accumbens (NAcc). In AMY, acute treatment showed decrease in the hevin´s expression in comparison to saline treatment. Interestingly, chronic ethanol treatment followed by a single dose challenge after withdrawal increased hevin expression levels in NAcc, compared to both saline treatment and acute ethanol treatment. At the same time, in NAcc lower levels of hevin expression were detected after ethanol withdrawal in comparison to the challenge after chronic administration of ethanol. We did not detect significant differences in hevin expression in FC, HIP or CPu. Thus, these results together with the alterations observed in humans suggest that alcohol intake could alter hevin´s expression, suggesting a role for hevin in the neurobiology of alcoholism.

THE OVEREXPRESSION OF NRG1-TYPE III DOES NOT AMELIORATE ALS CLINICAL OUTCOME IN HSOD1G93A MOUSE MODEL.

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Amyotrophic Lateral Sclerosis (ALS) is an adult onset disease that affects motor neurons (MNs) in the cerebral cortex, brainstem and spinal cord. Most of ALS cases (~90%) are sporadic, but ~10% of the cases are inherited. In approximately 20% of familial cases, the disease is caused by mutations in the gene encoding Cu/Zn-superoxide-dismutase1 (SOD1). Transgenic rodents overexpressing this mutated gene develop a neuromuscular disorder similar to human ALS.

Afferent inputs to MNs are crucial in regulating their excitability. Among different types of synaptic afferents, MNs receive prominent cholinergic C-type ("C-bouton") inputs from spinal interneurons. C-boutons modulate MN excitability, and synaptic transmission throughout C-boutons is involved in the regulation of MN vulnerability.

Some C-bouton-associated molecules appear to be relevant in ALS, like the sigma 1 receptor (S1R) (which mutations cause a juvenile familial form of ALS and its pharmacological activation prolongs lifespan of SOD1G93A mice) or the NRG1 receptor ErbB4 (which mutation causes another type of FALS). We have previously observed that neuregulin-1 (NRG1) accumulates in C-boutons, and described C-bouton alterations in a mouse model of ALS.

NRG1 signaling has been directly targeted in SOD1-ALS mice by virus-mediated delivery of NRG1 typeIII to the spinal cord, resulting in extended survival time and reduced C-bouton loss, and gene therapy based on intrathecal administration of adeno-associated virus to overexpress NRG1-III in SOD1G93A mice has therapeutic role.

By cross-breeding hSOD1G93A mice and NRG1-type III overexpressor mice (transgenic mice overexpressing human influenza hemagglutinin [HA])–tagged full-length NRG1 typeIII [HA-NRG1FL]), we created here a double transgenic mouse line. In this, we examined changes in body weight and survival, and performed behavioral and histopathological studies in spinal cord and skeletal muscles showing no improvement in either motor phenotype or lifespan. Our results indicate that the endogenous overexpression of NRG1-typeIII the does not ameliorate the SOD1G93A mouse phenotype.

THE ROLE OF BRAIN SYNAPTIC DYSFUNCTION IN THE PROGRESSION OF C9orf72-ALS/FTD

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are fatal neurodegenerative diseases seen in comorbidity in up to 50% of cases. The clinical overlap between the two diseases is also reflected in their genetics and neuropathology, with ALS and FTD sharing a number of key pathological characteristics. One of which is protein aggregation. Accumulation of protein aggregates leads to synapse loss and dysfunction, which has been associated with the development of cognitive deficits. Given that a hexanucleotide repeat expansion in the C9orf72 gene is the most common genetic cause of ALS and FTD, we used a transgenic mouse model C9orf72-BAC with up to 450 repeats, to study C9-ALS/FTD-related synaptic dysfunction. Using Golgi and immunofluorescent staining, we investigate how dendritic spines rearrange and neuronal and dendritic morphology change from 1 to 24 months in the cingulate cortex, hippocampus, cerebellum, and spinal cord of C9orf72-BAC mice. We used two groups: C9orf72-BAC transgenic (n=6) and non-transgenic (n=6) animals. Sholl analysis was used for quantification of morphological features in Golgi staining. Our results showed an increase in immature spines such as thin and stubby types, in the transgenic mice from 3 to 6 months of age, accompanied by an increase in soma size. Ageing effects were shown from 18 months in both groups. Those changes were accompanied by neuronal loss in hippocampus, cerebellum at early stages. Additionally, the dipeptide repeat protein (DPR) PR was found to stain axonal projections in the hippocampus and formed greater numbers of plaques in the hippocampus and cerebellum of transgenic mice. The results of our study indicate that early changes related to neuronal degeneration and DPR accumulation can be detected at the pre-onset stages of the disease.

THE ROLE OF EXTRACELLULAR VESICLES IN ALZHEIMER'S DISEASE: MECHANISTIC INSIGHT INTO INTRINSIC PROTECTION

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Alzheimer's disease (AD) is the leading cause of dementia worldwide (>30 million people affected). The cause of the disease is unknown, and there is no causal treatment. The gradual deterioration of cognitive functions in AD is paralleled by a hierarchical progression of amyloid-plaques (Aβ) and hyperphosphorylated Tau protein in the brain, which suggests that propagation of these aggregates has a role in the pathophysiology of the disease. Extracellular vesicles (EVs), including exosomes and microvesicles, are membranous nano/micro-structures released by most, if not all, cells. EVs carry proteins, lipids, and nucleic acids (DNA, mRNA, and miRNA), and they participate in cell-to-cell communication. However, the functionality and regulation of EVs (secretion, uptake, and cargo delivery) are not fully understood in the brain, where EVs have been related to essential functions such as neurotransmission and myelin maintenance. Recently, EVs emerged as relevant factors in neurodegenerative diseases, especially in AD, and they were described as disease biomarkers in the patient's fluids. Interestingly, they seem to have a dual role in AD: spreading pathological aggregates (Aβ and p-Tau) on the one hand and neuroprotection against the progression of the pathology on the other hand.

Here we will describe the biology of EVs in AD in detail. We will characterize the composition and cellular source of EVs and their uptake mechanisms throughout AD progression. EVs from the brains of AD patients and age/sex-matched controls will be obtained, and their RNA and protein profiles will be characterized by novel transcriptomic and proteomic work-ups. Moreover, hiPSCs-derived neuronal cultures will be used as a model to elucidate the role of these AD-derived EVs.

Our main goal is to generate mechanistic insight into EVs' neuroprotection in AD by combining deep phenotyping of patient-derived EVs with hypothesis-driven experiments in hiPSC to open the door to new EV-based AD treatment options.

THE ROLE OF THE STRIATOPALLIDAL INDIRECT PATHWAY IN THE GENERATION OF L-DOPA INDUCED DYSKINESIAS

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Parkinson's disease (PD) is mainly characterized by dopamine depletion in the striatum due to a loss of dopaminergic neurons in the Substantia Nigra pars compacta. Nowadays, the most efficacious treatment for PD is the dopamine precursor 3,4-dihydroxyphenyl-L-alanine (L-DOPA). Nevertheless, abnormal orofacial, limb and trunk involuntary movements known as L-DOPA-induced dyskinesias (LIDs) remain a major complication. Striatal outputs are composed of medium spiny neurons (MSNs), which inhibit the downstream nuclei of the basal ganglia via the direct and indirect pathways. Direct MSNs innervate the Substantia nigra pars reticulata and the internal segment of the Globus pallidus, while indirect MSNs project to the external segment of the Globus pallidus (GPe). Whereas the role of direct MSNs when mediating LIDs has been established, the contribution of indirect MSNs is still elusive. Anyhow, it is known that during dyskinesias, indirect MSNs display a decreased activity.
Our main objective is to determine the role of indirect MSNs in the LIDs of a mouse model of PD. Our hypothesis is that the activation of indirect MSNs will reduce LIDs. To that end, D2-cre recombinase expressing adult mice are lesioned with 6-hydroxydopamine (6-OHDA) in the medial forebrain bundle, promoting Parkinsonian symptoms. Channelrhodopsin expression is selectively induced in indirect MSNs by injection of adeno-associated cre dependent virus in the dorsolateral striatum. Being that direct and indirect MSNs are connected to each other at striatal level, in order to avoid unspecific effects, the optic fibers are implanted on the GPe. Dyskinetic symptoms are induced by repetitive administration of L-DOPA. Our results show the behavioural effect of the optical stimulation of indirect MSNs' terminals in the GPe, performed before and after three daily injections of L-DOPA, at subthreshold and suprathreshold dyskinetic doses. We also examine the combined effect of light stimulation and an acute L-DOPA treatment.

THE VPA MURINE MODEL OF AUTISM: DIFFICULTIES AND ACHIEVEMENTS RELATED TO ITS OBTAINMENT AND ANALYSIS

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Autism is a neurodevelopmental disorder with a multifactorial origin and diverse manifestations. Although many progresses have been made in the knowledge of this condition, there is still a long way to understand its cellular, neuroanatomical, and behavioural bases. In addition, it is mandatory to explore possible treatments that might ameliorate the symptomatology of people with this disorder. In this sense, biomedical research using animal experimentation has generated significant advances in the knowledge of autism. Nowadays several animal models that mimic autism in humans are used, either generated from exposure to environmental factors or carrying identified human genetic mutations.

One of the most validated and worldwide employed animal models for autism research is the valproic acid-induced rodent model of autism (VPA). However, we have detected multiple difficulties to obtain it and, unfortunately, the existing literature does not expose these arising hindrances. In the present work we describe the path that our team followed to generate the VPA model. Several problems emerged related to difficulties on the set of the optimal dosage of valproic acid: sedative and/or epileptogenic signs in dams, abortion in pregnant mice and mortality in dams and offspring.

Once solved these obstacles, we started to investigate the olfactory bulb histopathology of the animal model by histochemistry and immunohistochemistry, and its olfactory capabilities through a variation of the Marble Burying Task using odorants. Our preliminary results suggest variations at both levels in the VPA model. The analysis of the olfactory system will shed light on the autism spectrum disorders research field, as it has been shown that the sensory alterations in this condition could be affecting its general pathogeny.

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TRANSCRIPTOMIC ANALYSIS IN A FRAGILE X SYNDROME MOUSE MODEL AFTER CB1 RECEPTOR TARGETING REVEALS TREATMENT-ASSOCIATED CHANGES IN RNA SPLICING MACHINERY.

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Fragile X syndrome (FXS) is the most common monogenic cause of inherited intellectual disability and autism caused by the silencing of the FMR1 gene. The fragile X mental retardation 1 (Fmr1) knockout mouse shows cognitive impairment and some of the synaptic alterations observed in patients because of the loss of expression of the fragile X mental retardation protein (FMRP). Altough these alterations observed in the FXS mouse model have been restored by blocking the cannabinoid type-1 receptor (CB1R) with the systemic antagonist/inverse agonist rimonabant, the molecular hallmarks involved in these improvements are not yet fully understood. Since FMRP modulates local RNA translation at synapses to maintain synaptic activity and plasticity, we focused our analysis in a synaptoneurosome enriched fraction obtained from mice treated for seven days with rimonabant (0.1 mg/kg). We performed high-throughput RNA sequencing to analyse differential expression and splicing patterns between Fmr1 KO mice and WT after rimonabant or vehicle administration. Differential expression analysis at transcript level in Fmr1 KO synaptoneurosomes revealed the up-regulation of transcripts implicated in axon development and the down-regulation of transcripts related with synapse structure and organization, and mRNA processing. Notably, rimonabant treatment induced the upregulation of transcripts related with mRNA splicing. Furthermore, alternative splicing analysis of the same data showed a relevant number of transcripts producing different isoforms by exon skipping mechanisms in Fmr1 KO samples. Interestingly, these exon skipping events appeared to be regulated in opposite ways when Fmr1 KO mice were treated with rimonabant at low doses. Together, our transcriptomic analysis identifies a set of transcripts that may contribute to the aberrant synaptic phenotype in the FXS mouse model. Moreover, this analysis proposes that alternative splicing events described in Fmr1 KO mouse may be reverted after CB1 receptor inhibition under conditions that re-establish synaptic plasticity and memory perforamance.

TRAUMATIC BRAIN INJURY INDUCES A BIPHASIC LONG-TERM EFFECT ON ADULT HIPPOCAMPAL NEUROGENESIS

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Several important cognitive functions affected by traumatic brain injury (TBI) depend on the hippocampus, which harnesses several forms of neural plasticity, among them adult neurogenesis, the generation of new neurons throughout life. Adult hippocampal neurogenesis is a process involved in memory, learning and control of anxiety, cognitive functions which result impaired after TBI. We hypothesize that TBI induces fast and long-term changes in both neural stem cells (NSCs) and newborn neurons which could subsequently alter hippocampal and brain functioning. Using a model of controlled cortical impact (CCI) we have found that TBI has a dual effect on neurogenesis: In the short term (up to two months) it causes an increase in the number of newborn neurons but with aberrant migration, increased soma size and altered electrophysiological properties; in the long term, neurogenesis results impaired by a reduction in the number of immature neurons. We also suggest that the alteration in the expression of Rho Family GTPase 2 (Rnd2) could be causing some of the morphological changes in the immature neurons as well as their aberrant migration and thus could be a target to prevent TBI-induced aberrant neurogenesis, a hypothesis that we are currently investigating at the cellular level. In addition, we have found that NSCs get activated in higher numbers early after TBI, a result that could explain the later reduction in neurogenesis.

TROPHIC DEPENDENCE OF ABDUCENS MOTONEURONS ON MUSCLE VEGF

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Although initially discovered by its angiogenic properties, vascular endothelial growth factor (VEGF) has recently been shown to act as a neuroprotective molecule after different types of lesion, particularly in motoneurons. In the present work, we applied VEGF to axotomized abducens motoneurons to unravel whether this factor could recover the synaptic loss and firing alterations induced by axotomy. In addition, we applied VEGF neutralizing antibody in the muscle to study the role of this factor in the physiology of uninjured motoneurons.
Adult cats were prepared for the chronic recording of abducens motoneuron discharge activity simultaneously with eye movements. For the study of the effects of axotomy, the VIth nerve was cut in the orbit and its proximal stump inserted in a home-made chamber for the administration of PBS+0.1% BSA. VEGF administration was performed through either the nerve or the fourth ventricle, and the application of the neutralizing antibody was made directly on the muscle. We also carried out an immunocytochemical study of motoneuronal synaptic boutons at the confocal level, by using antibodies against synaptophysin, VGAT and GFAP, and against GLUT-1 for the study of the vasculature.
Axotomy led to an overall low firing rate and a decrease in motoneuronal sensitivities to eye movement parameters. These physiological changes were accompanied by the withdrawal of afferent synaptic boutons and an intense astrocytic reactivity. VEGF treatment recovered both the firing alterations and the synaptic stripping in axotomized motoneurons, and reverted the astrocytic reaction to control values without an increase of blood vessels. Moreover, the administration of VEGF neutralizing antibody rendered uninjured motoneurons into an axotomy-like state.
This is the first work demonstrating that VEGF acts as a powerful synaptotrophic molecule for injured motoneurons, restoring firing and synaptic inputs to the control situation. The present data reinforce the therapeutical potential of VEGF for motoneuronal disorders.

UNDERSTANDING THE ROLE OF PRE-CONDITIONING INFLAMMATION ON THE ONSET OF ALZHEIMER'S DISEASE

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Microglial cells are self-renewing macrophages of the brain with a highly regulated system of turnover (1) and are the first line of defence in the CNS. In the onset of Alzheimer's disease, microglial cells become activated and proliferate (2) and we recently identified that the prolonged engagement of microglia in proliferation induces replicative senescence, accelerating the disease (3). We now hypothesise that early life insults could promote an accelerated proliferation of microglial cells, pre-conditioning them to become senescent. Senescent microglia may show a phenotypic change characterised by cell cycle arrest and primed inflammatory response, which later in life could have a negative impact on the onset of AD. We investigated the effect of repeated sub-threshold inflammatory challenges in early life, similar to those seen in the human life course such as bacterial infection. We model this by injecting low-doses of LPS to young mice and then studying the effect that has on the severity of AD-like pathology later in life, using an inducible APP model (line 102) (4). Our results indicate that sub-threshold inflammation promotes the proliferation of microglial cells driven by the CSF1R pathway, which might be pre-conditioning them to become senescent. The outcome of this research could support future preventative approaches to delay the onset of clinical symptoms.

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UNRAVELLING THE DISTRIBUTION AND FUNCTION OF THE LIPID TRANSFER PROTEIN VPS13A IN THE BRAIN TO UNDERSTAND CHOREA ACANTHOCYTOSIS PATHOLOGY

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Chorea-acanthocytosis (ChAc) is caused by a VPS13A gene mutation leading to marked reduction or absence of VPS13A protein. ChAc patients show progressive movement disorders such as chorea and dystonia. The main neuropathologic feature in VPS13A mutations is a selective degeneration of the striatum, however, little is known about the VPS13A expression in the brain. There is also a poor knowledge about VPS13A function in neural cells. Thus, the objectives of this work are a) to assess the time course and the regional expression of vps13a gene in the mouse brain and b) to study the vps13a interaction partners. Single cell RNA showed that vps13a is present in mature neurons and qPCR revealed that vps13a expression is stable over time. Then, we used fluorescence in-situ hybridization and immunohistochemistry to determine the distribution of vps13a mRNA and protein in mouse brain from embryonic stages to adulthood. In the adult mouse brain, we found a widespread distribution of vps13a, with different staining intensity profiles between nuclei. In general, the mRNA localization resembled that of the protein one with an enrichment in the pons, cerebellum and hippocampus. We found moderate staining in the cortex and in the most thalamic and hypothalamic nuclei. Interestingly, we found weak staining in the basal ganglia nuclei. We observed vps13a staining in glutamatergic, GABAergic and cholinergic neurons. Not only neurons but also some glial cells expressed chorein. The levels of vps13a protein were not modulated neither by pilocarpine, amphetamine nor ketamine treatments, suggesting that VPS13A has structural and stable role in neural cells. We also evaluated the vps13a interactome through a specific protein immunoprecipitation from mouse cerebral cortex followed by mass spectrometry. Vps13a interacts with lipid metabolism proteins. Understanding the brain tissue distribution, expression and protein interacting partners can provide novel insights toward the knowledge of ChAc pathophysiology.

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UPREGULATION OF TLR4 SIGNALLING PATHWAY AND BEHAVIORAL DISHINIBITION IN WERNICKE-KORSAKOFF SYNDROME: EVIDENCE FROM AN ANIMAL MODEL AND HUMAN POST-MORTEM TISSUE

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Wernicke-Korsakoff syndrome (WKS) is a neuropsychiatric disorder induced by thiamine deficiency (TD) whose main causal factor is alcoholism. WKS patients show significant mood and executive function alterations which are even more devastating than memory problems. A dysfunction in the prefrontal cortex (PFC) has been associated to impulsivity and disinhibition in WKS patients. This pathology occurs with neuroinflammation as one of the mechanisms responsible of brain damage, but specific mechanisms have not been understood yet. Here, we explored the innate immune receptor Toll-like 4 (TLR4) and its signaling pathway in postmortem human tissue and in a WKS animal model and its relationship with behavioral disinhibition.

WKS was induced in rats by chronic consumption of 20% (w/v) alcohol during 9 months along with a TD hit (TD diet + pyrithiamine 0.25 mg/kg, i.p. daily injections) during the last 12 days of experimentation. Rats were evaluated on behavioral tasks that are highly dependent on the PFC, as the elevated plus maze and open field test at the end of treatments. Rat PFC was dissected and analyzed for TLR4, MyD88, IκBα and HSP70 expression. Additionally, immunohistochemical studies were carried out in postmortem brain of an alcohol-induced WKS diagnosed subject.

WKS animals showed a clear disinhibited-like behavior, which correlated with the upregulation of the TLR4 signaling pathway in the PFC. Interestingly, postmortem PFC samples of a WKS diagnosed patient showed an upregulation of TLR4 and its co-receptor MyD88 both in gray and white matter structures compared with a paired healthy control.

Our results show alterations in the TLR4 pathway in WKS postmortem human brain and suggest a key role for cortical TLR4 in the disinhibition-like behavior, as evidenced by the WKS animal model.

USE OF FLUOROPHORE-CONJUGATED PEPTIDES TO ASSES PUTATIVE TARGETING TO GLIA OF PEPTIDE-DRUG HYBRID MOLECULES AS A NEW THERAPEUTIC APPROACH FOR MULTIPLE SCLEROSIS

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Demyelinating diseases such as Multiple Sclerosis (MS) are a collection of pathologies that involve the degradation of the myelin sheath of the neurons, resulting in severe handicaps for neurological physiology. Oligodendrocyte precursor cells (OPCs) are the cells that upon differentiation and maturation (re)generate the myelin necessary for the correct function in central nervous system (CNS). Thus, finding pharmacological approaches to stimulate OPCs maturation into myelinating oligodendrocytes (OLs) is considered one the of the great challenges in MS treatment. Previous work from our group has shown the effect of compounds that enhance this (re)myelinating process [1].

However, one of the additional key challenges on systemically administered drugs is finding strategies to deliver them into their specific target organs or cells. In this sense, small peptides have previously exhibited promising features as target-specific interactors [2], thus becoming interesting putative carriers in hybrid molecule (carrier-drug) treatment strategies.

In this work, we utilized ad hoc designed small peptides, CTB2.20 and CTB 2.21 labelled withfluorophore Cy3 to validate the specificity of those peptides delivering small molecules into OPCs, both in vivo and in different cell cultures.

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WHY THE LOBULE X OF THE CEREBELLUM RESISTS THE NEURODEGENERATION OF THE PCD MOUSE?

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The Purkinje Cell Degeneration (PCD) mouse suffers a mutation in the Ccp1 gene, which codifies a carboxypeptidase responsible for the stability of the cytoskeleton. Under its absence in the PCD mouse, the Purkinje cells (PC) of the cerebellum collapse and at 30 post-natal days (P30) just a few PC remain alive, mainly in the lobule X. It is not fully understood why this region maintains its cell population longer than the other lobules.

We have studied several genes related with Ccp1 and the equilibrium of the cytoskeleton, like Ccp4, Ccp6 and Ttll1 by qPCR and by Western Blot; the Ccp family deglutamylates the cytoskeleton, the Ttll family polyglutamylates it, and both families must stay in equilibrium. Besides, we have studied with immunohistochemistry techniques the expression of HSP25, a Heat-Shock-Protein responsible for the neuroresistance of the lobule X in other animal models of ataxia. Moreover, we have also analysed the expression of the phosphorylated version of HSP25 (HSP25-P), which has even more anti-apoptotic properties.

We demonstrated that the lobule X of the wild type cerebellum has a lower expression of Ccp1, so it is possible that this lobule is less dependent of this gene, its absence being less harmful in the PCD mouse. Similarly, Ttll1 is less expressed in the lobule X than in the rest of the vermis, which could imply that the cytoskeleton of this lobule is less glutamylated, so that is why the deglutamylating function of Ccp1 is less required. Finally, we have seen that both HSP25 and HSP25-P are more expressed in the lobule X of the PCD mouse than in the wild type one. Therefore, HSP25 and HSP25-P could be also responsible of the neuroresistance of the lobule X in the PCD mouse. Altogether our data suggest a multifactorial origin for the neuroresistance of this cerebellar region.

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Δ9-TETRAHYDROCANNABINOL PROMOTES FUNCTIONAL REMYELINATION IN THE MOUSE BRAIN

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Δ⁹-Tetrahydrocannabinol (THC), the most prominent active constituent of the hemp plant Cannabis sativa, confers neuroprotection in animal models of multiple sclerosis (MS). However, the possible effect of THC on oligodendrocyte regeneration and myelin repair has never been studied. Here, by using oligodendroglia-specific reporter mouse lines in combination with 2 models of toxin-induced demyelination, we show that THC administration enhanced oligodendrocyte regeneration, white matter remyelination, and motor function recovery. Interestingly, THC also promoted axonal remyelination in organotypic cerebellar cultures ex vivo. THC remyelinating action relied on the induction of oligodendrocyte precursor cell cycle exit and differentiation via CB1 cannabinoid receptor activation. Overall, our study identifies THC administration as a promising pharmacological strategy aimed to promote functional CNS remyelination in demyelinating disorders as MS.

Topic 7

Homeostatic and Neuroendocrine Systems

Posters

ASTROCYTIC GLUT1 ABLATION IMPROVES SYSTEMIC GLUCOSE METABOLISM AND PROMOTES COGNITION

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Glucose supply from the blood to the brain is controlled by the glucose transporter GLUT1, highly expressed in astrocytes, which coordinate brain glucose supply, metabolization and storage. Ablating GLUT1 at the blood-brain barrier (BBB) endothelial cells leads to BBB breakdown, brain glucose hypometabolism and impaired cognition, but this approach cannot discriminate between insufficient glucose supply and BBB breakdown-derived effects. Such question is the focus of the present work, which aims to elucidate the relevance of astrocytic GLUT1 to cellular, brain and systemic glucose metabolism, and to cognition.

To address these questions, GLUT1 was ablated from primary astrocytes. Real-time cellular metabolism was examined using an extracellular flux analyzer (Seahorse) and gene expression studies. In vivo, astrocytic GLUT1 was ablated using a tamoxifen-inducible Cre/LoxP approach (GLUT1ΔGFAP mice). 18F-FDG PET, glucose and insulin tolerance, insulin secretion and fasting-induced hyperphagia were characterized. Cold exposure, histology and gene expression were used to study brown adipose tissue (BAT) activity. BBB integrity was examined by vessel immunostaining and capillary-depleted brain analysis. Recognition and spatial memory were assessed using Novel Object Recognition and Morris Water Maze tasks.

GLUT1-ablated astrocytes showed reduced glucose uptake and glycolysis, although preserving total ATP production. Unexpectedly, postnatal astrocytic GLUT1 deletion increased CNS glucose utilization. GLUT1ΔGFAP mice showed an improved metabolic status from which obese animals especially benefited. Specifically, GLUT1ΔGFAP mice were more efficient at suppressing hyperphagia and readjusting systemic glucose levels after hyperglycemia, exhibiting markedly increased insulin secretion. These effects were coupled with enhanced BAT activity, and reduced BAT adiposity. In parallel with this improved systemic homeostasis, GLUT1ΔGFAP mice performed both recognition and spatial memory tasks properly, even outperforming control mice.

Overall, this study demonstrates that astrocytic GLUT1 ablation impairs astrocytic glucose availability but enhances brain glucose utilization, reprograms systemic glucose metabolism towards a more efficient glucose-handling phenotype and promotes cognitive abilities.

CELLULAR PLASTICITY OF NEUROPEPTIDERGIC SYSTEMS IN THE MOUSE HYPOTHALAMUS.

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The neuropeptides oxytocin (OXT) and arginine vasopressin (AVP) play critical roles in regulating complex animal behaviors and homeostatic functions. Both neuropeptides are mainly synthetized at specific hypothalamic nuclei, such as the paraventricular and suprachiasmatic nucleus (PVN and SON). Our previous work (Madrigal & Jurado, 2021) revealed a significant number of neurons co-expressing OXT and AVP during early postnatal stages (PN7) coinciding with a critical period for social interaction. This mixed population drastically decline in the adult brain suggesting that a switch in neuropeptide expression is required for the maturation of the system. Here, we have analyzed the plastic properties of OXT and AVP circuits in the adult brain using brain clearing techniques (iDISCO+) and 3D imaging. Our study has revealed region-dependent cellular plasticity in the SON and the retrochiasmatic area (RCH) in response to sexual experience and motherhood. Our observations indicate a prevalence of AVP neurons in the SON of virgin females that turns into an increase of OXT neurons after giving birth. We explored the hypothalamic neuronal subtypes more susceptible to undergo changes in neuropeptide expression by expanding our analysis to additional markers associated to OXT and AVP neurons. To this aim, we combined interneuron marker GAD67 and monoamine indicator tyrosine hydroxylase (TH) with OXT and AVP. Our results indicate the presence of GAD67 positive neurons in the hypothalamus but minimal co-localization with neither OXT nor AVP cells, suggesting that oxytocinergic and vasopressinergic interneurons may express different markers like GAD65. On the other hand, we found that sexual experience induces TH expression in a subpopulation of AVP and OXT neurons in the RCH. Our findings provide new information to understand the specification of neuropeptidergic systems during development and their plastic properties upon critical life events in the adult animal.

ENHANCED NEURONAL GLYCOLYSIS CAUSES COGNITIVE IMPAIRMENT AND METABOLIC SYNDROME IN MOUSE

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In contrast to the predominantly glycolytic nature of astrocytes, neurons use very little glucose to obtain energy through glycolysis (1). This is a critical metabolic signature of the brain that is dictated by differences in the abundances of the pro-glycolytic enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3). Thus, PFKFB3 is highly expressed in astrocytes, but repressed in neurons due to a continuous proteasomal degradation after ubiquitination by the E3-ubiquitin ligase anaphase-promoting complex/cyclosome-Cdh1 (2). However, the in vivo physiological significance of the limited glycolytic activity of neurons is intriguing. To address this matter, here we generated a genetically engineered mouse to express PFKFB3 in neurons in vivo (CaMKII-PFKFB3). We found that neuronal PFKFB3 upregulated glycolysis, causing NAD depletion, dysfunctional mitochondria, redox stress and impairment of the autophagic flux in several brain regions, including the hippocampus and the mediobasal hypothalamus. Phenotypic characterization of these CaMKII-PFKFB3 mice revealed cognitive deterioration, motor discoordination, glucose intolerance and obesity. Interestingly, these biochemical and phenotypic alterations were rescued by abolishing redox stress via genetically expressing a mitochondrially-tagged isoform of catalase (mCAT) in neurons. Mechanistically, the increase in glycolysis shifted down glucose consumption through the pentose-phosphate pathway, causing redox stress that impaired mitochondrial respiration, triggering a signaling pathway leading to an aberrant positive loop of glycolytic activation. These data indicate that the existence of a low glycolytic activity in neurons is a natural mechanism aimed to sustain organismal welfare. [Funded by the Agencia Estatal de Investigación PID2019-105699RB-I00/ AEI / 10.13039/501100011033]. References: (1) Nat. Cell Biol. 6:45-51 (2004); (2) Nat. Cell Biol. 11:747-752 (2009).

ESTRADIOL REGULATES PSA-NCAM EXPRESSION AND CONNECTIVITY OF O-LM INTERNEURONS IN THE HIPPOCAMPUS OF ADULT FEMALE MICE

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17β-Estradiol is a sex hormone with well-reported effects on excitatory neurons and networks. However, our understanding on how this hormone affects inhibitory circuits and interneurons, namely O-LM cells, is still scarce. The somata of these cells are placed in the stratum oriens of the hippocampus and they innervate pyramidal neurons in the stratum lacunosum moleculare. They express somatostatin, display dendritic spines, and show high structural plasticity and dynamics modulated by the plasticity-related molecule PSA-NCAM. Furthermore, they contribute to the modulation of theta oscillations and receive direct input from the entorhinal cortex, altogether highlighting their importance. GIN mice are a great tool to study these neurons because they express constitutively GFP in a Golgi-like manner. Therefore, to study the effect 17β-Estradiol has on their structural plasticity and its regulation by PSA-NCAM, we used adult female ovariectomized mice of this strain. We show that the replacement treatment decreases the expression of PSA-NCAM in strata oriens and lacunosum moleculare, where these interneurons are located, as well as increases the density of inhibitory markers around these cells. Furthermore, it also increases the density of their axonal boutons, as well as lowers the density of their dendritic spines only in O-LM interneurons lacking PSA-NCAM expression. To further research these effects, we also performed entorhino-hippocampal organotypic cultures in order to image in real time these cells and study their structural dynamics being modulated by this hormone. Here we show a decrease of the appearance rate of dendritic spines, while the disappearance and stability rates remained unchanged. Altogether our results underscore the effect 17β-Estradiol has on the structural plasticity of these interneurons and how it is modulated by PSA-NCAM.

FUNCTIONAL PROPERTIES AND MOLECULAR MACHINERY UNDERLYING OXYTOCIN RELEASE

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The oxytocinergic system regulates key brain functions and its dysregulation leads to neurological disorders. However, it is still unknown how oxytocin (OXT) exocytosis is regulated in the Central Nervous System (CNS).
Oxytocinergic neurons in the hypothalamus are responsible for modulating OXT levels in the CNS mostly through release events occurring at their soma and dendrites. Immunostaining experiments revealed the expression of two SNAP isoforms: SNAP-47 and SNAP-23 in the somatodendritic compartment of oxytocinergic neurons, suggesting a role in OXT exocytosis. Interestingly, the expression of SNAP-23 becomes apparent during the first postnatal week suggesting a tight developmental regulation. We generated specific viral-based knockdowns (shARNs) to eliminate endogenous SNAP-47 and SNAP-23 to determine their role in OXT-vesicle secretion and dynamics. To this aim, we implemented staining protocols and imaging techniques to unambiguously identify OXT-vesicle trafficking. We observed KCl stimulation significantly increased the probability of OXT release. However, this effect is only observable during short stimulation protocols since long stimulation times seem to deplete the OXT releasable pool during the first seconds of KCl application. Visualization of somatodendritic OXT-vesicles revealed heterogeneous dynamics in response to neuronal stimulation. Four main vesicle pools were identified: i) dynamic vesicles mobilized during the first 10 s of stimulation, ii) stable vesicles, iii) uncoupled vesicles and iv) delayed vesicles, the most abundant type, with observable movement after 20-40 s of stimulation. We performed electrophysiological recordings to determine how OXT-vesicles kinetics relates to the excitability of oxytocinergic neurons. Our observations indicate oxytocinergic neurons exhibit slow depolarization that might underlie the delayed dynamics of OXT-vesicles and their partial dependence on calcium. These results suggest the existence of different pools of OXT-vesicles which are distinctly mobilized in response to the same stimulus, a feature that may be relevant for orchestrating complex behavioral responses.

HYPOTHALAMIC ANOREXIGENIC AND OREXIGENIC GENE EXPRESSION AFTER MORNING OR EVENING FORCED WHEEL EXERCISE IN ADOLESCENT RATS

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Physical activity became an effective way to modify the body composition. Changes in body composition during adolescence can affect manifestation of diseases such as obesity or diabetes later in life. In a previous work we found that males showed decreased adipose tissue contents after an exercise program with morning and evening sessions (2 sessions a day). Here, were analyzed the training effects during either morning or evening sessions in body composition and their molecular hypothalamic changes.

In this study P20 adolescent rats (n=24) were trained in a forced program until P60. Different groups trained at ZT13 (n=6 AM exercise, n=6 AM sedentary) or at ZT23 (n=6 PM exercise, n=6 PM sedentary). On P24 and P57, the whole body of the rats was analyzed through computerized tomography. Food and water intake were measured every 24 hours. On P60, the hypothalamic region was removed, snap-frozen in under 10 minutes after sacrifice, and stored at -80C until the mRNA isolation, cDNA synthesis and qPCR study. All the procedures regarding the qPCR methodology were performed by following the MIQE guidelines. Statistical analysis and graphs were performed with GraphPad Prism 9.

Only the PM exercise group showed lower adipose tissue content ($p<0.05$). Both exercise groups showed higher lean content than the respective control groups ($p<0.05$). No differences were observed regarding body weight or body volume between the groups ($p>0.05$). No differences were observed in the food intake or the orexigenic/anorexigenic genes expression Pomc, Agrp, Npy, Cartpt ($p>0.05$).

Our results suggest that the effects of morning or evening exercise on the body composition are not related to intake differences. Further studies are required to elucidate the molecular mechanisms responsible of these differential effects of exercise, dependent on the time of the day.

IGF1 MODULATES INFLAMMATION AND PHAGOCYTOSIS IN REACTIVE ASTROCYTES THROUGH PI3K(P110A) IN A SEX-SPECIFIC MANNER

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During the last years, increasing evidence suggests that male and female brains react differently to insults. However, little is known about the mechanisms underlying this differences. Insulin-like growth factor-1 (IGF1) is a neuroprotective factor involved in regulating processes such us neurogenesis, synapse formation, anti-inflammation and phagocytosis in brain damage situations.

To investigate the role of IGF-1 in regulating neuroinflammation, we use a systemic treatment with Lipopolysaccharide (LPS) that induced an increase of GFAP expression, which was abrogated by IGF-1 treatment only in males. In primary astrocyte cultures treated with LPS and IGF-1, we measured the levels of pro-inflammatory molecules by q-RTPCR and astrocyte engulfment of CY3-conjugated neuronal debris. LPS induced an increase of mRNA levels of TLR-2 and 4, iNOS, IP-10, IL-1β, IL-6 and IL-10 in both sexes and a decrease of IRAK4 mRNA expression specifically in male astrocytes. The treatment with IGF1 was able to counteract the effect of LPS on mRNA expression of TLR4 in both sexes and the expression of IRAK4, iNOS, IL-6 and IL-10 mRNA only in males. Furthermore, also in male astrocytes stimulated with LPS, IGF-1 induced an increase in neural debris phagocytosis.

To evaluate the involvement of PI3K/AKT pathway in IGF-1 regulation, specific inhibitors of PI3K catalytic subunits p110α, p110β and p110δ, were tested. Only p110α inhibitor counteracted the effects of IGF-1 in male astrocytes, with no significant effects on females. Although all the catalytic subunits interact physically with IGF1-R in both sexes, the level of expression of p110α is higher in male reactive astrocytes treated with IGF-1 than in females.

Taken together, our results reveal that IGF-1 affects the inflammatory and phagocytic function of astrocytes through a specific and sexually dimorphic interaction between IGF-1R and p110α.

IGF-I MITIGATES POST-TRAUMATIC STRESS THROUGH OREXIN NEURONS

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Maladaptive coping behaviors are probably involved in post-traumatic stress disorders (PTSD), but underlying mechanisms are incompletely understood. We previously documented that insulin-like growth factor I (IGF-I) is associated to vulnerability to stress both in mice and humans. Since hypothalamic orexin neurons express IGF-I receptors and are involved in responses to stress, we analyzed their role in the modulatory actions of IGF-I on stress.
Anxiolytic actions of IGF-I were measured after exposure to a predator using osmotic minipumps implanted icv in mice lacking IGF-I receptors in orexin neurons (Firoc mice). Firoc mice were submitted to fear conditioning and thereafter to c-Fos immunostaining in orexin neurons and noradrenergic neurons of the locus coeruleus nucleus. Chemogenetic (DREADD) inhibition of orexin neurons was carried out in fear conditioning and in an extended protocol including context recall and anhedonia tests. Molecular changes related to PTSD were determined by qPCR at different time points. Moreover, excitatory/inhibitory (E/I) balance in orexin neurons was analyzed by immunocytochemistry.
We found that Firoc mice are unresponsive to the anxiolytic actions of IGF-I and develop PTSD-like behavior that is ameliorated by inhibition of orexin neurons. Further, systemic IGF-I treatment ameliorated PTSD-like behavior in a wild type mouse model of PTSD. In addition, systemic IGF-I increased the E/I ratio in orexin neurons of naïve wild type mice by increasing the dephosphorylation of GABA(B) receptor subunit through inhibition of AMP-kinase (AMPK). Significantly, pharmacological inhibition of AMPK mimicked IGF-I, normalizing fear behavior in PTSD mice.
Collectively, these results suggest that IGF-I enables coping behaviors by balancing E/I input onto orexin neurons in a context-dependent manner. These observations provide a novel therapeutic approach to PTSD through modulation of AMPK.

INSULIN-LIKE GROWTH FACTOR I COUPLES METABOLISM WITH CIRCADIAN ACTIVITY THROUGH HYPOTHALAMIC OREXIN NEURONS

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Uncoupling of metabolism from circadian activity is associated with increased risk of various pathologies, including neurodegeneration. Recently, insulin and the closely related insulin-like growth factor I (IGF-I) were shown to entrain feeding patterns with circadian rhythms. Moreover, both hormones act centrally to modulate peripheral glucose metabolism; however, whereas central targets of insulin actions are intensely scrutinized, those mediating the actions of IGF-I remain undefined. We analyzed whether IGF-I targets orexin neurons in the lateral hypothalamus, as these neurons are involved in circadian rhythms and energy allocation, and are modulated by IGF-I. Mice with disrupted IGF-IR activity in orexin neurons show phase shifts in circadian feeding behavior, loss of circadian orexin expression, and gradually develop sex-dependent metabolic alterations. We also found that central modulation by IGF-I of hepatic KLF transcription factors involved in peripheral glucose metabolism is mediated by orexin neurons. Thus, IGF-I entrains energy metabolism and circadian rhythms through hypothalamic orexin neurons.

MITOCHONDRIAL FISSION FACTOR (MFF) REGULATES MITOCHONDRIAL DYNAMICS AND EXCITABILITY OF AGOUTI RELATED PEPTIDE (AgRP)-EXPRESSING NEURONS

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Tight regulation of whole-body metabolism is essential in maintaining energy homeostasis and for preventing several diseases such as obesity. AgRP neurons in the arcuate nucleus of the hypothalamus (ARC) promote food intake and control systemic insulin sensitivity. Mitochondria are highly energetic dynamic organelles, which can undergo fission and fusion events also in adaptation to the energy state. Mitochondrial fission factor (MFF) has a key role during the initiation of mitochondrial fragmentation, where it serves as an adaptor for the dynamin related GTPase (DRP)-1. Recently, mitochondrial dynamics was identified as a critical regulator of synaptic transmission. Thus, we aimed to define the role of MFF in AgRP neurons and its possible role in the regulation of whole-body metabolism.

To this end, we generated mice with AgRP-neuron specific MFF inactivation. When crossed to a mouse line, which express YFP at the outer mitochondrial membrane, this allowed us to monitor mitochondrial morphology and dynamics in control mice and those lacking MFF in AgRP neurons (AgRPΔMFF-mice). While control exhibited a more fragmented mitochondrial network during fasting, this effect was attenuated in AgRPΔMFF-mice. Interestingly, electrophysiological recordings performed in AgRP neurons of AgRPΔMFF mice indicated an increase in the neuronal excitability due to differences in the spike frequency adaptation. Nevertheless, these animals retain an unaltered ability to respond to orexigenic hormones such as ghrelin. Moreover, despite altered AgRP neuron excitability, AgRPΔMFF animals showed no overall changes in body weight and glucose homeostasis. Collectively, partially impairing mitochondrial fragmentation in AgRP neurons increases their neuronal excitability without major differences in metabolism.

NATURAL **IgMs THAT BIND TO THE NEO-EPITOPES PRESENT IN CORPORA AMYLACEA OF THE HUMAN BRAIN RECOGNIZE CARBOHYDRATE STRUCTURES**

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Corpora amylacea (CA) are spherical polyglucosan bodies that accumulate primarily in the periventricular, perivascular and subpial regions of the human brain during aging and some neurodegenerative diseases. Recent studies indicate that CA gather waste substances from the brain and act as waste containers. It has been reported that CA contain some neo-epitopes (NEs) recognized by natural IgMs, but these NEs are still undefined. Here, we performed IgM preadsorptions with increasing concentrations of specific monosaccharides to find out if the preadsorption produced a partial or total inhibition of CA staining by the IgMs. As controls, the same studies were performed by staining CA with concanavalin A (ConA), a plant lectin that binds to particular carbohydrate structures, and with an antibody directed against the p62 protein (anti-p62). ConA and anti-p62 were preadsorbed with the mentioned monosaccharides under the same conditions as IgMs. As expected, CA staining with ConA was blocked by the preadsorption with the appropriate monosaccharides. On the other hand, CA staining with anti-p62 was not affected by any monosaccharide tested, which demonstrated that the preadsorption with the sugars did not block the antibodies directed against the proteins. Regarding IgMs, we observed that the binding between the natural IgMs and the NEs sited on CA become interfered in vitro by the preadsorption with certain monosaccharides, particularly by glucose. These findings point out the carbohydrate nature of the NEs located in CA. Moreover, the present study indicates that, in vitro, the binding between certain natural IgMs and certain epitopes may be disrupted by some monosaccharides. Whether these inhibitions may also occur in vivo will be addressed in future experiments. In this regard, further studies will be carried out to assess the possible in vivo effect of glycemia on the reactivity of natural IgMs and, by extension, on natural immunity.

NEO-EPITOPES FROM CORPORA AMYLACEA IN THE HUMAN BRAIN DO NOT HAVE A PEPTIDIC NATURE

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Aging and neurodegenerative processes induce the formation of waste substances in the brain. Some of these substances accumulate in corpora amylacea (CA), a type of polyglucosan bodies constituted primarily by polymerized hexoses. CA act as waste containers, since they contain waste products from the human brain, are released from the brain into the cerebrospinal fluid, and reach the cervical lymph nodes where they are phagocytosed. In recent work, we found out that CA contain some neo-epitopes (NEs) that are recognized by natural IgMs, revealing a possible link between them and natural immunity. Natural antibodies, always present in the blood, help to maintain homeostasis by interacting with the NEs formed in old or damaged structures. Although some of these NEs are of carbohydrate nature, the precise nature of the NEs contained in CA is still unknown. In order to shed light on their nature and to discard that they are of peptidic nature, we stained the CA with IgMs after their digestion with pepsin. As controls, digested CA were stained with an antibody directed against the p62 protein (anti-p62), with an NHS ester probe, which binds to proteins, and with concanavalin A (ConA), a carbohydrate-binding protein. Predictably, the digestion of CA proteins prevented the CA staining with both anti-p62 and NHS ester probe, but the staining with ConA, which is directed against sugar structures instead of proteins, was unaltered. Similarly, CA staining with IgM was maintained after pepsin treatment. This evidence consistently rejects the protein nature of the NEs sited on CA while supports their carbohydrate nature. Moreover, the possible presence of carbohydrate NEs in CA reinforces that CA are structures involved in the entrapment of damaged and non-degradable products and their role in protective or cleaning mechanisms.

SIMULTANEOUS RECIPROCAL CHEMOGENETIC REGULATION OF AgRP AND POMC NEURONS REVEALS NON-OVERLAPPING FUNCTIONS IN CONTROL OF METABOLISM.

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Hypothalamic neuronal circuits control energy metabolism, adjusting food intake and metabolic demands to match internal and external cues. Two neuronal populations reside in the arcuate nucleus of the hypothalamus with opposite roles: GABAergic AgRP neurons respond to negative energy balance, promoting rapid food intake and insulin resistance, while POMC neurons are activated in positive energy balance states, resulting in reduced food intake and increased energy expenditure. The coordination of these two population is critical for the metabolism homeostasis. Their feeding-state dependent opposite regulation has thus far not been modulated in vivo, since current approaches have focused on the isolated chemo- or optogenetic modulation of either cell population.

To simultaneously modulate the activity level of both neuron subtypes in opposing directions, as observed during their natural regulation, we employed the use of non interacting recombinases to simultaneously express the activatory DREADD receptor hM3DGq in a Cre-dependent manner in AgRP neurons, while POMC neurons expressed inhibitory hM4DGi receptor dependent on POMC-neuron specific Dre recombinase expression. This approach created a novel transgenic mice line that allows isolated or combined antagonistic chemogenetic modulation of both neuronal subtypes. These experiments revealed that food intake and substrate utilization are driven mainly by AgRP circuits, without further effects of simultaneous POMC neuron inhibition. However, systemic insulin sensitivity, liver sympathetic nerve activity, liver transcriptome profiles and gluconeogenic capacity are differentially modulated by the interaction of POMC and AgRP neurocircuits in comparison to their separated effects. All together, these results demonstrate that the interplay between the independent activation levels of AgRP and POMC circuits controls the metabolic regulation in peripheral tissues, suggesting non-overlapping functions governed by AgRP and POMC neurons.

Topic 8

New Methods and Technologie

Posters

A NOVEL MODULAR TOOLBOX FOR PRECISE NEURONAL EPIGENOME EDITING

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Targeted editing of the neuronal epigenome has become a key methodology to improve our understanding of epigenetic function and regulation. These methods may also lead to the development of novel therapies to treat epigenome-associated diseases. Among epigenome editing systems, the use of CRISPR (Clustered Regularly InterSpaced Palindromic Repeats) stands out for its versatility, ease of engineering, and cost-effectiveness. The chimeric fusion of the nuclease-deficient dCas9 with epigenetic enzymatic activities enables locus-specific rewriting of epigenetic information by guide RNAs. However, the resulting chimeric proteins are often large in size and beyond the packaging capacity of the viral vectors most commonly used in neuroscience. To overcome this limitation, we have developed a novel, modular toolbox in which chimeric proteins are split into two smaller constructs taking advantage of nanobodies' ability to bind with high affinity to the recognized epitope. More precisely, the dCas9 protein was fused to a nanobody that specifically recognizes GFP and GFP was fused with the catalytic domain of different effector proteins. We targeted our tool to unique promoters of the gene encoding the brain-derived neurotrophic factor, Bdnf, which due to its complex transcriptional regulation and pivotal role in synaptic plasticity and memory is a particularly relevant candidate for epi-editing. Results combining our tool with synthetic and epigenetic effector modules highlight the potential of this novel toolbox for precise neuronal epigenome editing and open new opportunities to elucidate the role of epigenetic mechanisms in the regulation of gene expression in both physiological and pathological conditions.

ADJUSTING AND VALIDATING A PROCEDURE FOR PARENTERAL ANAESTHESIA IN NEONATAL MICE

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In rodents, parenteral anaesthesia is only approved for pups over 7 days of age. By contrast, for neonatal pups only hypothermia and gas anaesthesia (halothane or isofluorane) can be used, as parenteral agents are said to cause high mortality rates. However, for experiments requiring long-term anaesthesia and a certain kind of interactions with the pups, the use of parenteral anaesthesia becomes necessary.
Here we aim at modifying the parenteral anaesthesia doses and procedures approved for pups >7 days , to anaesthetize pups for a long period with reduced mortality rates. The experiment was performed on postnatal day-3 (P3) and P4 pups and the anaesthetic doses used were 37.5 mg/kg+3.75 mg/kg ketamine+xylazine and 50 mg/kg+5 mg/kg. These doses are lower than the ones employed for >P7 pups, thus ensuring reduced mortality.
Anaesthetic was injected intraperitoneally, pups were placed in a box maintained at 37ºC, and their behaviour was video-recorded for 70 minutes. We measured the latency to absolute immobility, the latency to the first movement and the latency of awakening (continuous movement). For those pups not moving at the end of the experiment, we applied pressure to the paw and registered if they responded (yes/no). Pups were then returned to their nest and their survival was checked the next morning.
The results show that both anaesthetic doses are optimal for P3 and P4 pups, ensuring as much as 3000-to-3500 seconds of complete immobility for 50% of the pups. As expected, the latency of complete immobility shows significant, negative correlation with the duration of anaesthesia. In addition, the latency of awakening is similar in P4 pups independently of the dose, but differs significantly between doses in P3 pups. This indicates that younger pups are more sensitive to the dose of anaesthetics. Mortality is low (6%) and does not depend on the dose or age.
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DESIGN AND IMPLEMENTATION OF A METHOD TO STUDY LARYNGEAL RESISTANCE DURING THE STIMULATION OF CUNEIFORM NUCLEUS (CnF) IN SPONTANEOUSLY BREATHING ANAESTHETIZED RATS

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Background: abduction and adduction of the vocal folds are performed by motoneurons located in the loose formation of the nucleus Ambiguus (nA) innervating the laryngeal muscles. In previous studies we have demonstrated a functional interaction between hypothalamic (DMH-PeF), mesencephalic (dlPAG) and pontine nuclei (PBc, A5 region) involved in cardiorespiratory control and in changes of laryngeal caliber (López-González et al., 2020; Lara et al., 2002). The Cuneifom nucleus (CnF) of the mesencephalon has afferent and efferent connections with all these nuclei. The aim of this study was to characterize the electrophysiological relationships between the CnF and those pontine-medullary neuronal circuits to understand their role in laryngeal control and its effect on vocalization. To achieve this objective is necessary to develop a variation of the classical technique of the "isolated glottis in situ" for the recording of subglottic pressure in rats.

Methods: experimental basic preclinical study in non-inbred male rats [SPF, Sprague-Dawley (250-300 grams)]. Animals were anesthetized with sodium pentobarbitone (60 mg/kg i.p., initial dose, supplemented 2 mg/ kg, i.v., as necessary). A double tracheal (upwards for the "glottis isolated in situ" technique, and downwards in the direction of the carina). Vagus and laryngeal recurrent nerves were isolated and stimulated with bipolar electrodes (Ag/AgCl). Electrical stimulation of the CnF using concentric bipolar electrodes was performed (1 ms pulses, 20-40 µA, 100 Hz for 5 s). Subglottic pressure, respiratory flow, pleural pressure, blood pressure, heart rate and unitary neuronal activity were also recorded.

Results: subglottic pressure was recorded in rats with an aneroid transducer (ADInstrument model FE141, ± 0,03 psi) by passing a stream of humidified medical air upwards through the larynx at a constant rate of 50-100 ml/min with a thermal mass digital air flow meter controller (Bronkhorst Hi-Tec F-201CV-AGD-22-V)

Conclusions: our variation of the classical technique for the recording of the "isolated glottis in situ" in rats shows good dynamic responses and can be perfectly used as an index of subglottic pressure and laryngeal activity.

DISENTANGLING MICROGLIA AND ASTROCYTES ACTIVATION AND NEURODEGENERATION NON-INVASIVELY USING DIFFUSION MRI

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Neuroinflammation is emerging as a cause of the pathogenesis of neurodegenerative diseases [1-4]. Recently we proposed an innovative strategy to image microglia and astrocyte activation in grey matter using diffusion-weighted Magnetic Resonance Imaging (dw-MRI) by building a microstructural multi-compartment tissue model based on glial morphology [5]. However, the capability of the framework to tease apart inflammation with and without neuronal damage was still to be evaluated. Therefore, our objective is to probe the feasibility of the framework for characterizing the inflammatory tissue state under neurodegeneration. To this end, we used a hippocampal rat neurodegeneration model through injection of ibotenic acid where dw-MRI and immunohistochemistry data were compared.

Nine rats were injected bilaterally in the dorsal hippocampus with 1 µl Ibotenic acid 2.5 µg/µl in one hemisphere, and 1 µl vehicle in the contralateral. After 14 days post-injection, the rats were scanned in a Bruker 7T MRI scanner using a dw-MRI sequence to extract the imaging biomarkers defined in [5] and immediately perfused for ex-vivo quantitative immunohistological analysis of microglia, astrocytes, and neurons.

As expected, neuronal staining (NeuN+ cells) was strongly reduced in the ibotenic injected hippocampus ($P<0.001$). Concomitant with the neurodegeneration, we found a strong microglia reaction (Iba1+ cells) characterized by retraction and dispersion reduction of cell processes ($P<0.0001$; $P<0.05$, respectively), together with an increase in cell density ($P<0.0001$). Notably, distinct water diffusion in the different compartments of the developed microstructural tissue model was able to detect and quantify all aspects of the microglial reaction (increased density; $P<0.05$ and decreased processes number; $P<0.001$ and dispersion; $P<0.01$) regardless of neuronal degeneration and identified a neurodegeneration fingerprint when it occurred.

This framework has the potential to disentangle glial activation with and without neurodegeneration and holds great promise to become a sensitive and specific non-invasive tool for characterizing neuroinflammation longitudinally and non-invasively.

EXPERIMENTAL AND MODELING STUDY OF NEAR INFRARED-LASER STIMULATION IN SINGLE AND ELECTRICALLY COUPLED NEURONS

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Near-infrared laser stimulation is a non-invasive stimulation technique for studying neural dynamics. This optical stimulus elicits changes in single neuron dynamics with little or no cell damage. However, the detailed biophysical basis of the effect of this stimulation is not yet known. In this work, we quantified the outcome of infrared laser stimulation both on single cells and on electrically coupled neurons. To explain the effect of the stimulation we employed a combined experimental and modeling approach in the nervous system of Lymnaea stagnalis. We first characterized the activity recorded with intracellular electrodes comparing the amplitude, duration, depolarization and repolarization slopes of individual spikes before, during and after the infrared-laser stimulation. In these experiments, the laser beam was focused on one of the neurons being recorded. Spike duration was reduced under the laser stimulation, along with a change in the repolarization slope. The effect was reversible after termination of the stimulation. The analysis was repeated on well-known electrically-coupled cells of the right parietal and visceral ganglia of Lymnaea. For electrically coupled cells, the activity was simultaneously recorded in both neurons while the laser was illuminating only one of them. The experimental protocols were reproduced in a conductance-based model that considered multiple explanations for the observed effect of the laser stimulation, and we tested which ones best reproduced all observations. Our study unveiled several factors underlying the source of the infrared-laser effect and supports the use of this stimulation in multiple experimental protocols.

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GENE THERAPY WITH VMAT2 REDUCES AGE-DEPENDENT NEUROMELANIN ACCUMULATION AND PREVENTS PARKINSON'S DISEASE PHENOTYPE IN NEUROMELANIN-PRODUCING RATS

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In Parkinson's disease (PD), there is a preferential degeneration of neurons that contain the pigment neuromelanin, especially dopaminergic neurons of the substantia nigra (SN), the loss of which leads to classical motor PD symptoms. We recently developed the first rodent model of human-like neuromelanin production based on the viral vector-mediated nigral expression of melanin-producing enzyme tyrosinase (AAV-hTyr). This has revealed that neuromelanin can trigger PD pathology when accumulated above a specific pathogenic threshold. Because neuromelanin derives from the oxidation of free cytosolic dopamine, we hypothesized that enhancing dopamine vesicular encapsulation with vesicular monoamine transporter 2 (VMAT2) will decrease cytosolic dopamine that can convert to neuromelanin. This approach should slow down the intracellular buildup of neuromelanin that occurs with age and thus prevent, delay or attenuate PD pathology.

Adult male Sprague-Dawley rats received single unilateral stereotaxic co-injections of AAV-hTyr and AAV-VMAT2 immediately above the SN. Control animals received equivalent amounts of either vehicle, AAV-hTyr or AAV-VMAT2, separately. At selected times post-injection animals were assessed for motor asymmetry and their brains processed for histological analyses.

VMAT2 overexpression in hTyr-expressing rats reduced intracellular neuromelanin to levels below its pathogenic threshold by lowering the production of dopamine-oxidized neuromelanin precursors. In these animals, reduction of neuromelanin levels was associated with a marked attenuation of Lewy body-like inclusion formation, nigrostriatal degeneration, extracellular neuromelanin accumulation, microglia/macrophage activation and PD-like motor deficits.

Our results demonstrate the feasibility and therapeutic potential of modulating intracellular neuromelanin levels in vivo.

GENERATION OF AN IN VITRO ASSAY TO EVALUATE ANTIPSYCHOTIC DRUG EFFECTS ON SYNAPTOGENESIS

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Atypical antipsychotics (AAPs) drugs, such as clozapine, remain the current standard treatment for schizophrenia in clinical practice. Although they directly recognize the orthosteric binding site of G-protein coupled receptors, these drugs have a very high affinity for the serotonin 5-HT2A receptors expressed in the central nervous system. Preliminary results from our group suggest an intrinsic activity of clozapine when interacting with 5-HT2A receptors aside from the activation of signaling effectors depending on G-protein coupling. We have also found that chronic clozapine treatment negatively regulates synaptic remodeling and cognition in experimental animals. However, further investigations are needed for understanding the molecular mechanism of action of AAPs.
In order to study the effects of AAPs in synaptogenesis beyond the classical heterologous expression systems, we have generated an in vitro model based on the culture of neural stem (NS) cells obtained from murine fetal forebrain tissue for the subsequent differentiation into cortical neurons. Fetal frontal cortex is obtained at embryonic day 12 (E12) and the resulting cellular suspension is cultured using a specific culture medium. These NS cells grow as adherent monolayer cultures which can be easily differentiated into neurons using selective culture mediums. Neurons derived from fetal NS cells represent an ideal in vitro model mirroring the physiological system where AAPs perform their action through 5-HT2A receptors.
Using this in vitro model, we have established an assay based on the rabies virus technology, which constitutes an unique monosynaptic tracer to unambiguously label directly connected neurons, in order to explore the AAPs effects on neuronal synaptogenesis and neuronal plasticity. Using the selective and retrograde spread of the rabies virus we are able to identify the initial rabies-infected cells and the presynaptically connected neurons, constituting an ideal approach to study synaptogenesis and mechanism of action of APPs.

HIPPOCAMPAL-TARGETED, CELL TYPE-SPECIFIC MANIPULATION OF NFKB ACTIVITY TO TREAT BRAIN INJURIES AND DISEASES

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NFκB is a major transcription factor that regulates a large number of genes during various biological processes, such as early development, cell survival, synaptic plasticity, memory functions, and various diseases, including brain damage, neuroinflammation and neurological diseases. In the central nervous system, many of those processes, such as memory formation, learning, control of anxiety, and cognitive functions, depend on the hippocampus. This brain region is profoundly affected in mesial-temporal lobe epilepsy (MTLE) and traumatic brain injury (TBI) models, where hyperexcitation and neuronal excitotoxicity cause gliosis, cell death, and aberrant neurogenesis. In those models, increased NFκB expression levels have been detected. In other models, as ischemic animal models, downregulation of NFκB activity reduces brain damage, whereas inhibition of NFκB activity promotes the severity of disease expression in models of spinal cord injury.

In order to evaluate the importance of NFκB in diseases that cause hyperexcitation in the hippocampus, we have developed adeno-associated viruses equipped with tetracycline controlled genetic switches selectively targeting astrocytes, microglia and neurons. By cell type specific inducible control of NFkB gene expression, we aim to investigate the role of NFkB in disease onset and progression, and possibly also protection.

IMAGING OF SYNAPSES IN 3D WITH NON-DESTRUCTIVE SYNCHROTRON X-RAY PTYCHOGRAPHY

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Wiring diagrams of neural circuits are of central importance in delineating mechanisms of computation in the brain. Hereby, the individual parts of neurons - axons, dendrites and synapses - need to be densely identified in 3-dimensional volumes of neuronal tissue. This is typically achieved by volume electron microscopy, which requires ultrathin physical sectioning or ablation, using high precision slicing techniques or ion beams, either before or during the image acquisition process.

Here, we employed cryogenic X-ray ptychographic tomography, a coherent diffractive X-ray imaging technique, to acquire 3-dimensional images of metal-stained mouse neuronal tissues with sufficient resolution to densely resolve axon bundles, boutons, dendrites and synapses without physical sectioning.

We show that a tissue volume of 10-20 µm in diameter can be imaged with X-ray ptychographic tomography and subsequently with focussed ion beam-scanning electron microscopy (FIB-SEM). This suggests that metal-stained neuronal tissue can be highly radiation-stable. Using FIB-SEM as ground truth, we show that X-ray ptychographic tomography resolves 60% of the synaptic contacts in the mouse olfactory bulb external plexiform layer with an 80% precision.

We demonstrate that synapses can be detected using X-rays. Ongoing improvements in synchrotron, X-ray and detector technologies as well as further optimization of sample preparation and staining procedures could lead to substantial improvements in acquisition speed. Combined with laminography and nano-holotomography it could allow for non-destructive X-ray imaging of synapses and neural circuits in increasingly larger volumes.

IMMUNOSELECTIVE NANOPHERESIS OF AB IN CEREBROSPINAL FLUID AS A TREATMENT FOR ALZHEIMER'S DISEASE

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Beta amyloid peptide (Aβ) is one of the main promoters of Alzheimer's disease. Originally, this protein is soluble, but it tends to self-aggregate, forming increasingly complex and insoluble structures that give rise to the characteristic senile extracellular plaques. Thus, Aβ is one of the main therapeutic targets, though none of the tried treatments to date has been effective.

Soluble Aβ is in constant equilibrium between the cerebrospinal fluid (CSF) and the interstitial fluid (ISF) in the brain parenchyma. We have previously proposed a new therapeutic strategy based on the removal of Aβ from the CSF, displacing the equilibrium, and decreasing the Aβ concentration in the brain parenchyma. To achieve this, we have designed tailored nanomembranes for Aβ nanopheresis. In this study, we evaluated nanomembranes permeability to Aβ and their impermeability to specific therapeutic agents against Aβ, such as anti- Aβ antibodies, albumin and neprilysin.

To this end, the nanomembranes were attached to a permeation chamber separating two cells: a donor cell, containing either the diffusible Aβ or the therapeutic agent dissolved in artificial CSF (aCSF), and an acceptor cell, containing only aCSF. The system was left for a maximum period of 72 hours and the levels of Aβ, and therapeutic agents were determined by ELISA. Our results showed that Aβ peptide diffuses through the nanomembrane, from the donor to the acceptor cell, whereas none of the therapeutic agents appeared in the acceptor cells. These observations support the use of nanomembranes for Aβ nanopheresis in the CSF, thus avoiding the side effects described for therapeutic agents when delivered systemically.

IMPROVING THE EFFICIENCY OF HUMAN BRAIN ORGANOID GENERATION FROM PLURIPOTENT STEM CELLS

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Brain organoids were first generated by Lancaster et al. following a protocol which set the basis for human brain organoid generation. Since then, other groups have made modifications over this protocol in order to improve the organoids features or to get specific brain regions. Pașca et al. developed a protocol to get a cortex-like phenotype. They used SB-431542, an inhibitor of Transforming Growth Factor-β (TGF-β); and Noggin, an antagonizer of bone morphogenetic protein (BMP) in order to double-inhibit the TGF-β pathway and achieve a rapid neural induction. Later, other key factors have been described: Ascorbic Acid (AA) to prevent oxidation and CHIR99021, a Glycogen Synthase Kinase3β (GSK3β) inhibitor, which activates WNT signaling and promotes neural induction and neuroepithelial proliferation. We wonder whether the combination of these factors could improve and accelerate the neuroepithelium formation in order to get a more efficient and reproducible protocol. The organoids were generated from a human Embryonic Stem Cell (hESC) line, AND-2. We obtained a rapid neural induction, avoiding the Embryoid Body stage, which resulted in a direct and efficient neuroepithelium formation. With this purpose, we performed IHC and Q-RT-PCR assays for the typical neural precursor markers. We also quantified the number of organoids that formed this neuroepithelium and almost the 100 percent of them were positive, which means that our protocol permits to get homogenous organoids of similar sizes. We also performed IHC and Q-RT-PCR for additional neuronal markers at later stages and we obtained the typical Ventricular Zones (VZs) with the characteristic radial structure. All these results show that our protocol improves the efficiency and reproducibility of the traditional protocols used so far and simplifies the tedious and costly methodology that have been developed until now.

LONGITUDINAL CALCIUM IMAGING OF NEURAL ACTIVITY IN EPILEPTIC NETWORKS: IN-VITRO AN IN-VIVO APPROACH

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Epilepsy is a neurological disorder characterized by recurrent epileptic seizures generated by dysfunctions in the neuro-glial circuitries. Seizures trigger a positive-feedback cascade of events that generate and perpetuate in one side gliosis, inflammation, cell death, in conjunction with neural patho-topological connections, aberrant synchronizations and hyperexcitability. Mechanisms are still poorly understood and the efficacy of treatments has not improved for decades.

We are working in complementary in-vitro/in-vivo experimental murine models which allow us to visualize and analyze neuronal activity in a longitudinal manner to monitor and deepen the mechanisms, in the same circuits and over time, behind the switch from physiological to pathological conditions, and additionally to check potential therapeutic approaches on the induced epileptic conditions.

In both systems, longitudinal monitoring of neural circuits' activity with single-cell resolution follows the initial genetic encoding of calcium sensors (specifically Gcamp6f) through viral vector injections.

In the in-vitro model, organotypic hippocampal cultures are cultured over weeks in control conditions and under temporary epileptogenic hyperexcitable conditions. Synchronizations, neural firing (frequency of calcium events) and functional connections can be characterized at different time points. Preliminary quantifications highlight increased neural firings and synchronizations in the neural circuits exposed to hyperexcitable conditions.

In the in-vivo model, we image longitudinally, in awake and behaving mice, hippocampal (or neocortical) circuit's activity through a state-of-the-art endoscope allowing single-cell resolution (Miniscope). Epilepsy is induced by a single injection of kainic acid (KA) into the contralateral hippocampus or the amygdala. Imaging over the same cells and circuit is performed chronically from physiological conditions (pre-KA-injection) to stabilized status epilepticus conditions (about ten days after KA-injection). Preliminary observations highlight the emergence of a variety of new patterns of functional connections and synchronizations in epileptic conditions.

The above methodology possibly opens new perspectives for testing mechanisms and the impact of treatment on the epileptic brain.

MODULATING CORTICOSTRIATAL ACTIVITY WITH TRANSCRANIAL STATIC-MAGNETIC-FIELD STIMULATION

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Modulating corticostriatal activity with non-invasive brain stimulation (NIBS) is appealing for treating neurological diseases. Corticostriatal activity would be studied with fMRI as functional connectivity (FC) estimated as correlation, but NIBS-induced local perturbations are confounders to this metric: FC changes between two regions can be observed without interaction changes, or unnoticed in their presence. We combined transcranial static-magnetic-field stimulation (tSMS) with resting-state fMRI and developed an analysis framework to disambiguate between-region FC and assess corticostriatal modulations.

We disambiguate FC by integrating several descriptive metrics of within-region (Hom: homogeneity; Var: variance) and between-region (DCorr: distant correlation) activity, as well as with an inferential metric that uniquely represents between-region interactions (SVar: shared variance). SVar is extracted from a model-based variance decomposition, Monte-Carlo validated ($y \approx x$; $R^2 > 0.94$). Classical FC (correlation of mean activity) was also assessed. We first studied corticostriatal connectivity on a sample from the Human Connectome Project (N=100) and then corticostriatal effects of tSMS with data from a study stimulating the supplementary motor area (Pineda-Pardo et al, Commun. Biol. 2018) (N=20). Significance was assessed by 2-way repeated-measures ANOVA (time x treatment).

Regions in the putamen (motor and ventral attention regions) are strongly connected to the SMA, and so is the paracentral lobule (PCL) to the motor region of the striatum. In the cortex tSMS increased Var in the right SMA proper ($F_{1,19}=9.6$; PFDR<0.01), and Var ($F_{1,19}=10.2$; PFDR<0.01) and Hom ($F_{1,19}=8.2$; PFDR<0.05) in the right PCL. In the striatum only the left motor region was modulated, increasing Hom ($F_{1,19}=11.6$; PFDR<0.05). Neither classical FC nor DCorr were affected by tSMS, but the modulation of corticostriatal activity was detected as an increased SVar between the left motor striatum and the right PCL ($F_{1,19}=18.0$; PFDR<0.001).

In conclusion, we provide direct evidence of non-invasive modulation of corticostriatal activity by tSMS.

MODULATING NEURONAL ACTIVITY USING PHYTOCHROMES

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Optogenetics is a promising approach for the precise spatiotemporal control of biochemical processes in cells and animals. Recently, modulation of brain activity has been used to treat neurological diseases in human patients. However, opsin-based stimulation induces acute changes in neuronal activity but lacks long-term modulation. Instead, phytochromes, which are soluble photoreceptors present in plants and bacteria, can act as adenylate cyclase and produce cAMP in cells upon photoactivation. Within neurons, cAMP modulate metabotropic responses and induce many intracellular signalling pathways, including synaptic plasticity, leading to long-term cellular changes. Thus, light-induced modulation of cAMP might allow for a more sustained modulation of brain circuitry. Our main goal is to evaluate the potential of phytochromes to induce long-term neuronal activity in specific brain circuits. First, we explored in mouse primary cortical neuronal cultures how changes in cAMP levels modify spontaneous neuronal activity by performing calcium-imaging recordings before and after Forskolin application. Forskolin-induced cAMP caused an overall increase in calcium levels and modulated the network. We also evaluated the changes of cAMP levels induced by Forskolin in STHdhQ7/Q7 neural cells using cAMP fluorescent sensors Flamindo 2 and Pink Flamindo. Then, we infected 7 DIV primary cortical neurons with a custom made AAV-CamKII-DdPAC-Flag-tag and investigated the dynamics of phytochrome activation and deactivation, with 670 nm and 780 nm light, respectively. We used NETCAL software to analyze the Ca2+ changes and decode calcium-dependent neuronal activity. Our results indicate that we can successfully activate phytochromes in neurons and modulate their activity. We are currently implementing fiber photometry tools to study phytochrome effects in vivo. Altogether, these results contribute to the development of new approaches towards modulating brain activity and establishing phytochromes as a novel tool for optogenetic applications.

NON-VIRAL VEHICLES AND MODIFIED OLIGONUCLEOTIDES FOR RNAI-BASED THERAPIES FOR CNS DAMAGE

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Damage to the Central Nervous System (CNS) usually results in dramatic deficits due to the loss or alteration of neural circuits. Gene therapies can modulate key proteins and pathways to reduce damage, increasing the chances of enhancing functional recovery. MicroRNAs (miRNAs) are endogenous short oligonucleotides that regulate the expression of hundreds of proteins to control cell function in physiological and pathological conditions. Despite their potential, applying miRNA-based therapies is limited by difficulties to deliver RNAs to neural cells, due to their poor stability, poor efficiency of delivery, and off-target effects. Within this context, we have compared the potential of different polymer-based vehicles as well as different oligonucleotide modifications for miRNA-based therapies.

Polymeric vectors are an attractive approach due to their improved safety profiles, easy and cheap production, ease of synthesis and chemical versatility, and unlimited possibilities of modification. In this study, we have compared commercial vehicles with various functionalized poly-ethyleneimine (PEI) polymers and N-ethyl-pyrrolidine-methacrylamide copolymers. Our in vitro analyses demonstrate that functionalized PEI vehicles show the best performance when considering together RNA protection against nucleases, delivery to neural cells, endosomal escape, toxicity, and efficacy in Neuro-2a cells and rat E18 primary hippocampal neurons. On the other hand, analyses with modified oligonucleotides reveal that siRNA-like duplexes (i.e. fully complementary sense strand) incorporating 2-O-methyl and 1, 3- propanediol present increased stability against nucleases and better transfection performance than endogenous miRNA duplexes. Moreover, their conjugation with specific cyclic peptides can provide additional advantages, particularly eliminating the need for vehicles by conferring protection and providing cell-targeting specificity.

OBTENTION AND CHARACTERIZATION OF EXOSOMES FOR NON-INVASIVE EPILEPSY MONITORING

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Exosomes are a type of small (30-200 nm) extracellular vesicles, which have a single membrane and a cytosol filled with proteins, DNA, RNA and other molecules. These vesicles play a fundamental role in intercellular communication, since they are synthesized by all types of cells and can transport molecules, even changing the phenotype of a receptor cell to a phenotype similar to the cell that has synthesized the vesicle. Such is their importance that they have already been related to several biological processes such as homeostasis, angiogenesis or the immune response; and to several pathologies such as cancer, neurodegenerative diseases and epilepsy. The aim of this project has been to perfect a method for isolating and characterization of blood exosomes from the GASH/Sal model of epilepsy using different techniques. The GASH/Sal constitutes an experimental model of reflex epilepsy of audiogenic origin derived from an autosomal recessive disorder. Differential centrifugation and size exclusion chromatography were the two isolation techniques chosen for this project and those that allowed the isolation of exosomes from GASH/Sal hamsters. Regarding the characterization techniques, electron microscopy and Nanosight particle tracker were useful to characterize the size and concentration of exosomes. We obtained particles ranging in diameter from 50 to 180 nm and a concentration of 1.8-2 x 108 particles/ml. However, flow cytometry and western blotting did not identified exosomes in the samples, because commercial antibodies against CD63, one of the exosomal typical markers, did not work for Syrian hamster. Future challenges should focus on designing these types of antibodies and, above all, on analyzing the content of the exosomes to find molecules that may be related to epilepsy.

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SELF-ASSEMBLED HYBRID HYDROGELS BASED ON GRAPHENE DERIVATES AND CERIUM OXIDE NANOPARTICLES AS THREE-DIMENSIONAL SUBSTRATES FOR NEURAL STEM CELLS

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A major challenge in utilizing stem cells for regenerative therapies is the poor control over the survival, differentiation and functional integration of the transplanted cells. The combination of stem cells with scaffolds has been proposed to overcome this problem. In the present work, graphene-based hydrogels were developed as a substrate for neural stem cells (NSCs). These hydrogels were further functionalized with cerium oxide nanoparticles, thus providing a multifunctional platform for cells that combines the intrinsic physico-chemical, electrical and mechanical cues provided by graphene derivatives with the antioxidant and cytoprotective properties from cerium oxide.

The graphene/cerium oxide hydrogels were fabricated via the self-assembly of graphene oxide (GO) in the presence of ascorbic acid (AsA) at 1:1, 1:4 or 1:10 proportion. Mechanical properties (rheology), electrical conductivity, antioxidant capacity, morphology (scanning electron microscopy-SEM) and physico-chemical properties (Raman spectroscopy and X-ray diffraction) were characterized. NSCs were seeded on the hydrogels and fixed for immunostaining at different time-points (1, 3, 7 and 14 days). Several markers (Nestin, MAP2, NeuN, DCX, S100B, GFAP, Olig2) were used to distinguish between the undifferentiated and the differentiated cells found in the CNS.

NSCs were able to attach and integrate into hydrogels without need of coating. Furthermore, the presence of AsA or cerium oxide was permissive with the differentiation of NSCs towards neuronal, astroglial and oligodendroglial lineages. The increasing the amount of AsA, which acted as a reducing agent, resulted in a more collapsed structure with smaller pore size and higher shear modulus and electrical conductivity. The incorporation of cerium oxide nanoparticles endow the hydrogels with antioxidant properties reducing both their shear modulus and electrical conductivity.

In this study, we present a simple and versatile method for the fabrication of graphene-based hydrogels with tunable mechanical, electrical, physico-chemical and morphological properties, supporting the adhesion and neurodifferentiation of NSCs, thus constituting a promising tool for future cellular therapies including nerve tissue regeneration.

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TRANSCRANIAL STATIC MAGNETIC STIMULATION OVER VISUAL CORTEX OF HEALTHY SUBJECTS

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Non-invasive brain stimulation (NIBS) techniques have been used for more than a decade to treat various diseases by the modulation of electrical activity in the cerebral cortex. NIBS could employ electrical stimulation or electromagnetic fields, such as static magnetic stimulation (tSMS). Recently, several studies have been reported that the application of tSMS on different cortical areas modulates its excitability and generally causes inhibitory effects on neuronal activity.
Our experimental hypothesis is that tSMS application over visual cortex (VCx) changes neuronal excitability in this area. For the evaluation of tSMS effects, we obtained the threshold for the generation of phosphenes by single pulses of transcranial magnetic stimulation (TMS) which has been largely employed as a measure of VCx excitability.

Twelve healthy volunteers participated in this study (eight females; mean and SD age 36.8 ± 10.6 years, range 22-57 years), recruited by local advertisement. Experiments were carried out in a real-sham, double-blinded randomized crossover study. The intervention was tSMS (or sham) applied over the occipital cortex (centered over the Oz position of the EEG 10-20 system). Intervention was performed by using a magnet (strength 120-200 mT at 2-3 cm from the scalp) or non-magnetic cylinder for sham intervention. The effect of the tSMS intervention (real vs. sham) was tested in two experimental sessions. In each session, subjects were seated in a chair in a dark room and blindfolded, and they were habituated to darkness for at least ten minutes. We assessed VCx excitability by single-pulses of TMS (≤ 0.1 Hz, circular coil) over VCx to calculate the intensity threshold for phosphenes generation. Phosphenes threshold was calculated before and immediately after tSMS intervention (or sham).
Subjects were not able to identify any difference between the magnet and sham sessions. No effects were found in the intensity threshold for phosphenes production after 10min tSMS.

USE OF BIORESORBABLE NANOPATTERNED POLYMER SCAFFOLDS AS A STRATEGY TO GUIDE THE MIGRATION OF NEURAL AND DENTAL STEM AND PROGENITOR CELLS

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Injuries in the central nervous system (CNS) and nerve lesions have a strong impact on high financial expenses and quality of life for the patients. We present a nanostructured polymer scaffold based on bioresorbable elastomeric co-polyesters functionalized with graphene derivatives to promote the attachment, alignment and migration of stem and progenitor cells of neural (murine origin, mNSC, control) or dental pulp stem cells (human origin, hDPSCs). hDPSCs present substantial advantages with respect to other types of stem cells for CNS therapy, as hDPSCs express neural markers and neurotransmitter receptors and have an excellent capability to be differentiated towards neural lineage due to its neural crest origin. hDPSCs are resistant to hypoxic conditions & highly accessible, secrete neurotrophins and anti-inflammatory factors.

Scaffolds of lactide and caprolactone based copolyesters were first nanopatterned with gratings of 300 nm linewidth and subsequently functionalized with polydopamine, which acted as an adlayer for the final immobilization of graphene oxide (GO). mNSCs or hDPSCs were seeded and videorecorded on these scaffolds for 72h. After 3, 7 and 10 days, cells were fixed and immunostained for neuronal and glial markers. Furthermore, the interactions, cell-to-cell contacts and synaptic connections were analyzed by SEM.

Both type of stem cells instead of grow forming neuro / dentospheres, sedimented attached and elongated following the nanograting axis generating chains of cellular migration. Immunohistochemistry analyses showed the persistence of both neuronal and glial markers when seeded on GO-functionalized nanostructured scaffolds compared with the control. Furthermore the scaffolds were compatible with intracranial transplantation allowing to connect brain with olfatory bulb in a partially bulbectomized murine model.

The combination of a nanostructured bioresorbable polymeric scaffold together with the functionalization of the surface with GO enables a simple and scalable method to align and guide the migration of neural and progenitor stem cells for future neuroregenerative therapies.

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VIRTUAL WATER MAZE FOR HUMAN MEMORY ASSESSMENT SYNCHRONIZED WITH TRANSCRANIAL MAGNETIC STIMULATION

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The Morris Water Maze task has been used for many years to assess memory in rodent model studies. Many of these studies consisted of correlating synaptic plasticity mechanisms with behavioral memory changes. To date, Transcranial Magnetic Stimulation (TMS) technology induces plastic changes in the human cortex, therefore, we set the aim to develop a Virtual Water Maze (VWM) task to conduct experimental research on human memory and design translational studies.

This work presents the development of an interactive graphical interface in which the user can navigate through a round pool in search of a hidden platform guided by four symbols presented in the pool's wall. During the different trials, the symbols and the platform remain in the same position, while the user appears in a random place within the pool. The user can move through the space using the arrow buttons on the keyboard and orientating the camera using the mouse. The data recorded from the videogame are: player's position coordinates (50 fps), time to complete each trial and time each signal is viewed.

We performed an experimental pilot study (2 male subjects, real/sham) in which we paired the VWM with simple TMS pulses towards the motor cortex following a Paired Associative Stimulation (PAS) protocol. While the subject is searching the platform oriented by the symbols, we programmed the trigger of a single-pulse TMS each time the user guides the camera towards a signal and it enters the field of vision. Therefore, we synchronized an activation of the motor cortex with the cortical endogenous activation of spatial memory process. We measured the baseline of memory capacity using VWM and the cortical excitability using Motor Evoked Potentials (MEPs), before, immediately after and 30-minutes after PAS.

The results unveiled a significantly potentiated spatial learning and motor corticospinal excitability in the experimental subject.

We have developed a VWM task to measure spatial memory in humans both for evaluation and induction of cortical plasticity.

Topic 9

History, Teaching, Release and Ethics

Posters

SITUATION OF UNIVERSITY BIOTHERIUMS AND RESEARCH CENTERS USING MURINE SYSTEMS IN PERU

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The use of animals for scientific purposes represents a historical practice in human civilization. In Peru, laboratory animal science has reached a higher level of development in recent years. However, the legal and regulatory framework for laboratory animal science is still embryonic. The "Guide for the care and use of laboratory animals" in its eighth edition is not adequately disseminated, as were the previous versions. There are 143 public and private universities in Peru. To date, public and private universities do not have accreditation of institutions that promote the use of animals without unnecessary suffering in scientific activities. On the other hand, a high percentage of universities do not have an "Institutional Ethics Committee for the Use of Animals in Research" (equivalent to the Institutional Committee for the Care and Use of Laboratory Animals - CICUAL), restricting the existence of these committees to the main universities in the country. In Peru, there are also around 173 research centers, most of them in the field of basic and applied sciences. Furthermore, animal production centers for research purposes are scarce in the country, with this responsibility - for the murine model - falling on four public and two private institutions.

THE WOMEN NEUROSCIENTISTS DISCIPLES OF PÍO DEL RÍO-HORTEGA SPREAD THE CAJAL SCHOOL THROUGH EUROPE AND AMERICA

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Pío del Rio-Hortega discovered microglia and oligodendroglia and he was, possibly, the most prolific mentor among all the direct disciples of Santiago Ramón y Cajal (Nobel laureate in Physiology or Medicine 1906, and considered as the father of modern Neuroscience). Among Río-Hortega´s mentees, there are three women, chronologically: 1) Pio's niece Asunción Amo del Río, technician specialized in the study of nervous system and specially neural tumours, who worked with Río-Hortega at Madrid, Valencia, Paris and Oxford from 1922 to 1939; 2) the distinguished Australian-born British pathologist Dorothy Russell, who also worked with Río-Hortega at Oxford (1939-40), where she completed her technical formation in the metallic impregnations, that started under Wilder D. penfield (the most distinguished disciple of Don Pío) to become one of the leaders in the field of Neuropathology and brain tumours after the death of Río- Hortega; and 3) Amalia Pellegrino de Iraldi, the last mentee in Río-Hortega´s career, at Buenos Aires, who develop most of her career as professor at the Universidad de Buenos Aires, after being the most distinguished collaborator of Eduardo De Robertis, and Arvid Carlsson (Nobel laureate in 2000), making fundamental contributions to the synaptic vesicles, cytoskeleton, and different `Hortegian´ subjects; among her disciples accounts Dr. Claudio Cuello (McGill University).

In the present work, we introduce and discuss the research and contributions of these women at Río-Hortega´s laboratory and thereafter for better comprehension of the History and its frame. The present work completes the contribution of women neuroscientists that worked with Cajal and his main disciples of the Spanish Neurological School.

Topic 10

Others

Posters

ACETYLCHOLINESTERASE IN CORTICAL NEURONS DERIVED FROM PATIENT-DERIVED iPS

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Acetylcholinesterase is the enzyme in charge of the hydrolysis of acetylcholine in the cholinergic synapses. It is also one of the multiple proteins affected in Alzheimer's Disease (AD), finding maintained levels of protein whereas the enzymatic activity is reduced in the brain cortex of AD patients. In addition, AChE has non-cholinergic roles like favouring neurite outgrowth or amyloid beta deposition. iPS-derived cortical neurons provide a good model for studying this disease since it can reflect specific changes from patients suffering AD. In this context, the aim of this study is to characterise AChE in patient-derived iPS and neurons when neurons are cultivated alone, co-cultured with astrocytes or/and microglia. Preliminary results indicate that there is an increase of AChE activity when neurons are co-culture with microglia or alone but in BrainPhys, a media that favours neuronal maturation. Further studies are being carried out to test whether this changes are common to control and AD patient-derived cortical neurons or specific to either and if amyloid beta treatment influences AChE similarly to what has been describe in the literature.

AGE-RELATED CHANGES IN THE NEUROMUSCULAR JUNCTION AND SKELETAL MUSCLE OF C57BL/6J MICE

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Aging is accompanied by a reduction of muscle mass and strength, known as sarcopenia, and an important impairment of motor abilities.

To elucidate mechanisms leading to sarcopenia, we undertook a detailed characterization of pathophysiological changes occurring in the mouse neuromuscular system over the course of aging. Motor behavioral and electrophysiological tests, and histological and immunocytochemical analyses were performed in slow- and fast-twitch muscles of young, adult and old C57BL/6J mice. Aging was associated with a reduction in body weight and a gradual decline in locomotor activity of mice. In muscles from old mice, neuromuscular junctions showed higher numbers of both polyinnervated and denervated endplates, signs of endplate fragmentation and ectopically innervated acetylcholine receptor clusters throughout myofibers. In relation to adult animals, skeletal muscles of old mice exhibited increased expression of different molecules related to neuromuscular junction stabilization and plasticity, including: CGRP, GAP-43, FGBP1, TGF-β1 and agrin. Moreover, a higher proportion of myofibers showing increased size, central nuclei (indicating a process of degeneration and regeneration), and lipofuscin aggregates were found in muscles from old mice when compared with those in adult animals. Changes in fiber type composition, a decrease in the number of satellite cells and a reduction of both PGC-1α and ATP5A in old muscles were also found. In relation to young-adult mice, old animals had a significant reduction in the nerve conduction velocity and the amplitude of the compound muscle action potential in distal plantar muscles. Although the distinct type of old muscles examined share some common features indicative of the aging process, the profile of some alterations differed between muscles. This variability was noticed even between muscles located in close vicinity and having similar type composition. These data suggest that the degree of activity and specific function of muscles, rather than topography and fiber typology, have greater impact on muscular changes occurring with aging.

APOLIPOPROTEIN D FUNCTION IN MICROGLIAL RESPONSES TO OXIDATIVE STRESS AND AMYLOID BETA-TRIGGERED DAMAGE

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The brain is surveyed by microglia, resident phagocytes that show complex phenotypes. Microglia degrade injury-related cell debris, and their secreted factors modulate the immune response and tissue repair. Apolipoprotein D (ApoD) is secreted by astrocytes and myelinating glia upon injury, altered proteostasis or oxidative stress (OS). ApoD helps to maintain lysosomal functional integrity, contributes to cell survival, and optimizes macrophage phagocytosis.

Microglial cells do not express ApoD, neither in homeostatic conditions nor upon experimental OS. By adding exogenously ApoD, we found that it rapidly internalizes into BV2 microglial cells and exerts a pro-survival effect upon acute challenges of OS or β-amyloid oligomers. Following internalization, ApoD locates in vesicular cell compartments. We found a partial colocalization of ApoD with the lysosomal/endosomal marker Lamp-2, prompting further investigations on the protein traffic within microglial cells and its function in phagocytosis. We evaluated the phagocytic activity of BV2 cells upon exposure to myelin purified from wild type and ApoD-KO mouse brains. The presence of ApoD in phagocytosed myelin, or pre-exposure of microglia to exogenous ApoD, conditions phagocytosis efficiency and rate of myelin degradation, which was quantified by immunofluorescence, flow cytometry and immunoblot. ApoD influences the process in different ways depending on whether the cell is already "primed" with internalized ApoD, or ApoD enters the cell associated to the phagocytosed myelin. Multiplex analysis of cytokine production by primary microglial cells reveals that ApoD stimulates TNFα response to OS and Aβ oligomers, but not to LPS. Modulation of IL-4 production is stimulus- and sex-dependent. ApoD inhibits IL-4 secretion by male microglia in control and OS situation, but not upon Aβ exposure, while it has no influence on IL-4 secretion by female microglia. Understanding how ApoD acts on microglia, modulating its polarization and phagocytic activity upon disease-related stimuli is key to assess its neuroprotective potential.

DEVELOPMENTAL NEUROTOXICITY EFFECTS OF NANOPLASTICS IN ZEBRAFISH EMBRYO AND HUMAN NEURAL STEM CELL MODELS

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Plastic production has increased exponentially and a significant proportion ends up in aquatic and terrestrial environments. Mechanical, physical, and biological processes degrade this material producing micro (< 5 mm) and nanoplastics (< 1000 nm; NP). Exposure routes include ingestion (food and water), inhalation, and dermal absorption. In addition, microplastics have recently been discovered in human placentas. Some NP health effects have been reported, and developmental neurotoxicity (DNT) is one of the most important ones due to the smallest NP potential capacity to penetrate the blood-brain barrier. Therefore, our main objective was to investigate possible DNT effects of NP at a cellular and organism level.

To accomplish this, we selected an in vivo model, the developing zebrafish embryo, and an in vitro model, a human neural stem cell line, both widely used to study DNT. The two models were exposed to polystyrene NP of 30 nm, fluorescently labeled and pristine, at concentrations that have been reported in nature, ranging from 0,2 - 10 mg/L. Embryos were exposed from 1 - 120 hours post-fertilization, and cells for a total of 16 h - 4 days.

Our results show that 30 nm NP can penetrate both embryo and cell models but are not able to enter the cell nuclei. Accumulation was dependent on concentration. Developing neural cells suffered from morphological changes that were reflected in proliferation and differentiation markers, and increased mortality. NP particles penetrated zebrafish organs, blood vessels, and were observed in larvae brain, eyes, and otoliths. DNT was indicated by smaller head and eye sizes, and alterations in locomotion, anxiety, and acetylcholinesterase assays.

In conclusion, 30 nm polystyrene NP are able to accumulate in cells and organs and induce DNT effects in both in vitro and in vivo models.

DOES RTP801/REDD1 PARTICIPATE IN tRNA METABOLISM?

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RTP801/REDD1 is a stress responsive protein overexpressed in neurons of patients with neurodegenerative disorders such as Parkinson's and Huntington's diseases. Its main function is to inhibit the mTOR pathway and therefore has a pro-apoptotic effect in differentiated cells like neurons. Nevertheless, RTP801 might have other functions not yet elucidated. In preliminary results from our laboratory, RTP801 was found to interact with HSPC117 and DDX1, two proteins that are part of the tRNA splicing ligase complex, which performs the ligation of the tRNA fragments generated during splicing. Since alterations in tRNA metabolism have recently been associated to the development of some neurodegenerative diseases, we aimed to deeper study the relationship between RTP801 and these tRNA-processing enzymes.

Here, we confirm by immunoprecipitation that RTP801 interacts with DDX1, which in turn interacts with HSPC117. Interestingly, we found that HSPC117 subcellular localization differs between wild type (WT) and RTP801 knockout (KO) mouse embryonic fibroblasts, being predominantly nuclear in the latter. Finally, we found that the levels of a neuron-specific tRNA are significantly reduced in the cortex of RTP801 KO mice compared to WT. These results suggest a novel role of RTP801 in tRNA processing, which must be further studied, as RTP801 could be a potential target to prevent altered tRNA metabolism in neurodegenerative diseases.

GENE VARIANTS INVOLVED IN THE GLUTAMATE AND CALCIUM PATHWAY IN THE EPILEPTIC MODEL HAMSTER GASH/SAL

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The GASH/Sal hamster is a genetic model of audiogenic epilepsy, in which the inferior colliculus (IC) is the epileptogenic focus. The GASH/Sal exhibits numerous gene variants that might contribute to the genetic influences for seizure susceptibility. The goal of this work was to determine the molecular significance of some gene mutations related to glutamate and calcium pathways detected when comparing the exome of the GASH/Sal hamster with that of the wild type (control). Sequencing techniques, RT-qPCR, three-dimensional protein structures, immunohistochemistry and Western blot allowed us to detect and validate single nucleotide polymorphisms (SNPs) in the genes coding the kainate receptor (Grik1) and the α2β3 subunit of the calcium channel CaV2.1 (Cacna2d3).

In silico analysis of the three-dimensional protein modeling revealed that the SNPs carry amino acid substitutions (S/P and H/Y) that might affect the intramolecular contact and stability of both proteins. The RT-qPCRs, immunohistochemical and Western-blot analyzes showed an increase in the gene and protein expression of Grik1 in the IC, as well as an increase in immunostaining for Grik1 and Cacna2d3 in the facial nucleus when compared the GASH/Sal with the counterpart controls. On the contrary, a significant decrease in gene and protein expressions was detected for both proteins in the cerebellum, hippocampus, and cortex. In sum, our study supports that the mutations detected in the Grik1 and Cacna2d3 genes are most likely to result in an excitatory unbalance in the IC that facilitates the status epilepticus.

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GENOMIC IMPRINTING OF Dlk1 IS ALTERED DURING ADULT NEURAL STEM CELLS (NSCs) REPROGRAMMING INTO PLURIPOTENT STEM CELLS (iPSCs)

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Genomic imprinting is an epigenetic process leading to parental-origin-specific expression of certain genes – imprinted genes – resulting their monoallelic expression. This specific mechanism is essential for normal mammalian embryonic development, metabolism and adult behaviour (1). Alteration of the expression of imprinted genes has been commonly associated with perturbation of their imprinting state, leading to a loss of imprinting (LOI) (2). LOI implies the disruption of the monoallelic expression pattern of imprinted genes and has been involved in several disorders including malignant transformation (2). To understand the dynamics of genomic imprinting during acquisition of a pluripotent state, we have used neural stem cells (NSCs) from reprogrammable mice carrying a doxycycline-inducible polycistronic cassette encoding the transcriptional factors Oct4, Sox2, Klf4 and c-Myc (3), to generate induced pluripotent stem cells (iPSCs). Our results show alterations in the expression levels of most of the imprinted genes analyzed, which are partially reverted after iPSCs-differentiation into neuroprogenitors (NPs), suggesting that the plasticity in this epigenetic process might be an important mechanism during neural differentiation. These alterations in gene expression were accompanied with changes in the methylation levels at the imprinting control regions (ICRs), which control the imprinted status of imprinted genes. However, only the imprinting state of the paternally expressed gene Dlk1 varies during the acquisition of a pluripotency state and during neural differentiation.

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IN VIVO AND IN VITRO STUDIES REVEAL A SEX-DEPENDENT ROLE FOR THE INSULIN DEGRADING ENZYME (IDE) IN MEMORY TASKS AND IN MICROGLIAL CELLS

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The insulin-degrading enzyme (IDE) is a metalloprotease highly expressed at major sites of insulin degradation, but surprisingly also markedly expressed in the brain. IDE has been described to cleave not only insulin but also amyloid beta (Aß) peptides, which makes this enzyme a good candidate acting as a pathophysiological link between Alzheimer's disease and Type 2 diabetes.

To address the role of IDE in vivo we performed a comprehensive analysis of metabolic, behavioral and molecular parameters on a cohort of 12-month-old wild-type, heterozygous and knockout mice for the Ide gene. The open field test indicated that the partial or total absence of IDE does not produce significant abnormalities in the behavior of mice, while memory tests revealed sex- and genotype-dependent differences. We are currently performing a histological analysis to assess gliosis in the hippocampi of these mice and a multivariable analysis integrating all variables measured to construct a model that accounts for differences between genotypes. We then moved to in vitro studies to decipher the role of IDE specifically in primary microglial cells, master regulators of the neuroinflammatory response associated with brain degeneration and the main Aß-degrading cells. IDE absence significantly decreased microglial proliferation and delayed its response to the mitogen M-CSF. Cytokine production by Luminex assay revealed that IDE-KO microglial cells have impaired polarization under both pro-/anti-inflammatory stimuli, are more sensitive to oxidative stress, and exhibit a sex-specific pro-inflammatory response to Aß oligomers. Regarding Aß managing, amyloid phagocytosis was unchanged, but Aß degradation was diminished in IDE-KO microglia.

Our results indicate that IDE plays significant sex-dependent roles in memory tasks, and in microglial cells. IDE shows prominent functions in inflammatory polarization, microglial proliferation and Aß oligomers degradation, which makes IDE a potential therapeutic target for neurodegenerative processes.

SEX DIFFERENCES OF ACUTE AND CHRONIC ADMINISTRATION OF CANNABIDIOL IN THE GENETICALLY AUDIOGENIC SEIZURE-PRONE HAMSTER GASH/SAL

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Despite evidence supporting the use of cannabidiol (CBD) as an anticonvulsant agent, controversy remains related to dosage, sex-dependent efficacy, and negative health effects. Here, we aimed to investigate the potential anticonvulsant and adverse effects of CBD in the genetic audiogenic seizure hamster from Salamanca (GASH/Sal). Male and female GASH/Sal hamsters received acute and chronic intraperitoneal injections of CBD (200 mg/kg) or the vehicle and seizures were induced by loud sound stimulation. Animals were evaluated for seizure severity and neuroethology with the Ethomatic software, body weight variations as well as hematological and biochemical parameters (14-days post-treatment). Blood levels of CBD were assessed using HPCL in all experimental groups. Animals treated with the vehicle exhibited the maximum values in the categorized seizure index after acute and chronic administrations. Acute effects of a single CBD administration were the complete elimination or significant reduction of seizures in females, whereas males were not affected. Chronic treatment with CBD showed absence of seizures in 40% of the females and a significant decrease in seizure severity in another 40%. On the contrary, 12% of the males presented fully absence of seizures and 50 % reduced the seizure severity, whereas no effects were noticed for the remaining males. All GASH/Sal animals showed a steady weight as well as normal hematological and biochemical parameters after chronic administration, without statistically significant differences as compared to the baseline pre-treatment conditions. Higher blood levels of CBD correlated with reduced seizure scores. In sum, acute and chronic CBD treatments exert sex-dependent anticonvulsant effects on the GASH/Sal model. No adverse effects on body weight, hematological parameters and liver function were observed following repeated daily administration.

SMALL RNA IN PLASMA EXTRACELLULAR VESICLES AS EARLY BIOMARKERS IN HUNTINGTON´S DISEASE

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Despite the toxicity of the mutant HTT protein in Huntington´s disease (HD) has traditionally been considered the main cause of the disease, growing evidence indicates that small CAG repeated RNAs (sCAG) and other perturbed small non-coding RNA (sRNA) are implicated in the pathogenesis. Extracellular sRNAs (exRNA) in body fluids can be found encapsulated in extracellular vesicles (EVs) and could act as toxic carriers. In HD, early diagnosis and prognosis biomarkers are needed for optimal clinical management, facilitating patients' stratification and characterization along the disease course and treatments. Specifically, the study of exRNA in plasma supposes a promising approach for harbouring biomarkers, as reflection of disease status. Here, we describe an optimal method for plasma-EVs purification by Size-exclusion chromatography (SEC) and Ultrafiltration (UF). We also explored EV-sRNA content providing a deep exRNA analysis. Profiling of plasma-EVs from three different cohorts, including manifest HD, premanifest HD and controls, revealed no differences in size and morphology of EVs. Instead, regarding EVs-exRNA, we show heterogeneous proportions of sRNAs biotypes distributions and distinct differential expression patterns profiles between groups. Using SeqCluster tool for sRNA analysis, we highlight that most sRNA-clusters in HD-EVs are downregulated in comparison to Control-EVs, with many changes occurring at premanifest stages. These findings suggest that alterations in circulating EV-sRNAs may reflect early clinical and pathological changes in HD patients.

THE NEUROPROTECTIVE LIPOCALIN APOLIPOPROTEIN D INTERACTS WITH SPECIFIC SUBTYPES OF DETERGENT-RESISTANT MEMBRANE DOMAINS IN A BASIGIN-INDEPENDENT MANNER

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Repair mechanisms of cell membranes are critical for maintaining their roles as selective barriers and for an efficient communication and transduction of biological messages between and within cells. Plasma and lysosomal membranes contain specialized detergent-resistant domains (DRMs) rich in sphingomyelin, cholesterol and gangliosides. Maintenance of these membranes is of special relevance for the nervous system, where most neurons are long-lived cells, with a membrane-centered physiological role, and continuously challenged by oxidative stress and toxic catabolites.

The Lipocalin Apolipoprotein D (ApoD) is expressed by glial cells, secreted to the extracellular milieu, internalized by glia and neuronal cells, and targeted in a finely controlled way to the subset of lysosomes most sensitive to oxidative stress.

In this work we use membrane and isolated DRM preparations from whole brain, primary astrocytes, and glial and neuronal cell lines. Binding of purified ApoD to membranes of non-expressing neurons in vitro and ApoD detection in DRMs allow us to assay biochemical parameters on which ApoD-membrane interactions depends. We use fluorescence immunocytochemistry and confocal microscopy to test protein subcellular localization and the MTT assay to quantify cell viability.

We demonstrate that ApoD is stably associated to a particular subset of DRMs with specific buoyancy properties, co-fractionating with both plasma and lysosomal membrane markers. The association of ApoD with isolated neuronal and glial membranes is stable under metabolic and acute oxidative stress conditions. We have tested if Basigin (Bsg), a transmembrane glycoprotein reported to be an ApoD receptor, is required for ApoD-membrane association and endocytosis-dependent uptake. Using a Bsg-KO astrocytic cell line, we conclude that neither ApoD interaction with DRMs, nor its internalization, are dependent on Bsg in astroglial cells. Our current analysis centers on the dependency of membrane lipid composition, since the molecular nature of ApoD-membrane interaction is an important issue to fully understand its neuroprotective mechanism.

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