ABSTRACT BOOK

Basque Neuroscience Meeting

Neurogune

Bilbao 2016

June 27th 2016
Bizkaia Aretoa (Abandoibarra 3, Bilbao)

Neurogune 2016
Basque Neuroscience Meeting
2016 June 27th
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Abandoibarra 3, Bilbao
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Organised by:
Achucarro Basque Center for Neuroscience
University of the Basque Country (UPV/EHU)
BCBL – Basque Center on Cognition, Brain and Language
BioDonostia Health Research Institute

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PROGRAMME

Time	Activity	Duration (min)
9.00 - 9.30	*Registration (and poster arrangement)*	*30*
9.15 - 9.30	*Opening*	*15*
9:30 - 10:20	*Role of redox regulation and neuroinflammation in prefrontal excitatory-inhibitory balance and myelin maturation in schizophrenia: a human and mice study* Opening Keynote by **Kim Q. Do** (Laussane, Switzerland)	*50*
10:20 - 11:00	*Coffee Break (and Posters)*	*40*
11:00 - 13:00	Oral Communications (4 talks x 30 minutes each) • Track 1: Cellular and Molecular Neuroscience / Physiology • Track 2: Cellular and Molecular Neuroscience / Pathology • Track 3: Behaviour & Cognition, Imaging and Psychiatry	*120*
13:00 - 15:00	*Lunch (and Posters)*	*120*
15.00 - 15:50	*Targeting alpha-synuclein for treatment of synucleinopathies* Closing Keynote by **Wassilios Meissner** (Bordeaux, France)	*50*
15:50 - 16:00	*Closure*	*10*

ORGANIZATION

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Additionally, Neurogune 2016 has received financial support from the "*Congress Organisation Calls*" of the Basque Government and the University of the Basque Country.

KEYNOTES

Opening Keynote
9:30 – 10:20

Kim Q. Do

Director of the Center for Psychiatric Neuroscience and head of the Unit for Research in Schizophrenia of the University of Laussane (Switzerland)

The opening keynote of this edition of Neurogune will be on the *"Role of redox regulation and neuroinflammation in prefrontal excitatory-inhibitory balance and myelin maturation in schizophrenia: a human and mice study"*

Closing Keynote
15:00 – 15:50

Wassilios Meissner

Professor of Neurology at the University Bordeaux and the University Hospital Bordeaux, and an expert for diagnosis and treatment of multiple-system atrophy and Parkinson's disease

Prof. Meissner will be closing the congress with his talk about *"Targeting alpha-synuclein for treatment of synucleinopathies"*.

ORAL COMMUNICATIONS

Cellular and Molecular Neuroscience / Physiology

ID: O01

Title

Regulation of neurogenesis by phagocytic microglia-derived factors

Authors

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During adult hippocampal neurogenesis, the majority of the newborn cells undergo apoptosis. To prevent disturbing the surrounding neurons, these apoptotic cells are quickly and efficiently removed by phagocytosis by resident microglia. Here we propose that phagocytosis is not merely a process to remove corpses but it has an active role in maintaining the homeostasis of the adult hippocampal neurogenic cascade by producing neurogenic regulators. To test this hypothesis, we first set up an in vitro model of phagocytosis in which primary cultures of postnatal microglia were fed with apoptotic SH-SY5Y (a human neuronal line) cells over a time course. Using this model, we performed a full genome-wide transcriptomic analysis of the phagocytic microglia using gene expression arrays comparing naïve vs phagocytic microglia. Gene ontology analysis revealed that, in addition to major changes in different cellular pathways, phagocytosis triggered a pro-neurogenic program in microglia. We found a significant upregulation of the neurogenesis function and we identified significant changes in 209 genes of genes involved in functions related to different stages of the neurogenic cascade. Afterwards, the expression of relevant genes with significant changes was validated by RTqPCR. To confirm the upregulation of the pro-neurogenic genes in phagocytic microglia *in vivo,* we resorted to utilize single-cell RNA sequencing comparing microglia isolated from dentate gyrus (DG; enriched in phagocytic cells) with that of CA (where there is no neurogenesis and therefore no apoptosis nor phagocytosis). Using bioinformatic tools we expect to discriminate changes in the profile associated to phagocytosis. In conclusion, these results give us a novel insight into the changes initiated by microglial phagocytosis in the neurogenic niche and strongly suggest the existence of a pro-neurogenic/repair program triggered by phagocytosis in microglia.

ID: O02

Title

Role of neuronal nitric oxide synthase and reactive oxygen species in the development of cellular tolerance to different opioid agonists in the rat locus coeruleus

Authors

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Nitric oxide (NO) is involved in the neuroadaptations observed following chronic opioid use, such as tolerance and -opioid receptor (MOR) desensitization in the locus coeruleus (LC). However, the role of NO and NO-derived reactive oxygen species (ROS) in the development of cellular tolerance to different opioids in the LC, the main noradrenergic nucleus in the brain, remains to be studied. Herein, we examined the effect of the selective neuronal nitric oxide synthase inhibitor 7-nitroindazole (7-NI) and the antioxidants Trolox + ascorbic acid (TX +AA) and U-74389G on the development of cellular tolerance induced by morphine, methadone and fentanyl in rat brain slices containing the LC. For induction of morphine tolerance, animals were treated with a slow release emulsion containing free base morphine (200 mg/kg, 3 days, s.c). Methadone (60 mg/kg/day, 6 days) and fentanyl (0.2 mg/kg/day, 7 days) tolerance was induced by subcutaneous implantation of osmotic pumps. Sham animals were implanted with the vehicle of the opioid. To study the cellular tolerance, we performed concentration-effect curves for the inhibitory effect of Met5-enkephalin (ME; 0.05-12.8 M, 2x, 1 min) on the neuronal activity of LC cells. Morphine, methadone and fentanyl treatments shifted to the right concentration-effect curves for ME and increased the EC50 (concentration needed to obtain the 50% of the maximal response) by 4, 2 and 3 folds, respectively. Co-administration of TX+AA (40 and 100 mg/kg/day, respectively, i.p.) or U-74389G (10 mg/kg/day, i.p.) in morphine-treated animals prevented the development of cellular tolerance. Conversely, co-treatments with U-74389G or 7-NI (30 mg/kg/12 h, i.p.), failed to affect the induction of cellular tolerance after methadone or fentanyl treatments. Our results suggest that MOR agonists with different intrinsic efficacies cause variable degrees of cellular tolerance in LC cells. Moreover, NO/ROS pathways are differentially involved in opioid tolerance after prolonged treatments with morphine, methadone and fentanyl.

ID: O03

Title

Identification of a new partner and regulator of the dopamine transporter: snapin

Authors

Amaia M. Erdozain[1,2], Stéphanie De Gois[1], Véronique Bernard[1], Victor Gorgievski[1,3], Nicolas Pietrancosta[4], Carlos E. Macedo[1], Peter Vanhoutte[1], Jorge E. Ortega[2,5], J. Javier Meana[2,5], Eleni T. Tzavara[1], Vincent Vialou[1], Bruno Giros[1,3]

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Dopamine (DA) is a major regulator of sensorimotor and cognitive functions. The importance of DA neurotransmission is emphasized by its direct implication in devastating neurological and psychiatric disorders. The DA transporter (DAT) is the key protein that regulates the spatial and temporal activity of DA release into the synaptic cleft via the rapid reuptake of DA into presynaptic terminals. There is strong evidence suggesting that DAT-interacting proteins may play a role in its function and regulation.
Methods: We used yeast-2-hybrid screening, first, and then different molecular, cellular and in vivo approaches to identify new partners of DAT and understand the role of these interactions.
Results: We have identified snapin, a SNARE-associated protein implicated in synaptic transmission, as a new binding partner of the carboxylic terminal of DAT, and determined the domains required for this interaction in both proteins. We also characterized the DAT-snapin interface by the generation of a 3D modelleing. In situ hybridization assays showed that snapin is expressed in vivo in dopaminergic neurons along with DAT. By different approaches we demonstrated that both proteins colocalise in cultured cells and brain, and are present in the same protein complex. With functional studies we have shown that snapin produces an important decrease in DAT activity. Finally, using a shRNA lentivirus directed against snapin, we have demonstrated that the downregulation of snapin produces an increase in DAT levels and activity in vivo, which is accompanied by a higher locomotor behavioural response to amphetamine and higher DA levels.
Conclusions: Our data show that snapin is a new direct partner and regulator of DAT, and we provide evidence for the relevance of this regulation in vivo.

ID: O04

Title

The effects of - 9-tetrahydrocannabinol in dorsal striatum structural plasticity

Authors

Mónica R. Fernández- Cabrera[1], Alejandro Higuera-Matas[1], Isabel Fernaud-Espinosa[2], Abraham Selvas[1], Javier DeFelipe[2], Emilio Ambrosio[1], Miguel Miguéns[2,3]

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Derivatives from the *Cannabis* plant are the most commonly abused illegal substances in the world. Its main psychoactive component -9-tetrahydrocannabinol (THC) exerts its effects through the specific activation of CB1 cannabinoid receptors. These receptors show a distinct lateral-medial gradient expression in the dorsal striatum, which is involved in the anatomical circuits that support goal-oriented behavior and habit formation. It is known that dendritic spines exhibit important synaptic functional attributes and a potential for plasticity which is thought to mediate long lasting changes in behavior. In the present study, adult, male C57BL6/J mice were intraperitoneally injected with THC or vehicle for 15 days. Using single cell intracellular injections and confocal microscopy 3D reconstruction of labeled neurons, we studied the effects of chronic treatment with THC in dendritic and spine morphology in medium spiny neurons (MSNs) of the anterior dorsolateral (aDLS) and posterior dorsomedial (pDMS) areas of the striatum. Data analysis showed that THC treatment is related to a slight increase in dendritic spine density in the distal part of the dendrites of the posterior dorsomedial striatum, but no changes were found in the rest of the parameters analyzed in either region studied. We also observed a difference in spine density, area and volume between the aDLS and the pDMS in the THC group which was not present in the control group. Considering that the distal part of MSNs dendrites show regenerative activity that produces somatic potential changes resembling sustained depolarization, our results seem to indicate that the effects of THC in the transition from goal directed behavior to habitual learning might be due to alterations of structural plasticity in the circuits involving the posterior dorsomedial striatum MSNs.
Key words: -9-tetrahydrocanabinol, CB1 receptors, anterior dorsolateral striatum, posterior dorsomedial striatum, spine morphology.

Cellular and Molecular Neuroscience / Pathology

ID: O05

Title

Myelinophagy: A Novel Mechanism for Schwann Cell Mediated Myelin Breakdown

Authors

Marta Palomo, Encarni Perez-Andres, Daniela Medrano, Marta Iruarrizaga-Lejarreta, Marta Varela-Rey, Ashwin Woodhoo.

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In peripheral nerves, myelin breakdown, or demyelination, is a universal outcome of a remarkably wide range of conditions that involve disturbance to Schwann cells or the nerve environment, whether due to genetic or acquired disease, toxicity, or nerve transection/crush. It has also become clear from studies on cut nerves that, perhaps surprisingly, Schwann cells themselves have the ability to turn against their own myelin and initiate myelin breakdown, destroying about 40–50% of the myelin during the first 5–7 d after injury. In spite of the central position of myelin breakdown in Schwann cell biology and pathology, the cellular and molecular mechanisms that make Schwann cell–mediated myelin digestion possible have not been established. In this talk, I will present evidence that autophagy, a mechanism by which many cells digest their intrinsic cellular components, plays a central role in Schwann cell myelin breakdown. We show that nerve injury triggers strong activation of Schwann cell autophagy, find myelin debris in autophagosomes, and demonstrate a strong requirement for autophagy in myelin digestion, revealing a novel form of selective autophagy of the myelin sheath, myelinophagy.

ID: O06

Title

Axon-to-soma degeneration by local translation of transcription factors

Authors

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Alzheimer´s disease (AD) spreads through the brain in a non-random manner indicating propagation along connecting fiber tracts. The molecular mechanisms driving the spread of the pathology remain largely unknown. We have recently discovered an unexpected mode of long-range transmission of the pathological effects of A oligomers from axons to cell bodies that relies on intra-axonal translation of ATF4. These findings support a model in which retrograde transport of locally translated proteins leads to pathological, transcriptional changes in the neuronal cell bodies.

Local translation enables axons to react to extracellular stimuli in an acute manner. Intra-axonal protein synthesis is best understood in the context of neurodevelopment where it plays crucial roles in growth cone behavior, axon pathfinding and retrograde signaling. On the other hand adult axons have long been thought to be translationally inactive. However, high-throughput analyses have revealed that mature axons have a more complex and dynamic transcriptome than expected, especially under pathological conditions, and local translation is required for axonal regeneration upon nerve injury, it improves motor function in a mouse model of spinal muscular atrophy (SMA), and we have recently found that it mediates A-induced neurodegeneration in vitro and in vivo.

ATF4 mediates the cellular responses upon activation of the integrated stress response (ISR) by inducing the transcription of genes involved in cell death or survival, but it can also repress long-term potentiation under normal conditions acting as a CREB-1 antagonist. Our results establish that Aβ application selectively to axons triggers retrograde somatic degeneration through ATF4 axonal translation. Thus axonally-synthesized ATF4 could be targeted in order to prevent or slow down the spread of AD pathology throughout the brain. However, ATF4 is also translated in the neuronal soma of granule cells in the dentate gyrus (DG) following Aβ infusion in the mouse hippocampus in vivo. Atf4 knockdown increases Aβ-mediated neurodegeneration in the DG, suggesting that in this particular case ATF4 would rather be involved in a protective, adaptive response to Aβ exposure. This raises the intriguing possibility that ATF4 elicits distinct responses based on its translation at the subcellular level. Such differential responses could be explained by the availability of potential ATF4 binding partners in axons and cell bodies upon Aβ exposure. Here we characterize the role of ATF4-related transcription regulators in axons, whose interaction with ATF4 could be targeted in order to prevent axon-to-soma degeneration triggered by amyloid peptides.

ID: O07

Title

Analysis of the chr5q11 autoimmune risk locus points to a role for CD4+ T lymphocyte-expressed ANKRD55 in multiple sclerosis and neuroinflammation

Authors

Nerea Ugidos[*,], Ana Feliú, Jorge Mena[*,], Aitzkoa Lopez de Lapuente[*,], Miriam Mecha, Ianire Astobiza[*,], José Riera[*,], Francisco Carillo-Salinas, Manuel Comabella[¶], Xavier Montalban[¶], Iraide Alloza[*,§], Carmen Guaza, and Koen Vandenbroeck[*,§]

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An intronic variant in *ANKRD55*, rs6859219, is a genetic risk factor for multiple sclerosis (MS) but the biological reasons underlying this association are unknown. This chr5q11 region contains various plausible risk genes for MS including *IL6ST, IL31RA, DDX4, SLC38A9* and *ANKRD55*. We characterized the expression of these genes in human PBMCs and cell lines, and related their expression levels with genotype. For ANKRD55, three transcript variants (Ensembl isoforms 001, 005 and 007) could be detected in PBMCs and $CD4^+$ T cells, but were practically absent in $CD8^+$, $CD14^+$, $CD19^+$ and $CD56^+$ cells. Rs6859219 was significantly associated with ANKRD55 transcript levels in both PBMCs and $CD4^+$ T cells (*cis*-eQTL). The processed noncoding transcript 007 was the most highly expressed variant in $CD4^+$ T cells, followed by 001 and 005, respectively, but was not detected in Jurkat, U937 and SH-SY5Y cell lines. Homozygotes for the risk allele produced over 4 times more transcript copies than those for the protective allele. ANKRD55 protein isoforms 005 and 001 were predominantly located in the nucleus of $CD4^+$ T cells, Jurkat and U937 cells. ANKRD55 was induced in $CD14^+$-monocyte-derived dendritic cells and regulated by tolerogenic/inflammatory stimuli. ANKRD55 was produced by primary cultures of murine hippocampal neurons and microglia and by the murine microglial cell line BV2, and was induced by inflammatory stimuli. ANKRD55 protein was increased in the murine mouse model of experimental autoimmune encephalomyelitis (EAE). Flow cytometric analysis of CNS-infiltrating mononuclear cells showed that $CD4^+$ T cells and monocytes expressed ANKRD55 in EAE mice with the higher fluorescence intensity found in $CD4^+$ cells. A low percentage of microglia also expressed ANKRD55. Together, these data support an important role for ANKRD55 in MS and neuroinflammation.

ID: O08

Title

EGFR inhibition reduces aberrant cell proliferation in hyperexcitatory kainate model of epilepsy

Authors

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Epidermal growth factor receptor (EGFR) expression is upregulated in activated neural stem and progenitors cells (NSPCs) in the neurogenic niche of the subventricular zone, and its stimulation induces cell proliferation[1,2,3]. In addition EGFR inhibition impairs astrocytic differentiation.[4]. In the present work we demonstrate the presence of EGFR receptor in both *in vitro* and *in vivo* in hippocampal neural stem cells and progenitors. When EGFR signalling is blocked for 48h it reduces around 70% of cell proliferation *in vitro* even in presence of fibroblast growth factor 2 (FGF2).

Kainic acid (KA) infusion into the hippocampus trigger seizures mimicking mesial temporal lobe epilepsy (MTLE) and has been recently shown to induce hyperactivation and exhaustion of hippocampal (NSCs) by differentiation into reactive astrocytes.[5]. In the present work we block EGFR signalling in the KA-MTLE model using Gefitinib, a reversible inhibitor of the EGFR in clinical phase II for cancer treatment. When administered at 10mg/Kg twice a day for consecutive 3 days after induction of MTLE, Gefitinib reduces NSC hyperactivation, as measured by BrdU incorporation and KI67 expression, and ameliorates NSC-derived astrogliosis. At 14 days after the induction of MTLE the inhibition of the EGFR by Gefitinib rescues neurogenesis, increasing the numbers of doublecortin and NeuN-positive cells. However, these newborn immature neurons, mostly mislocalized to the hilar region.

Our results demonstrate that EGFR pathway is involved in the massive activation of hippocampal NSCs induced by seizures, and that the inhibition of the EGFR pathway is a good candidate to preserve neurogenesis and suggest that the preservation of the niche is crucial for the final settlement of newborn neurons.

References:

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5. - Sierra A. Cell Stem Cell. (2015) May 7; 16(5):488-503.

Behaviour & Cognition, Imaging and Psychiatry

ID: O09

Title

Brain Machine Interfaces coupled with rehabilitation orthoses: a promising therapy for motor restoration in stroke patients

Authors

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Paralysis caused by stroke is one of the leading causes of long-term disability. This loss of muscle control is given by an injury in brain areas or tracts associated with motor function, limiting the motor command signal generation and their transmission towards the muscles. In the past years Brain-Machine Interfaces (BMIs) have been established to control rehabilitation devices using brain activity from the sensorimotor cortex associated with intended or imagined execution of upper-limb movements [1].These systems provide proprioceptive feedback to patients by moving their paralyzed limb based on the inferred motor intention from their brain signals. In a recent study, we showed that BMIs can be used to control of rehabilitation orthoses and restore motor function in severely paralyzed stroke patients [2]. In this study, 32 chronic stroke patients with severe hand weakness were divided into two groups: 1) the experimental group underwent a BMI training in which their hand and arm were mobilized by rehabilitation orthoses developed by TECNALIA contingently to the desynchronization of ipsilesional oscillatory sensorimotor rhythms, 2) in the control group movements of the orthoses occurred randomly. Both groups also received behavioral physiotherapy. The experimental group showed a bigger and significant improvement in the score of the Fugl-Meyer (FMA) clinical assessment of the upper-limb motor function than the control group after the training period. This outcome measure indicates the importance of a contingent, real-time coupling of the motor intention of the lesioned area with the actual movement of the paralyzed limb facilitated by orthoses. Through the use of proprioceptive-BMIs, the damaged efferent connections between the lesioned brain area and the muscles can be reinforced, activating neuroplastic mechanisms to induce motor rehabilitation. These results suggest that BMIs coupled with orthotic devices constitute a promising therapy for motor rehabilitation in severely paralyzed patients who cannot benefit from conventional treatments.
At the present time, the Neuroprosthetics group of the Institute for Medical Psychology and Behavioural Neurobiology in the University of Tübingen works in close cooperation with the Neuroengineering group of TECNALIA for combining BMIs with robotic orthoses and functional electrical stimulation systems for paralyzed muscles in stroke rehabilitation. This collaboration focuses on the development of BMI systems able to accurately decode movement intention inferred from the patient's electrophysiological signals such as electroencephalography, electrocorticography or electromyography, and their use for the control of sophisticated body actuators able to functionally move or stimulate a paralyzed upper-limb. Improvements in the contingency and functionality of trained movements in proprioceptive-BMIs are believed to speed up functional neuroplastic processes and enhance motor learning. Moreover, the study of the neuroscience behind motor recovery is essential to discover the key factors that lead to the restoration of motor function after a brain lesion. For this purpose, this research group uses diverse neuroimaging techniques such as magnetic resonance imaging, transcranial magnetic stimulation or electroencephalography together with BMIs to explore the sensorimotor

integration and its link to behavior in patients with disorders of the central nervous system caused by diseases such as stroke or spinal cord injury.

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ID: O10

Title

The proactive bilingual brain: Using interlocutor identity to generate predictions for language processing

Authors

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Bilingual language activation is modulated by explicit linguistic cues (e.g., sentence context or lexical items), and bilinguals can also be trained to rely on non-linguistic cues, such as flags or colors, for language activation. Moreover, it has been recently demonstrated that a non-linguistic cue such as interlocutor identity (e.g., Spanish, Basque or bilingual interlocutor) also bias language activation: During language comprehension, bilinguals are faster in a lexical decision task when the language used by the interlocutor during the task is congruent with his identity (e.g., Spanish word pronounced by a Spanish versus a Basque interlocutor).

The current study was designed to explore whether bilinguals pre-activate the context-appropriate language even before the interlocutor produces language. We did so by using the technique of event-related potentials (ERPs) and by exploring bilinguals' brain activation between the onset of the visual presentation of the interlocutor and the onset of speech he produced. (1) Interlocutor identity might interact with language activation during speech processing itself. In that case, we would not expect any effect of interlocutor identity before speech onset. (2) Alternatively, bilinguals might pre-activate the expected language when presented with an interlocutor they know, before this interlocutor even starts to produce speech. In this case, brain responses between visual and audio onset should significantly differ between monolingual and bilingual interlocutors (no possible language pre-activation when facing a bilingual interlocutor who is using the two languages alternatively and randomly).

Twenty-three early proficient Spanish-Basque bilinguals took part in the ERP experiment. They were first familiarized, through video segments, with two Spanish, two Basque and two bilingual interlocutors. Then, they had to perform an audio-visual lexical decision task on items uttered by the six interlocutors. Participants had to decide if the words they heard were real or not in any of the languages (150 Basque and 150 Spanish words; 60 Basque-like and 60 Spanish-like pseudowords). There was an average of 350 ms gap between the onset of the video and the onset of the auditory signal.

The main outcome of the study was that the influence of interlocutor identity (bilingual versus monolingual interlocutor) on the bilingual's brain started around 100 ms after the onset of the video, even before the onset of speech. Thus, we showed for the first time that bilinguals can pre-activate a language in their mind, for further speech comprehension. This pre-activation can be performed in 'naturalistic' conditions, the cue for language pre-activation being the identity of a known interlocutor. This preparation for language mode seems to affect participants' behavior, since participants were faster to perform the lexical decision task on any type of lexical item when the interlocutor was monolingual versus bilingual. Our results have potential important implications for models of bilingual language control, which should take into account the possibility of selective language pre-activation even before any linguistic input, based on 'naturalistic' cues such as an interlocutor identity.

ID: O11

Title

Complex networks reveal structural-functional brain resting-state subnetworks: identification, description and application as biomarkers

Authors

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Elucidating the intricate relationship between brain structure and function, both in healthy and pathological conditions, is a key challenge for modern neuroscience. Recent progress in neuroimaging has helped advance our understanding of this important issue, with diffusion images providing information about structural connectivity (SC) and functional magnetic resonance imaging shedding light on resting state functional connectivity (rsFC). In this work, we adopted a complex networks approach, relying on modular hierarchical clustering, to study together SC and rsFC datasets gathered independently from healthy human subjects. By employing the template of hierarchical modular organization derived from structural data to represent the resting state functional one and vice versa, we searched for the optimal common partition shared by structure and function by maximizing a novel quantity, that we dub "cross-modularity". This procedure allows the extraction of an optimal partition that we uncovered divides the brain into distinct subnetworks that we refer to as common "structure-function modules" (SFMs), representing a coarse-grained skeleton of the brain, which is largely shared by structure and function. First, we describe the emerging common structure - function modules (SFMs) and compare them with commonly employed anatomical or functional parcellations. Secondly, we use SFMs to characterize aging impact on brain networks. Specifically, by looking at the variation of the inter- and intra- module connectivity as a function of age, we show how a multiple linear regression model can describe global brain networks aging.
In conclusion, our results show how the resting-state brain activity is shaped by the existence of structural-functional subnetworks whose interplay and connectivity varies as a function of age.

ID: O12

Title

Evaluation of spinophilin expression in postmortem prefrontal cortex of subjects with schizophrenia: effect of antipsychotic treatment

Authors

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Schizophrenia is a chronic incapacitating disease that affects 1% of the population. Today, schizophrenia is conceptualized as a neurodevelopmental psychiatric disorder with altered connectivity between different brain regions. Several studies have demonstrated reduced dendritic spine density and other dendritic abnormalities in schizophrenia. These spine deficits may be related to disturbances in the molecular mechanisms that underlie spine formation, pruning and maintenance. Spinophilin is a scaffold protein involved in multiple signaling pathways. Spinophilin modulates excitatory synaptic transmission and dendritic spine morphology. Besides, spinophilin has been shown to regulate G-protein coupled receptor signaling, including dopamine D2 receptors and alpha adrenoceptors, which play a role in the pathophysiology of the schizophrenia and are targets of antipsychotics. Previous studies have reported changes in spinophilin mRNA levels in different brain regions from schizophrenic subjects. For these reasons it is of great interest to study the spinophilin protein expression in the brain of subjects with schizophrenia.

Methods: Spinophilin protein density was determined by Western Blot in postmortem dorsolateral prefrontal cortex of subjects with schizophrenia. Forty eight subjects where included in the study: 24 with an antemortem diagnosis of schizophrenia, and 24 controls with no antemortem history of psychiatric disease, matched by age, gender, and postmortem delay. Twelve of the schizophrenia subjects were taking antipsychotic treatment at the moment of death (based on positive blood toxicological analysis), while the other 12 where antipsychotic-free at death (negative toxicology). Spinophilin was measured in a preparation of synaptosomes obtained by ultracentrifugation methods, and normalized for actin immunoreactivity as loading control.

Results: Immunoreactivity for spinophilin detected two bands at ~120 and ~95 kDa, which disappeared with the blocking peptide, demonstrating the specificity of both bands. Quantification for the ~120 kDa band, which is the entire protein, showed no significant differences between schizophrenia and control subjects. When subjects were divided in order to study the effect of antipsychotic treatment, there was a non-significant trend to a reduction (-8%) in spinophilin levels in antipsychotic-free subjects, which was not observable in treated subjects. However, spinophilin ~95 kDa band, supposed to be a cleaved form of the protein, was significantly reduced (-15%) in the dorsolateral prefrontal cortex of schizophrenia subjects ($p=0.0067$, $n=24$) when compared with controls. When subjects were divided in regard to the treatment ($n=12$), this form showed non-significant differences in antipsychotic-free subjects, while there was a significant reduction (-24%) in antipsychotic-treated subjects ($p=0.0028$).

Conclusions: No significant changes were observed for spinophilin ~120 kDa form in synaptosomes from postmortem prefrontal cortex of subjects with schizophrenia. Conversely, there was a significant reduction in the ~95 kDa form, which seems to be due to the antipsychotic treatment. Further research needs to be done to elucidate the nature and role of this cleaved ~95 kDa form.

POSTERS

Behavioural and Cognitive Neuroscience

ID: P01

Title

Clinical markers from language complexity patterns

Authors

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One of the first sources of information in psychological and psychiatric practice is language. Language can inform us about possible cognitive impairments, but also about atypical cognitive development. An example of the former is the logopenic progressive aphasia, attributed to either Alzheimer's disease or to frontotemporal lobar degeneration. An example of atypical cognitive development is the linguistic profile exhibited by people with an atypical genotype like Down syndrome or Williams syndrome. The way to approach the linguistic ability of patients with a potential linguistic impairment or atypical language development is usually based on tests (e.g., Boston test) and analyses of utterances by counting words (e.g., (in)capacity to perform a passive sentence or (in)capacity to say words that are semantically related like names of animals). However, as is well-known, sometimes the scores obtained are not fully informative, since some individuals reach scores at the edge of being classified as healthy, although the suspicion about a potential disturbing factor is evident from the speaker's spontaneous speech. There is the possibility to resort to other invasive techniques (e.g., lumbar puncture to detect abnormalities in the cerebrospinal fluid) and-or neuroimaging technologies (e.g. fMRI, DTI, EEG). We contend that there is a methodological gap between the basic tests and invasive/neuroimaging that can be filled by a data analysis technique that approaches speech data macroscopically (instead on focusing on single words/sentences).

We present a new technique for the extraction of more refined data from the language source using a combination of linguistic, morpho-syntatic analysis and network science. This methodology is able to extract complementary information about the speaker's linguistic system, that cannot be observed by the unaided eye. Complexity patterns of word combination emerge from a sample of spontaneous speech. These patterns take the form of a complex network made of words/morphemes (its nodes) and syntactic links (its edges), so that we can extract formal, objective indicators that speak about the cohesion of the speaker's linguistic system, which are the most connected words (hubs), which kind of links are crucial for the structure of her linguistic capacity. Therefore we consider that linguistic networks are good biomarkers – or endophenotypes – for language impairment or atypical language development.

We have applied this technique to 7 one-year-long corpora of typical children covering 6 different languages, 32 samples of Down syndrome, 20 cases of Specific language impairment and 20 cases of Hearing impariment. Moreover, we have the first data of a pilot study of Williams syndrome language network analysis. In sum, we observe that typical children develop their linguistic networks following a common developmental path, whereas the rest of atypical cases differ from that developmental path. The structure of atypical linguistic networks is different in many ways and the formal indicators support the idea that these speakers have a qualitatively different ability to combine words and information management.

The continued analysis by means of this technique provides a useful technique for tracking the (a)typical development of language or even its desintegration (in the cases of neurodegenerative diseases).

ID: P02

Title

The effects of reading acquisition on verbal and nonverbal skills

Authors

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Studies on literate and illiterate adults suggest that reading-induced brain changes are massive and they might not be limited to linguistic processes. However, it is still unclear whether these results can be generalized to normal reading development. The present study is aimed at identifying to which extent neural responses to verbal and nonverbal stimuli are reorganized while children learn to read.

MEG data were collected from two groups of Basque children (skilled and poor readers, 4-8y) while they were passively presented with written words, spoken words and visual objects. Children had to press a button whenever the current stimulus was identical to the previous one. The evoked fields elicited by the experimental stimuli were compared to their scrambled counterparts.

Visual words elicited left posterior (200-300 ms) and fronto-temporal (400-800 ms) activations in skilled readers only, suggesting a strong reorganization of children's visual word responses after reading acquisition. Specifically, after reading training the left language network was activated by written words.

Spoken words elicited greater left temporal activity relative to scrambles in both groups (300-700 ms), suggesting that reading acquisition does not have a strong impact on spoken word recognition within the first years of reading instruction. Lexical and semantic analysis of spoken words would not be specifically affected by reading expertise.

Brain responses to objects were greater than scrambles in posterior regions (200-500 ms), with skilled readers showing stronger left-lateralized responses than poor readers. Correlation values showed a greater involvement of the left hemisphere as reading performance improved, suggesting a strengthened verbal decoding of visual configurations after reading acquisition. Learning a new writing system would imply a stronger involvement of the left hemisphere due to an entrenched access of linguistic codes based on graphic information.

The present results provide evidence that learning to read not only influences written word processing in children, but also affects visual object recognition, suggesting a non-language specific impact of reading on children's neural mechanisms.

ID: P03

Title

Reading in deaf

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Most deaf people never achieve a good reading level compared with hearing peers. Preceding studies have shown that the skilled deaf readers might activate visual, orthographic and semantic codes during reading, but not phonological codes. In contrast, other studies have highlighted the importance of phonological coding and awareness for the development of reading skills in deaf individuals. In a series of experiments comparing skilled deaf and hearing readers we have investigated the role of phonological and orthographic processing in Spanish, a language with a transparent orthography. We carried out different behavioral tasks and explored the electrophysiological correlates of phonological and orthographic coding using different paradigms with varying demands of explicit and implicit reading mechanisms to study the similarities and differences between skilled deaf and hearing readers. The results of this line of research suggest that the main differences between deaf and hearing skilled readers are in phonological processing, while orthographic processing remains identical across groups. These findings suggest that the absence of phonological processing does not necessarily lead to reading difficulties. Hence, efficient phonological processing is not a prerequisite for word identification in languages with transparent orthographies.

ID: P04

Title

Myths about brain in education: Prevalence among Spanish teachers and an exploration of cross-cultural variation

Authors

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Enthusiasm for research on the brain and its application in education is growing among teachers. However, a lack of sufficient knowledge, poor communication between educators and scientists, and the effective marketing of dubious educational products, has led to the proliferation of numerous 'neuromyths'. The greatest concern of the dissemination of these misconceptions about brain involves not only economic costs for families and schools but also an opportunity cost for children who are deprived of effective, evidence-based interventions. The aim of this study was to explore the prevalence of these misconceptions among in-service teachers in Spain. As in previous studies, we observed that the belief in neuromyths was very high in this sample. In addition, teachers who responded correctly to general knowledge questions about the brain were more likely to believe in neuromyths. A meta-analytic synthesis of the present and previous studies revealed some consistencies across countries but also certain peculiarities in the type of neuromyths more widespread in each population. These results highlight the need for action to combat the increasing presence of pseudoscientific practises in schools worldwide.

ID: P05

Title

Hydroxylated DHA restores hippocampal memory function and adult neurogenesis in Alzheimer´s disease mouse model

Authors

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Alzheimer's disease (AD), the leading cause of dementia, impairs cognitive and functional abilities through a wide range of pathophysiological mechanisms. Among them, alterations in cholesterol, triglycerides or omega-3 fatty acids have been associated with the cognitive decline observed in AD. Docosahexaenoic acid (DHA), the most abundant n-3 polyunsaturated fatty acid in the brain, has been found to be substantially reduced in the brains of individuals with AD (1).

In previous works our research group showed that the novel hydroxyl-derivative of DHA (2-hydroxydocohexaenoic acid- OHDHA) has a strong therapeutic potential to treat AD improving behavioral deficits and A plaques in a mouse model of AD. Nevertheless, its potential use as neuroprotective agent in vivo it is yet to be confirmed (2, 3) To delineate its usefulness in AD treatment, in the present work, we performed oral administration of OHDHA and examined the impact on memory and neurogenesis disturbances exhibited by mice with AD-like pathology (APPswe/PS1dE9 mice).

We found that OHDHA significantly increased neurogenesis in 8 month old APPswe/PS1dE9 mice hippocampus. Furthermore, behavioral tests showed significant improvement of short-term memory (tested by the object recognition task), spatial learning and memory (by Morris Water Maze), as well as anxiolytic effect (performance on elevated plus-maze). These behavioral functions, spatial memory and anxiety, are hippocampal-dependent, suggesting that the increase in hippocampal neurogenesis is counterbalancing neuronal loss and improving cognitive function. Further experiments both *in vivo* and *in vitro* will validate this hypothesis and shed light on the mechanism of action.

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ID: P06

Title

Effect of intranasal administration of encapsulated IGF-1 in an animal model of Alzheimer's disease

Authors

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In the last decade there has been a dramatic increase in the prevalence of people suffering from neurodegenerative diseases (NDs), especially from Alzheimer's disease (AD). Unfortunately, at present, therapies are only symptomatic and there is no cure for this dramatic disease (1). A promising alternative to address the neurodegenerative process is the use of neurotrophic factors, such as insulin growth factor (IGF-1). However, after *in vivo* administration, these kind of growth factors present important limitations such as short-half life and rapid degradation rate, together with difficulties to cross the blood brain barrier (BBB). Indeed, this barrier is a limiting factor in brain drug development; that is why, in the past few years, intranasal drug delivery has appeared as an alternative non-invasive administration route to bypass the BBB and target drugs directly to the CNS.

In an attempt to overcome all these drawbacks, our research group is currently working in the encapsulation of different growth factors in biodegradable and biocompatible nanospheres, providing protection and a sustained delivery to the drugs. Moreover, in several works the intranasal administration has been investigated in order to prove new administration routes and avoid the associated problems with the BBB (2-3).

Thus, the aim of this work was to analyze the restorative potential of intranasally administered IGF-1, encapsulated in chitosan-coated nanostructured lipid carrier (CS-NLC-IGF1), after its administration to an animal model of Alzheimer's disease.

The CS-NLC-IGF1 formulation was prepared by a melt emulsification method. The nanospheres particle size was around 100 nm and negative zeta potential. The nanospeheres *in vitro* toxicity assay was conducted in primary neuronal cultures, maintaining the cell viability after the nanoformulation addition. *In vivo* studies were carried out in amyloid precursor protein/presenilin-1 (APP/Ps1) mice administering daily CS-NLC-IGF1 by intranasal route. The results obtained showed that CS-NLC-IGF1 were able not only to improve behavioral deficits, but also to decrease A deposits.

Taking all the above mentioned results into account, it may be concluded that the intranasal administration of IGF-1 encapsulated in NLCs may be a promising therapeutic approach to treat Alzheimer's disease.

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ID: P07

Title

The proactive bilingual brain: Using interlocutor identity to generate predictions for language processing

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Bilingual language activation is modulated by explicit linguistic cues (e.g., sentence context or lexical items), and bilinguals can also be trained to rely on non-linguistic cues, such as flags or colors, for language activation. Moreover, it has been recently demonstrated that a non-linguistic cue such as interlocutor identity (e.g., Spanish, Basque or bilingual interlocutor) also bias language activation: During language comprehension, bilinguals are faster in a lexical decision task when the language used by the interlocutor during the task is congruent with his identity (e.g., Spanish word pronounced by a Spanish versus a Basque interlocutor).

The current study was designed to explore whether bilinguals pre-activate the context-appropriate language even before the interlocutor produces language. We did so by using the technique of event-related potentials (ERPs) and by exploring bilinguals' brain activation between the onset of the visual presentation of the interlocutor and the onset of speech he produced. (1) Interlocutor identity might interact with language activation during speech processing itself. In that case, we would not expect any effect of interlocutor identity before speech onset. (2) Alternatively, bilinguals might pre-activate the expected language when presented with an interlocutor they know, before this interlocutor even starts to produce speech. In this case, brain responses between visual and audio onset should significantly differ between monolingual and bilingual interlocutors (no possible language pre-activation when facing a bilingual interlocutor who is using the two languages alternatively and randomly).

Twenty-three early proficient Spanish-Basque bilinguals took part in the ERP experiment. They were first familiarized, through video segments, with two Spanish, two Basque and two bilingual interlocutors. Then, they had to perform an audio-visual lexical decision task on items uttered by the six interlocutors. Participants had to decide if the words they heard were real or not in any of the languages (150 Basque and 150 Spanish words; 60 Basque-like and 60 Spanish-like pseudowords). There was an average of 350 ms gap between the onset of the video and the onset of the auditory signal.

The main outcome of the study was that the influence of interlocutor identity (bilingual versus monolingual interlocutor) on the bilingual's brain started around 100 ms after the onset of the video, even before the onset of speech. Thus, we showed for the first time that bilinguals can pre-activate a language in their mind, for further speech comprehension. This pre-activation can be performed in 'naturalistic' conditions, the cue for language pre-activation being the identity of a known interlocutor. This preparation for language mode seems to affect participants' behavior, since participants were faster to perform the lexical decision task on any type of lexical item when the interlocutor was monolingual versus bilingual. Our results have potential important implications for models of bilingual language control, which should take into account the possibility of selective language pre-activation even before any linguistic input, based on 'naturalistic' cues such as an interlocutor identity.

ID: P08

Title

A proposal for real time stress classification using F-State Machine technique by Analysing of the Nervous System Arousal

Authors

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Independence and personal autonomy are values linked to human beings. But in some collectives, these values are not entirely reached. This is the case of people with chronic diseases, people with intellectual disabilities, or elderly people.

In an effort to improve this condition, new research approaches focused on the study of human emotions offer outstanding results. In the case of people with disabilities, due to their lack of autonomy, unexpected emotional and behavioural changes may appear, leading to a blocking state during stressful events or situations. Therefore, a key issue to be investigated is how emotional responses to certain events happen in people. Thus, it is necessary to classify them and to observe how they affect their personal autonomy, and hence, their quality of life.

Considering the relationship between the body physiology and emotional states, a proper approach for identifying stressful situations appears to be the analysis of physiological signals and the search for correlations between them. To perform a detailed study on stress, one approach worthy of consideration is the analysis of bio-signals related to the Autonomic Nervous Systems activity.

This work presents a biophysical real-time stress classification system that is able to classify the sympathetic nervous system response and detected stress stages at three levels: low, medium, and high. Furthermore, the system identifies persistent stress situations or momentary alerts, depending on the subject's arousal.

This system uses two non-invasive physiological signals for preliminary processing and analysis (the galvanic skin response and the heart rate variability), and a finite state machine to identify and classify the stress level. Based on the identification process previously mentioned, 6 different arousal states are found, and the transitions between the states have been identified. For this purpose, a finite state machine has been built with the following parameters: Q={1-6} where 1=Low Alert, 2=Low Stress, 3=Medium Alert, 4=Medium Stress, 5=High Alert y 6=High Stress, q0={0}, without there being a final state because of its cyclical nature.

An experimental procedure was designed and configured in order to elicit a stress situation that is similar to those found in real cases. Tests were carried out with 166 voluntaries and showed that the proposed system is able to detect and classify the different stress stages, reaching an F-measure of 0.98 in the case of high stress level, 0.97 for medium level, and 0.94 for low stress level.

Based on the proposed system, a potentially useful tool for designing ergonomically adapted solutions will be made available. These solutions will be able to accurately identify, at the earliest possible moment, when an individual is facing an emotional block situation, and to generate the corresponding alert signals to his/her caregivers or relatives in these cases. Therefore, a significant improvement in the integration of these people in their work and social environments would be achieved.

ID: P09

Title

Bilingual Distance Effect. An ERP study

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Bilinguals show a preference for one of their languages in their arithmetic representations and learning; even when they are fully proficient balanced bilinguals (Spelke and Tsivkinm, 2001; Salillas and Wicha, 2012; Bernardo 2002; Salillas & Carreiras, 2014). We have suggested that this preferred language is the Language of Learning Math (LL^{math}), vs. the other language (OL), and this preference affects magnitude comparison. The comparison of single digits is robustly characterized by a Distance Effect (close numbers are more difficult to compare than numbers further apart). The distance effect has taken a prominent place in studies on numerical cognition, both at the behavioral (e.g., Dehaene, Dupoux, & Mehler, 1990), and the neuronal level (e.g., Nieder, Freedman, & Miller, 2002; Pinel, Piazza, Le Bihan, & Dehaene, 2004). But this effect has not widely been observed in bilinguals using Event Related Potentials (ERP's). The present tested a group of Spanish-Basque balanced bilinguals in a magnitude comparison task comparing Arabic numerals to measure the Distance Effect; this effect is usually obtained in the number comparison task where participants need to select the largest (or smallest) of two numbers. ERP's show a difference in magnitude processing at the P2 component for each language.

ID: P10

Title

Effectiveness of the rehacop cognitive rehabilitation program in aging

Authors

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Introduction

Normal aging can evolve through pathological cognitive impairment (MCI) to reach dementia. Given these data, we find it necessary to promote programs of cognitive and functional rehabilitation in order to improve functionality and quality of life and prevent their decline. The aim of the current study is to test the efficacy of REHACOP (Ojeda et al. 2012), an integrative rehabilitation program in normal aging.

Methods

Seventy-four patients with a mean age of 79 were randomized to the rehabilitation (REHACOP) or control group. REHACOP group (n=44) received cognitive rehabilitation for attention, processing speed, learning and memory, language and executive functions for 3 months (3 sessions/week of 60 minutes). The control group (n=30) they performed occupational tasks with the same time and duration. Both interventions were administered in group format.

Patients underwent an extensive neuropsychological assessment at baseline and after treatment that included the assessment of 6 different cognitive domains. For the analysis, we used the Z scores of attention, naming, apathy, fatigue and life satisfaction and the composite scores created for processing speed, verbal and visual memory, working memory, verbal fluency and executive functions.

Results

Both groups did not differ significantly in any score at base line. Group by time interactions of repeated measures ANOVA indicated that the experimental group improved significantly when compared to control group in verbal fluency (η^2_p=0.18. p‹0.001), verbal memory (η^2_p=0.12, p=0.004), apathy (η^2_p=0.07, p=0.03), and life satisfaction (η^2_p=0.07, p=0.03) as well as a tendency to significant improvement in fatigue (η^2_p=0.05, p=0.09).

Conclusions

The REHACOP group showed significant and large improvement in verbal fluency, verbal memory, apathy and life satisfaction, and medium-large improvement in fatigue. The results of this study support the idea that intervention programs structured as REHACOP improve cognitive abilities and functionality of the elderly, making it susceptible to health and social interest to be used as part of prevention programs and promoting gerontological population health.

ID: P11

Title

The prolyl oligopeptidase inhibitor IPR19 improves cognitive deficits in schizophrenia-like mouse models

Authors

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Schizophrenia is a chronic and disabling psychiatric disorder that affects about 1% of the population worldwide. Cognitive decline associated with this illness is considered a key feature since might precede the onset of the disease and continues after psychosis, being a predictor of the disorder evolution. Current antipsychotic drugs for positive or negative symptoms have little or no effect on cognitive deficits. Prolyl oligopeptidase (POP) is an 81-kDa monomeric serine protease that is expressed in brain and other tissues. Experimental data showed that POP inhibitors have neuroprotective, anti-amnesic and cognition-enhancing properties. In the present study, the pharmacological activity of IPR19 [(S)-1-((2S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl) pyrrolodine-2-carbonitrile], a new potent and selective POP inhibitor, was evaluated in three schizophrenia-like mouse models based on pharmacological subchronic phencyclidine (PCP) and acute dizocilpine (MK801) administration, and offspring of mothers with immune reaction induced by polyinosinic:polycytidylic acid (poly(I:C)) administration during pregnancy. Behavioral responses in novel object recognition (NOR) test, T-maze, eight-arm radial maze, Morris water maze and prepulse inhibition (PPI) were tested under basal conditions and after acute IPR19 (5 mg/kg ip) administration in C57/BL6 male mice. Data were analyzed using unpaired Student's t-test, and one-way ANOVA test followed by Dunnett´s post hoc test at $P<0.05$. The number of animals was previously estimated by sample size calculations and ranged from 6 to 23 animals per group.
In NOR test, MK801-treated animals and offspring of poly(I:C)-treated mothers showed a lower ratio of time devoted to the novel object than controls. IPR19 reversed the effect, restoring basal values. In T-maze test at 10 s and/or 40 s delay, PCP-, MK801 and poly(I:C) models displayed fewer correct choices than wild type animals. The effect was reversed by acute IPR19 in PCP and poly(I:C) models but not in MK801 model. In eight-arm radial maze test, PCP, MK801 and poly(I:C) models made less correct choices than the vehicle-treated counterparts. Acute IPR19 decreased the percentage of errors in PCP and poly(I:C) models whereas was ineffective in MK801 model. In Morris water maze test, mice under PCP and MK801 treatments, and poly(I:C) offspring spent less time in the target quadrant where the platform was located than control mice. IPR19 had the capacity to reverse this reduction in the three models. A strong decrease in the PPI at 81, 85, and 90 db was observed after acute MK801 administration and with less intensity in offspring of poly(I:C) mothers. In contrast, subchronic PCP treatment did not induce effects on PPI. IPR19 reversed the effects of acute MK801 across the different intensities to values that did not differ from those in controls whereas IPR19 did not modified the impaired PPI response in the poly(I:C) model.
In conclusion, the POP inhibitor IPR19 enhances cognitive performance and sensorimotor gating in different phenotype animal models of schizophrenia, suggesting that the compound may have therapeutic potential in the cognitive deficits associated to this psychiatric disorder. The finding becomes particularly valuable as a new option of pharmacological mechanism for the treatment of cognitive dysfunctions which represent the most treatment-elusive symptoms of schizophrenia.

ID: P12

Title

Exposure to Enriched Environment in Adulthood Reverts Cognitive Impairment and Interneuron Deficiency Induced by Early MK801 Administration

Authors

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Schizophrenia is a complex psychiatric disorder with a developmental component that compromises neural circuits. Understanding the neuropathological basis of schizophrenia remains a major challenge for establishing new therapeutic approaches. Causal factors for abnormal brain development in schizophrenia include NMDA receptor hypofunction and altered GABAergic circuit-mediated neurotransmission.

One of the most compelling findings in schizophrenia is the reduction of GABAergic markers, particularly the calcium-binding protein parvalbumin (PV) and 67-kDa isoform of the GABA synthesizing enzyme glutamic acid decarboxylase (GAD67). Similar reductions are observed in rodents after chronic exposure to NMDA receptor antagonist MK-801. Less attention has been paid to somatostatin-expressing interneurons (STT), although robust decreases of this neuropeptide have been found in human schizophrenic patients. In this work, we administered MK801 intraperitoneally to Long Evans rats from postanatal days 10 to 20 (0,5 mg/kg), and evaluated PV, GAD67 and STT expression in adulthood (P73). We showed by unbiased stereological methods that PV was significantly decreased in the dentate gyrus (DG) and CA1 region of the hippocampus compared to controls. In addition, a significant reduction of GAD67 and STT was found in CA1 of MK801-treated rats. In mPFC, only limbic regions were affected by the drug and the cell number of both PV and STT was diminished, although no concurrent loss of GAD67 was found.

Environmental Enrichment (EE) promotes sensory, motor and cognitive experience, and plays a major role in neuroprotection and neurorestoration. Nevertheless, effects of EE in adult animals after neurodevelopmental impairment with MK801 have not been studied so far. Our results confirm that a brief exposure to EE in late adolescence/early adulthood (P55-P73) completely reverted deficits of interneuron markers, except for PV expression in CA1 which was only partially recovered.

EE not only had beneficial effects on interneuron expression, but also improved cognition. Postnatal MK801 treatment led to spatial learning deficits in Morris Water Maze (MWM), and to a reduced Discrimination Index in Object-in-Place task. Object-in-Place performance was decreased on a short-term and on a long-term basis. Exposure to EE of MK801-treated rats enhanced MWM performance and improved long-term association memory.

Our results suggest that environmental intervention is an effective strategy to restore GABAergic immunoreactivity, and this might be sufficient to overcome long-lasting functional alterations relevant for schizophrenia research.

ID: P13

Title

Brain Machine Interfaces coupled with rehabilitation orthoses: a promising therapy for motor restoration in stroke patients

Authors

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Paralysis caused by stroke is one of the leading causes of long-term disability. This loss of muscle control is given by an injury in brain areas or tracts associated with motor function, limiting the motor command signal generation and their transmission towards the muscles. In the past years Brain-Machine Interfaces (BMIs) have been established to control rehabilitation devices using brain activity from the sensorimotor cortex associated with intended or imagined execution of upper-limb movements [1].These systems provide proprioceptive feedback to patients by moving their paralyzed limb based on the inferred motor intention from their brain signals. In a recent study, we showed that BMIs can be used to control of rehabilitation orthoses and restore motor function in severely paralyzed stroke patients [2]. In this study, 32 chronic stroke patients with severe hand weakness were divided into two groups: 1) the experimental group underwent a BMI training in which their hand and arm were mobilized by rehabilitation orthoses developed by TECNALIA contingently to the desynchronization of ipsilesional oscillatory sensorimotor rhythms, 2) in the control group movements of the orthoses occurred randomly. Both groups also received behavioral physiotherapy. The experimental group showed a bigger and significant improvement in the score of the Fugl-Meyer (FMA) clinical assessment of the upper-limb motor function than the control group after the training period. This outcome measure indicates the importance of a contingent, real-time coupling of the motor intention of the lesioned area with the actual movement of the paralyzed limb facilitated by orthoses. Through the use of proprioceptive-BMIs, the damaged efferent connections between the lesioned brain area and the muscles can be reinforced, activating neuroplastic mechanisms to induce motor rehabilitation. These results suggest that BMIs coupled with orthotic devices constitute a promising therapy for motor rehabilitation in severely paralyzed patients who cannot benefit from conventional treatments.

At the present time, the Neuroprosthetics group of the Institute for Medical Psychology and Behavioural Neurobiology in the University of Tübingen works in close cooperation with the Neuroengineering group of TECNALIA for combining BMIs with robotic orthoses and functional electrical stimulation systems for paralyzed muscles in stroke rehabilitation. This collaboration focuses on the development of BMI systems able to accurately decode movement intention inferred from the patient's electrophysiological signals such as electroencephalography, electrocorticography or electromyography, and their use for the control of sophisticated body actuators able to functionally move or stimulate a paralyzed upper-limb. Improvements in the contingency and functionality of trained movements in proprioceptive-BMIs are believed to speed up functional neuroplastic processes and enhance motor learning. Moreover, the study of the neuroscience behind motor recovery is essential to discover the key factors that lead to the restoration of motor function after a brain lesion. For this purpose, this research group uses diverse neuroimaging techniques such as magnetic resonance imaging, transcranial magnetic stimulation or electroencephalography together with BMIs to explore the sensorimotor integration and its link to behavior in patients with disorders of the central nervous system caused by diseases such as stroke or spinal cord injury.

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[2] Ramos-Murguialday A, Broetz D, Rea M, Laeer L, Yilmaz O, Brasil FL, Liberati G, Curado MR, Garcia-Cossio E, Vyziotis A, Cho W, Agostini M, Soares E, Soekadar S, Caria A, Cohen LG, Bibaumer N. Brain-Machine- Interface in Chronic Stroke Rehabilitation: A Randomized Study. Annals of Neurology 74(1), 100-108 (2013).

ID: P14

Title

Behavioural testing-related changes in the expression of Synapsin I and glucocorticoid receptors in standard and enriched aged Wistar rats

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Our aim was to assess the changes in the Synapsin I and glucocorticoid receptor (GR) expression induced by behavioural testing in the dorsal and ventral hippocampus of standard and enriched aged Wistar rats. The environmental enrichment (EE) was carried out 3h/day over a period of two months and then, the rats were tested in the elevated zero-maze (EZM) and radial-arm water maze (RAWM). Behavioural results showed that, even at an advanced age, EE was able to reduce anxiety-related behaviours and improve the performance in the RAWM. Regarding the neurobiological data, Synapsin I expression in the dorsal CA3, but not in the ventral, was enhanced both in enriched and standard rats when they performed the behavioural testing. Interestingly, the EE exposure was enough to increase Synapsin I in the ventral CA3. The analysis of GR in the dorsal hippocampus showed an increase of this receptor in the dDG both in enriched and standard rats when they performed the behavioural testing, whereas in the dCA1 and dCA3, the effect of the testing depended on the previous housing condition. In the ventral region, we found that the effects of EE were higher because on the one hand, the GR expression induced by the behavioural testing was enhanced in the dSUB, vCA1 and vCA3 when the rats were previously enriched and on the other hand, EE, regardless of the behavioural testing, increased the GR expression in the vDG and vSUB. Therefore, our results suggest that the effect of the behavioral testing on the neurobiological mechanisms studied is different depending on the previous housing condition of aged rats.

Imaging

ID: P15

Title

Complex networks reveal structural-functional brain resting-state subnetworks: identification, description and application as biomarkers

Authors

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Elucidating the intricate relationship between brain structure and function, both in healthy and pathological conditions, is a key challenge for modern neuroscience. Recent progress in neuroimaging has helped advance our understanding of this important issue, with diffusion images providing information about structural connectivity (SC) and functional magnetic resonance imaging shedding light on resting state functional connectivity (rsFC). In this work, we adopted a complex networks approach, relying on modular hierarchical clustering, to study together SC and rsFC datasets gathered independently from healthy human subjects. By employing the template of hierarchical modular organization derived from structural data to represent the resting state functional one and vice versa, we searched for the optimal common partition shared by structure and function by maximizing a novel quantity, that we dub "cross-modularity". This procedure allows the extraction of an optimal partition that we uncovered divides the brain into distinct subnetworks that we refer to as common "structure-function modules" (SFMs), representing a coarse-grained skeleton of the brain, which is largely shared by structure and function. First, we describe the emerging common structure - function modules (SFMs) and compare them with commonly employed anatomical or functional parcellations. Secondly, we use SFMs to characterize aging impact on brain networks. Specifically, by looking at the variation of the inter- and intra- module connectivity as a function of age, we show how a multiple linear regression model can describe global brain networks aging.
In conclusion, our results show how the resting-state brain activity is shaped by the existence of structural-functional subnetworks whose interplay and connectivity varies as a function of age.

ID: P16

Title

Inverse anterior frontal white matter asymmetry and its implications for cognition in patients with schizophrenia

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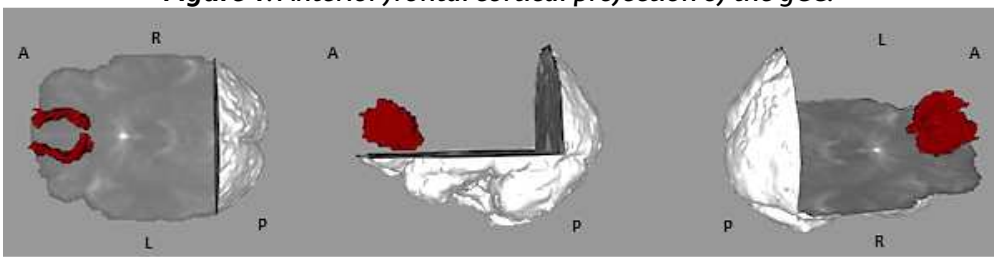
Background: Interhemispheric tracts are some of the most altered tracts in patients with schizophrenia (SZ). This damage affects the laterality of the brain of these patients, removing or even inverting the common pattern of white matter (WM) asymmetry found in healthy controls (HC) (greater WM integrity in left hemisphere). Furthermore, WM integrity in the corpus callosum (CC) has been reported to be decreased in SZ, especially in its anterior part (Genu of CC). Therefore, this study aimed firstly to compare the WM integrity of the genu of the CC (gCC) between patients with SZ and HC and secondly, to investigate the asymmetry related to a specifically determined frontal tract (anterior frontal cortical projection of the gCC) in patients compared to HC. In addition, implications of this asymmetry for neurocognition were assessed.
Methods: Ten patients with SZ (mean age=39.00) and 10 HC (mean age=45.80) underwent a diffusion weighted imaging study on a Siemens 3T MRI scanner. Seeds and waypoint masks of interest, gCC and anterior frontal cortical projection of the gCC, were manually generated for each hemisphere based on JHU-ICBM-DTI-81 White-Matter and Craddock atlases (Craddock et. al 2011) respectively, in order to perform probabilistic tractography. WM pathways of interest were obtained after applying BedpostX and ProbtrackX (implemented in FSL). WM tracts were normalized by dividing by the number of streamlines that went through the waypoint masks and thresholded at 5% (Figure 1). Overall and tract-specific fractional anisotropy (FA) indexes were obtained. Participants were assessed by a wide neuropsychological battery including processing speed and verbal memory (VM) measures among others. Data was tested for normality using Saphiro-Wilk analyses. Age and overall FA were added as covariates in a one way and a repeated measures MANCOVA designs and correlation analyses using SPSS.

***Figure 1**. Anterior frontal cortical projection of the gCC.*



Results: Patients showed significant lower mean FA in the gCC compared to HC ($F_{(1,16)}$=6.61; η_p^2=0.29; p=0.02). Repeated measures MANCOVA showed a statistically significant GroupXLaterality effect ($F_{(1,16)}$=7.43; η_p^2=0.32; p=0.02) indicating differences between patients and HC when we compared the mean FA from the left and right anterior frontal cortical projection of the gCC. As expected, HC exhibited a higher mean FA in the left hemisphere tract compared to right hemisphere whereas patients showed a greater mean FA in the right tract comparing with the left hemisphere. Moreover, in patients mean FA of the right tract inversely correlated with VM performance (r=-0.73; p=0.04). This result pointed out that the higher the FA in the right anterior frontal cortical projection of the gCC, the worse the performance in VM in patients. No significant correlations were found in HC.
Conclusions: To our knowledge, this is the first study that confirms the inverse asymmetry in a specifically determined anterior frontal cortical projection of the gCC in patients with SZ. Moreover, as a consequence of this altered asymmetry, a greater mean FA in the right tract seems

to be related with a worse performance in VM, highlighting the potential impact of this inverted asymmetry on cognition in SZ.

ID: P17

Title

Virtual Reality and 4D Holography as new immersive imaging technologies to simplify visual understanding of brain diseases

Authors

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Nowadays the combination of computation and digital imaging coming from MRI/MRT and confocal microscopy devices is indisputably the most accurate option for diagnosis in nuclear medicine. It allows the understanding and visualization of brain anomalies and shows a reliable representation of cerebral diseases, including the access to data images in real time.
At the moment, there is different software in the market that generates simulated 3D visualization of the images from the MRT systems. However, researchers and physicians generally carry out this visualization in 2D flat monitors and, in the best-case scenario, in a 3D screen, losing a great part of the advantages provided by the 3D spatial representation. To overcome this situation, new 3D visualization technologies must be developed.
In this field, new devices have recently appeared in the market, going beyond the state of the art, and transforming images into real 3D immersive experiences. Technologies such as virtual reality and holographic vision allow three-dimensional objects to appear in the surrounding environment of the observer and in combination with manipulation tools, can create interactive environments with stereoscopic, 3D visual displays and immersive interaction from a first person perspective, allowing physicians to enter the object of study and decide in the diagnosis.
These technologies make it possible to simulate an environment for brain research and clinical applications with better validity and control than ever before. Virtual reality is compatible with brain imaging methods and this allows researchers to obtain a more accurate diagnosis and a new use of brain imaging results for pre-surgical planning.
In this work, we've developed a new software for Virtual Reality HeadSets as 'Oculus Rift' and a new device for holographical vision, 'HoloDeck'. HoloDeck is a functional multipurpose 4D holographic immersive visualization hardware and software viewer, with two 3D screens, to improve the accuracy, speed and efficiency for clinical diagnosis in nuclear medicine, clinically validated. It includes a gesture controller device, instead of a mouse or a pad, for a natural hand movement in 3D space. Both systems are integrated in a workbench that generates interactive and gesturally operable 3D models, based on the 2D DICOM images coming from MRI or microscopy equipments.
These systems help professionals to get into the brain through a HD quality 3D-based 360º immersive environment of holographic appearance and, they also facilitate the interaction by the means of touch-less devices in order to enable their incorporation in sterilized environments. At present time, new collaborative features are being introduced to the system in order to allow professionals from different physical places to share and visualize information simultaneously for a cooperative and more precise diagnosis and evaluation.
Thanks to this new imaging workbench, medical, research and academic communities are able to take a tour into a patient's brain and interact with it in laboratories, classrooms and surgery rooms in hospitals.
Virtual Reality and Holographic Vision combined with MRI and brain imaging software is the key that opens the future to the simplification of the visual understanding of brain diseases.

ID: P18

Title

Resting-state functional connectivity in carriers of E46K-SNCA mutation

Authors

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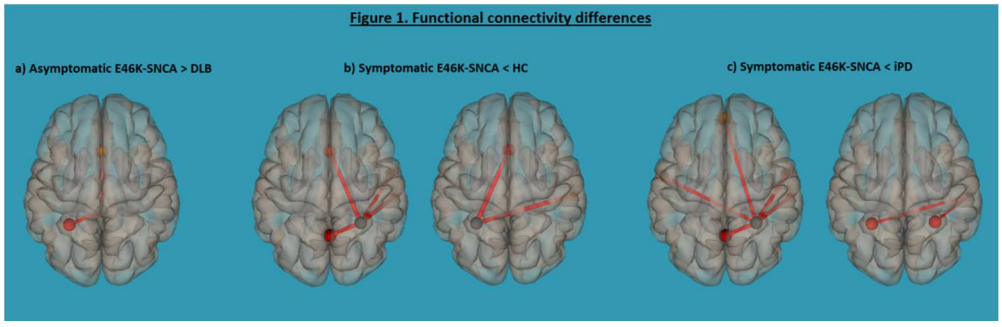
Background. E46K mutation of alpha-synuclein gene (E46K-SNCA) is a rare, highly-penetrant and clinically aggressive genetic model of pure Lewy body disease (LBD). Previous findings support the presence of a common clinical and cognitive pattern in the aggressive forms of idiopathic LBD and in E46K mutation carriers, suggesting a common pathophysiological process underlying Lewy body pathology and neuronal degeneration in both groups.
Objective. The objective of this research is to compare the functional connectivity profile of the default mode network (DMN) in carriers of E46K-SNCA mutation with healthy controls (HC), idiopathic Parkinson's disease (iPD) and dementia with Lewy bodies (DLB) patients.
Methods. We evaluated cross-sectionally 48 participants including n=7 E46K-SNCA [4 symptomatic (mean disease duration (dd): 9.1 years) and 3 asymptomatic], n=18 iPD (dd: 5.3 years), n=4 DLB (dd: 8.7 years) and n=19 HC, matched by sex, age and education. All participants underwent a 3D T1-weighted imaging and resting-state functional magnetic resonance imaging study on a Philips Achieva 3T. CONN-Functional Connectivity toolbox was used for analyses. ROI-to-ROI group comparisons were assessed using cluster-level inference at p(FDR)<.05. Seeds selected according to areas of DMN were: a) posterior cingulate cortex, b) anterior cingulate cortex, c) medial prefrontal cortex, d) bilateral medial temporal lobe, e) bilateral parietal cortex, and f) precuneus.
Results. Significant differences were found between asymptomatic carriers of E46K-SNCA and DLB patients, showing asymptomatic E46K-SNCA higher functional connectivity between posterior cingulate cortex and anterior cingulate cortex and left superior parietal lobe compared with DLB patients (see Figure 1a). No significant differences were found between asymptomatic E46K-SNCA and symptomatic E46K-SNCA, iPD or HC.
Symptomatic carriers of E46K-SNCA showed significant differences compared with HC, showing symptomatic E46K-SNCA lower functional connectivity between right superior parietal lobe and precuneus, anterior cingulate cortex and right medial temporal lobe, and between left superior parietal lobe and anterior cingulate cortex and right medial temporal lobe (see Figure 1b). Moreover, there were found significant differences between symptomatic E46K-SNCA and iPD patients. Symptomatic E46K-SNCA showed lower functional connectivity between right superior parietal lobe and medial temporal lobe, medial prefrontal cortex and precuneus, and between right medial temporal lobe and bilateral superior parietal lobe compared with IPD patients (see Figure 1c). No significant differences were found between symptomatic E46K-SNCA and DLB patients.



Conclusions. Resting-state functional connectivity of the DMN is altered in symptomatic carriers of E46K-SNCA. The results revealed that symptomatic E46K-SNCA were comparable to those with DLB. Moreover, the similarities between symptomatic E46K-SNCA and DLB may support the existence of a functional connectivity phenotype specific for alpha-synucleinopathies driven by parietal lobe abnormalities in the DMN.

ID: P19

Title

Lipid distribution in the rat brain

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Lipids are considered no longer mere molecules with structural or energy function. Moreover, they have been identified both as intracellular signalling molecules or binding to specific receptors, which are involved in cell proliferation, growth or neuroprotection. The development of new analytical techniques, such as the imaging mass spectrometry (IMS), is contributing to understand their involvement in physiological processes. IMS allows to obtaining a wide range of information about the distribution and intensity of the different molecules.

The aim of this study is to map the distribution of different lipid species in the rat CNS using IMS to find out a possible relationship between anatomical localization and physiology.

Analyses were performed in positive ionization mode and negative ionization mode, using a spectrophotometer LTQ-Orbitrap XL. The data were analysed with the ImageQuest and MSIReader software

As the results, we selected 88 peaks of each ionization mode. In the case of negative ionization mode assigned 34 molecular species were assigned to known molecules with an error of 5 ppm, and 51 in positive. These molecules were grouped by different lipid families, obtaining: Phosphatidylcholines (PC): PC (34: 1) + K^+ and PC (32: 0) + K^+ distributed mainly in grey matter, and PC (36: 1) + K^+ and PC (38: 1) + Na^+ distributed in white matter. Phosphatidic acid (PA): PA (38: 3) + K^+ in white matter, and PA (38: 5) + K^+ in grey matter but also in brain ventricles. Phosphoinositol (PI): PI (18: 0/20: 4) - H^- in grey matter, and PI (O-30: 1) or PI (P-30: 0) - H^- in white matter. Phosphatidylserines (PS): PS (34: 1) - H^- in grey matter, and PS (38: 1) - H^- in white matter. Sphingomyelin (SM) SM (d18: 1/16: 0) - H^- in ventricles and SM (d18: 1/18: 0) - H^- in grey matter. Sulfatides (ST): ST (d18: 1/24: 1) - H^- in white matter.

Therefore, the specific distribution of different lipids supports their involvement not only in structural and metabolic functions, but also as intracellular effectors or specific receptor ligands. The described specific localization in CNS will allow us to analyse their role in neurodegenerative pathologies, such as the Alzheimer's disease.

Neurology - Clinical Neurology

ID: P20

Title

Structural and functional brain alterations in patients with Parkinson's disease and mild cognitive impairment: a multimodal imaging study

Authors

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Background
Mild cognitive impairment (MCI) is common in Parkinson's disease (PD) patients since the early stages. The Movement Disorders Society Task Force Criteria has proposed a MCI classification of PD (PD-MCI). The objective of this study was to identify cerebral differences between PD-MCI patients and patients without MCI (PDnoMCI) combining both structural and functional magnetic resonance images.
Methods
Thirty-seven PD patients underwent a neuropsychological battery and T1-weighted structural, diffusion weighted and functional magnetic resonance images (fMRI) during resting-state and during a memory paradigm (with learning and recognition tasks) were acquired on a Phillips Achieva 3T. MCI diagnosis followed Level II of the Movement Disorder Society Task Force criteria (comprehensive assessment) and patients were classified as PD-MCI or PDnoMCI. Between-group cerebral differences were performed controlling for age, gender, years of education and scores on the Spanish version of the National Adult Reading Test (NART). The levodopa equivalent daily dose was also introduced as a covariate in fMRI analysis and the total intracranial volume in structural analysis.
Results
From the whole sample, 24 PD patients were classified as PD-MCI patients and 13 as PDnoMCI. Freesurfer analyses showed significant reduced volume in the left (F= 7.55; p=.01) and right (F=7.29; p=.01) hippocampus in the PD-MCI group compared to the PDnoMCI group. Moreover, PD-MCI patients showed increased radial diffusivity in the left corticospinal tract, the left superior longitudinal fasciculus and the left superior corona radiata compared to the PDnoMCI group (p<.05 FWE-corrected). In addition, PD-MCI patients showed significant higher positive functional connectivity between the posterior cingulate cortex and the parahippocampal gyrus compared to PDnoMCI (t= 4.26; p=0.02 FDR-corrected). No significant differences were found in whole-brain grey matter volume or during the memory fMRI paradigm between groups.
Conclusions
Cognitive impairment in PD patients is accompanied by early grey and white matter structural atrophy and functional cerebral changes. These findings suggest the existence of different neuroanatomical and neurofunctional substrates for PD patients with and without MCI diagnosis that could be detected since the early stages of the disease.

ID: P21

Title

Biomimetic Spiking Neural Network for Neurological Studies

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Millions of people worldwide are affected by neurological disorders which disrupt connections between brain and body causing paralysis or affect cognitive capabilities. Such a number is likely to increase in the next years and current assistive technology is still limited. Since last decades Brain-Machine Interfaces (BMIs) and generally neuroprosthesis have been object of extensive research and may represent a valid treatment for such disabilities. The development of these devices has and will hopefully have a profound social impact on the quality of life. The realization of such prostheses implies that we know how to interact with neuronal cell assemblies, taking into account the intrinsic spontaneous activity of neuronal networks and understanding how to drive them into a desired state or to produce a specific behavior. The long-term goal of replacing damaged brain areas with artificial devices also requires the development of neuronal network models. The hardware set-up that will be used to interface the biological component is a Spiking Neural Network (SNN) system. This SNN implements biologically realistic neural network models, spanning from the electrophysiological properties of one single neuron up to network plasticity rules. In this work, we present a biomimetic digital hardware implementation of Spiking Neural Networks (SNN). This part constitutes one main point of the FET7 European project Brainbow. This digital implementation computes in real-time biologically realistic cortical Izhikevich neurons and it requires few resources. To get the best trade-off between biocompatibility and low-resources, we decided to implement the Izhikevich model. Interneuron connections are composed of biomimetic synapses and synaptic plasticity. We also add the short-term plasticity described by Izhikevich that manages the depression or the facilitation of the synaptic strength. The architecture of the network implementation is based on RAM blocks (that store all parameters needed to define a network), two computation cores (one used for neurons and the other one for synapses), a block to manage the state machine and the addresses and a block “Communication RS232” (that will allow us to configure/change parameters of our system). It is freely configurable from an independent-neuron configuration to different neural network configurations with different options like the synaptic plasticity, the synaptic noise and the axonal delay. Furthermore, this implementation used a few part of resources and it uses pipeline implementation. This SNN is used for the development of a neuromorphic chip for neuroprosthesis, which has to replace or mimic the functionality of a damaged part of the central nervous system and to study neurological diseases. Several hybrid experiments with biological neurons have been done like CPG activities for spinal cord lesion, and replacement neural network for patterned culture with lesions.

Neurology – Neurogenetics

ID: P22

Title

Mutations affecting splicing process: high prevalence of cases within our cohort of patients with autosomal dominant retinitis pigmentosa

Authors

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Background: Retinitis pigmentosa (RP) is the most frequent group of inherited retinal dystrophies. It is highly heterogeneous with more than 80 disease-causing genes currently associated with RP, 27 of which have been ascribed to autosomal dominant RP (adRP). Several genes associated with adRP (PRPF3, PRPF4, PRPF6, PRPF8, PRPF31) or the RNA helicase (SNRNP200), are splicing factors which comprise the spliceosome multisubunit complex, termed as splicing-related adRP. *The spliceosome orchestrates two transesterification reactions, necessary to remove introns and to join the adjacent exons operating by step-wise formation of subcomplexes that recognize regulatory sequences and promote efficient splicing.* Considering that these genes are expressed ubiquitously in all tissues and are highly conserved in all eukaryotes, it is not clear why mutations in these genes are associated exclusively with autosomal dominant cases of RP. The most straightforward explanation is that splicing is inefficient in the retina, a high transcriptionally active tissue, where reduced levels of mRNA lead to a deficit in the amount of proteins. Another possible answer is related to co-transcriptional splicing, since it is well established that splicing and transcription are coordinated processes. Therefore, alterations in the spliceosome can affect the transcriptional machinery. A third hypothesis -compatible with the previous two ones- has recently been proposed: a reduction in the activity of splicing-related adRP proteins produces genomic instability that can lead to programmed cell death, specially in cells with high transcriptional activity. The impairment of the splicing machinery can form R-loops (RNA/DNA duplex) that expose the singlestrand DNA to DNA-damaging agents such as UV light at active transcriptional regions.
Purpose: To identify mutations responsible for RP in a cohort of 29 patients diagnosed with adRP.
Methods: 29 index cases were ascertained based on a family tree compatible with adRP at the Ophthalmology Service Donostia University Hospital, San Sebastian. A custom panel with 31 genes associated with adRP and other autosomal dominant inherited retinal dystrophies were analyzed by targeted next-generation sequencing using the Ion personal genome machine (IPGM; Life Technologies), in combination with Sanger sequencing.
Results: We were able to detect mutations likely causative of the disease in 14 out of the 29 families analyzed, resulting in a ratio of clinically relevant genetic diagnosis of 48,28%. It was remarkable that about 38% of total adRP cases analyzed showed mutations affecting splicing process, mainly due to mutations in genes coding for spliceosome factors, SNRNP200 and PRPF8, but also due to splice-site mutations in RHO gene.
Conclusion: Our results will help to give a more accurate genetic counseling and will contribute to a better characterization of the disease, and might have a therapeutic impact in the future, considering all undergoing studies based on targeting RNA splicing with therapeutic purposes.

Psychiatry - Molecular Psychiatry

ID: P23

Title

Evaluation of spinophilin expression in postmortem prefrontal cortex of subjects with schizophrenia: effect of antipsychotic treatment

Authors

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Schizophrenia is a chronic incapacitating disease that affects 1% of the population. Today, schizophrenia is conceptualized as a neurodevelopmental psychiatric disorder with altered connectivity between different brain regions. Several studies have demonstrated reduced dendritic spine density and other dendritic abnormalities in schizophrenia. These spine deficits may be related to disturbances in the molecular mechanisms that underlie spine formation, pruning and maintenance. Spinophilin is a scaffold protein involved in multiple signaling pathways. Spinophilin modulates excitatory synaptic transmission and dendritic spine morphology. Besides, spinophilin has been shown to regulate G-protein coupled receptor signaling, including dopamine D2 receptors and alpha adrenoceptors, which play a role in the pathophysiology of the schizophrenia and are targets of antipsychotics. Previous studies have reported changes in spinophilin mRNA levels in different brain regions from schizophrenic subjects. For these reasons it is of great interest to study the spinophilin protein expression in the brain of subjects with schizophrenia.

Methods: Spinophilin protein density was determined by Western Blot in postmortem dorsolateral prefrontal cortex of subjects with schizophrenia. Forty eight subjects where included in the study: 24 with an antemortem diagnosis of schizophrenia, and 24 controls with no antemortem history of psychiatric disease, matched by age, gender, and postmortem delay. Twelve of the schizophrenia subjects were taking antipsychotic treatment at the moment of death (based on positive blood toxicological analysis), while the other 12 where antipsychotic-free at death (negative toxicology). Spinophilin was measured in a preparation of synaptosomes obtained by ultracentrifugation methods, and normalized for actin immunoreactivity as loading control.

Results: Immunoreactivity for spinophilin detected two bands at ~120 and ~95 kDa, which disappeared with the blocking peptide, demonstrating the specificity of both bands. Quantification for the ~120 kDa band, which is the entire protein, showed no significant differences between schizophrenia and control subjects. When subjects were divided in order to study the effect of antipsychotic treatment, there was a non-significant trend to a reduction (-8%) in spinophilin levels in antipsychotic-free subjects, which was not observable in treated subjects. However, spinophilin ~95 kDa band, supposed to be a cleaved form of the protein, was significantly reduced (-15%) in the dorsolateral prefrontal cortex of schizophrenia subjects ($p=0.0067$, $n=24$) when compared with controls. When subjects were divided in regard to the treatment ($n=12$), this form showed non-significant differences in antipsychotic-free subjects, while there was a significant reduction (-24%) in antipsychotic-treated subjects ($p=0.0028$).

Conclusions: No significant changes were observed for spinophilin ~120 kDa form in synaptosomes from postmortem prefrontal cortex of subjects with schizophrenia. Conversely, there was a significant reduction in the ~95 kDa form, which seems to be due to the antipsychotic treatment. Further research needs to be done to elucidate the nature and role of this cleaved ~95 kDa form.

ID: P24

Title

Neurotrophin Signaling in First Episode psychosis: Relation to Response to Antipsychotics after 1 Year of Follow-Up

Authors

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Background: Previous studies have shown a pro/anti-inflammatory imbalance in patients with a first episode of psychosis (FEP), which continues 12 months after. Research in this area is increasingly focused on finding biomarkers that help us understand the pathophysiological mechanisms underlying the disease.
The aim of this study is to evaluate changes in the expression of neurotrophins BDNF and NGF and their receptors in peripheral blood to identify any potential correlation with levels of inflammation, clinical symptoms over time and response to antipsychotic treatment.
Methods: The study included 94 patients with a FEP and 80 healthy subjects. Blood samples andvclinical data were taken both at baseline and 12 months later. The expression of BDNF, NGF andvtheir receptors TrkB (functional and truncated) and TrkA was measured in peripheral mononuclearvcells. The pro/anti-inflammatory parameters evaluated were NFkB, COX-2, iNOS, PPARgamma andv15d-PG12. Patients' functionality was measured by the GAF scale.
Results: Expression of the functional isoform (FL) of the BDNF receptor increases 1 year after diagnosis, whereas the truncated form (T) (inactive) descends. The ratio of both forms FL/T increases during follow-up only in the group of non-affective psychosis, suggesting different mechanisms underlying different subgroups of patients with FEP. Expression of NGF receptor, TrkA, increased in patients during follow. After adjustment for confounding variables basal levels of proinflammatory variables were significantly associated with the ratio FL/T, suggesting that increased inflammation would be associated with a higher ratio. Besides, the FL/T ratio could have a predictive role of the functionality of patient after 1 year, depending on whether the patient is treated with antipsychotic treatment or not.
Discussion: Inflammatory processes, neurotrophins pathways and functional status of the patient appear to be related wich has important translational relevance. In particular, the level of expression of TrkB receptor isoforms (FL and T) should be considered before starting antipsychotic treatment in patients with a FEP.

BDNF and NGF Signalling in Early Phases of Psychosis: Relationship With Inflammation and Response to Antipsychotics After 1 Year.
Martinez-Cengotitabengoa M, MacDowell KS, Alberich S, Diaz FJ, Garcia-Bueno B, Rodriguez-Jimenez R, Bioque M, Berrocoso E, Parellada M, Lobo A, Saiz PA, Matute C, Bernardo M, Gonzalez-Pinto A, Leza JC; FLAMM-PEPs.
Schizophr Bull. 2016 Jan;42(1):142-51.

ID: P25

Title

A follow-up of biochemical parameters associated with neuroinflammation in depressed subjects: relationship with clinical response

Authors

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Recent findings have established a connection between inflammation and depression, and specifically the role of hypothalamic-pituitary-adrenal axis (HPA axis) in this pathology. A dysfunctional HPA axis accompanied by an increased pro-inflammatory cytokines has been described in depressed patients. The cytokines could activate the enzyme indoleamine 2,3-dioxygenase (IDO), contributing to kynurenine pathway activation. Enhanced tryptophan degradation via the kynurenine pathway could induce neurotoxic changes both in glia and neurons. In this adverse context, the S100 calcium binding protein B (S100B) is considered a peripheral marker of the glia damage.

The aim of this study was to analyze the time-course of alterations of different components of inflammatory response, HPA axis and CNS damage in blood plasma of patients with depression (n=24) using a naturalistic, longitudinal design. The relationship between biological and clinical status of patients was evaluated. Data were collected at baseline and after 3, 6 and 12 months of pharmacological treatment. Severity of depression was quantified using the Hamilton Depression Scale (HDS). Molecular analyses for the proinflammatory cytokine IL6, the HPA axis activity marker ACTH, the glial lesion marker S100B, the IDO enzyme expression and the inflammatory marker CRP (C-reactive protein) were performed by ELISA and particle-enhanced immuno-turbidimetric assay commercial kits. Statistical comparisons were performed by one-way ANOVA followed by Dunnet's multiple comparison test.

At baseline, HDS score in depressed patients was 24.4±1.3. There was significant differences between baseline and follow-up data from third month ($F[3,91]=13.48$; $p<0.0001$). At the 12th month, the HDS was 5.2±1.1, which was interpreted as remission score. Conversely, there was not statistical differences in plasma PCR concentrations over time in depressed subjects ($F[3,74]=0.07$; $p=0.98$). ACTH values progressively decreased ($F[3,71]=3.92$; $p=0.01$) being statistically lower than in baseline conditions at 12 months. No difference in IDO concentrations in plasma of depressed patients was observed between any of measured values ($F[3,66]=0.58$; $p=0.63$). Furthermore, proinflammatory IL6 plasma values were also similar after 3, 6 and 12 months compared to baseline values ($F[3,64]=1.13$; $p=0.34$). Similarly, there were not differences in glial lesion marker S100B plasma concentrations over the time in depressed patients ($F[3,71]=1.35$; $p=0.27$).

Recently our group has showed increased ACTH, CRP, IL6, IDO and S100B concentrations in plasma of depressed subjects when compared to matched healthy controls. According with the present results, ACTH decreases over time in parallel to HDS depressive symptoms severity whereas inflammatory parameters do not modify and remain higher than in controls. The elevated concentrations of the peripheral marker of CNS lesion S100B in depressed subjects are also maintained. These results suggest that increased neuroinflammation markers in depression do not respond to antidepressant treatment and remains hyperactive. In contrast, the hyperactive HPA axis normalizes in parallel to clinical response to antidepressant drugs.

ID: P26

Title

Pro-inflammatory state associated to elevated indoleamine 2,3-dioxigenase (IDO) expression and S100B concentrations in plasma of depressed subjects

Authors

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Multiple lines of evidence support the pathogenic role of neuroinflammation and hypothalamic-pituitary-adrenal (HPA) axis dysfunction in depressive disorders. Microglial activation by pro-inflammatory cytokines, which are elevated in blood and CNS of patients with depression, could contribute to activate the kynurenine pathway. Enhanced tryptophan degradation by indoleamine 2,3-dioxigenase induces toxic effects both in astrocytes and neurons. In this context, the S100 calcium binding protein B (S100B) is considered a peripheral marker of the glia damage. The aim of this study was to analyze the potential alterations of different components of inflammatory response, glial damage markers and HPA axis in blood plasma of patients with depression (n=42) and control subjects (n=61). Depression was quantified using the Hamilton Depression Scale (HDS). Molecular analyses were performed by ELISA commercial kits for proinflammatory cytokines (IL1beta, IL1alpha, IL6 and TNFalpha), the anti-inflammatory cytokine IL10, the neurotrophic factor BDNF, HPA axis activity markers (cortisol, ACTH and beta-endorphin [-end]), a glial lesion marker (S100B) and IDO expression. Inflammatory marker CRP (C-reactive protein) was quantified by particle enhanced immuno-turbidimetric assay commercial kit. The statistical comparison between means was performed by Student's t-test. Demographic and clinical characteristics were included as covariables in the analyses. Pearson's correlation coefficients were calculated to test possible association among variables.

The HPA axis activity biomarker ACTH was increased in depressed patients (44.6±2.6 ng/ml vs 38.0±0.9 ng/ml; $p<0.05$) but non-significant changes were obtained in cortisol and beta-endorphin concentrations of these subjects. IDO concentration showed higher levels (1.72±0.13 ng/ml; $p<0.05$) in depressed patients compared to controls (1.40±0.10 ng/ml). ACTH and IDO concentration were significantly correlated in depressed subjects ($r=-0.42$; $p<0.01$). IL6 was higher in subjects with depression (0.71±0.07 ng/ml; $p<0.05$) than in controls (0.53±0.05 ng/ml). When compared to controls, CRP concentration was increased in depressed patients (2.84±0.43 ng/ml vs 1.76±0.3 ng/ml; $p<0.05$). Plasma IL6 concentration and CRP concentration were correlated both in depressed patients group ($r=0.47$; $p<0.01$) and control group ($r=0.30$; $p<0.05$). The protein S100B was also increased in depressed patients (438±29 pg/ml vs 352±21 pg/ml in controls; $p<0.05$). In control group, cortisol concentration was negatively correlated with IL10 ($r=-0.25$; $p<0.05$) and positively with S100B concentration ($r=0.30$; $p<0.05$) but this correlation disappeared in depressed patients. No differences were obtained between groups for BDNF concentrations.

The present study demonstrates increased concentrations of proinflammatory factors and peripheral cytokine-inducible IDO enzyme in depressed patients. Elevated IDO could contribute to the over-stimulation of kynurenine pathway and neurotoxic metabolites production. These metabolites may induce brain glial damage, showed as an elevation of S100B concentration in blood. This increase could also be related to HPA axis dysregulation and the hyperactivation of immune system. Chronic HPA axis hyperactivation suggests a glucocorticoid resistance in depressed patients.

ID: P27

Title

Pharmacological hallmarks of the 5-HT2A receptor ligands ketanserin and altanserin in human frontal cortex: potential concern in schizophrenia studies

Authors

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Serotonin 5-HT_{2A} receptor (5-HT_{2A}R) is a G protein-coupled receptor involved in signaling mechanisms that in the central nervous system (CNS) affect fundamental processes such as cognition, perception, memory and mood. Moreover, the $5HT_{2A}$R is highly involved in the pathophysiology of certain psychiatric disorders such as schizophrenia and it is a main pharmacological target of atypical antipsychotic drugs, i.e. clozapine. Thus, it is of major interest to elucidate the signaling hallmarks of different ligands through 5-HT_{2A}R directly in the human CNS. The radio-labeled ligands [^{3}H]ketanserin and [^{18}F]altanserin have been used to evaluate the 5-HT_{2A}R brain density in schizophrenia with overall discrepant outcomes. A potential explanation is the relative selectivity of these ligands for the different structural conformations of 5-HT_{2A}R and their different functional signaling. This hypothesis was tested by evaluating the pharmacological properties of both [^{3}H]ketanserin and [^{18}F]altanserin in membrane homogenates of human postmortem prefrontal cortex (PFC).

In [^{3}H]ketanserin binding assays (0.03-10 nM), Gpp(NH)p (100 µM) –a GTP analogue that uncouples G-proteins from the receptor– produced a significant decrease in the radioligand affinity for the receptor (K_D=0.62±0.07 nM vs. K_D=1.47±0.13 nM; n=3; $p<0.001$). In competition binding assays the 5-HT_{2A}R agonist (±)DOI (10^{-12}-10^{-4} M) displaced [^{3}H]ketanserin binding (2nM) in a biphasic manner ($logK_{i\text{-}high}$=-9.46±0.42; $logK_{i\text{-}low}$=-6.82 ± 0.11; $fraction_{high}$=22±0.1%; n=3) and the presence of Gpp(NH)p removed the (±)DOI high-affinity site and significantly reduced (-33±2%, $p<0.05$) the basal binding of [^{3}H]ketanserin at 2 nM.

This effect of Gpp(NH)p on the pharmacological properties of [^{3}H]ketanserin led us to test the functional activity of ketanserin in terms of G protein coupling, comparing ketanserin functional properties with those of the closely related 5-HT_{2A}R ligand altanserin.

Scintillation proximity assays revealed that ketanserin (10 µM) induced a stimulation of [^{35}S]GTPS binding to $G_{q/11}$-protein subtype (E_{max}=119±4%; n=4; $p<0.05$) that was reversed by the 5-HT_{2A}R antagonist MDL11939 (10 µM). This effect of ketanserin was not observed when G_{i1} coupling was tested (99±2%; n=4; $p>0.05$). In contrast, altanserin was unable to induce activation of $G_{q/11}$ (101±2%; $p>0.05$), whereas the presence of altanserin (10 µM) induced a significant reduction in the basal activation of G_{i1} (88±1%; $p<0.001$). This inhibition was completely reversed by ketanserin or MDL11939 (10 µM). Moreover, when coupling to G proteins was tested in frontal cortex of 5-HT_{2A}R-KO mice as compared to wild-type littermates, both activation of $G_{q/11}$ coupling by ketanserin and inhibition of G_{i1} coupling by altanserin was undetectable.

In summary, these results indicate ketanserin and altanserin recruit specific 5-HT_{2A}R-mediated patterns of G protein coupling in human frontal cortex, suggesting preferences in terms of biased agonism with ketanserin as a 5-HT_{2A}R agonist of the $G_{\alpha q/11}$ pathway and altanserin as a 5-HT_{2A}R inverse agonist of the $G_{\alpha i1}$ pathway. Our findings may provide an explanation for the discrepancies in the radioligand binding studies of the 5-HT_{2A}R density in the PFC of schizophrenic subjects.

Cellular and molecular Neuroscience - Glia

ID: P28

Title

Microglial phagocytosis is severely impaired in a genetic model of progressive myoclonus epilepsy

Authors

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In physiological conditions, aging, inflammation and excitotoxicity microglial phagocytosis is fast and efficiently coupled to apoptosis in the adult hippocampus, but becomes impaired in a mouse model of epilepsy induced by intrahippocampal injection of kainate (KA). To further assess the mechanisms regulating microglial phagocytosis, we have now studied a genetic model of epilepsy, Progressive Myoclonus Epilepsy (EPM1). EPM1 is a hereditary neurodegenerative disorder characterized by severe myoclonus, seizures, and ataxia and is caused by loss-of-function mutations in the cystatin B (Cstb) gene, which encodes an inhibitor of cysteine proteases. We found that at the onset of myoclonus (1 month), Cstb KO mice showed a severe impairment in microglial phagocytosis, which only engulfed 15% of the apoptotic cells, very similarly to what we observed in the KA epilepsy model. This phagocytic impairment was not compensated by other cell types such as astrocytes. These changes were accompanied by a vast increase in apoptosis in the dentate gyrus of the hippocampus, a dramatic change in microglial morphology, and an increase in microglial numbers and proliferation. Importantly, this morphological change was previously described in microglia at postnatal day 15, before seizures arise, suggesting that microglial malfunction might be triggering the pathological events that lead to seizures. Our results further confirm the inhibition of microglial phagocytosis during epilepsy, and point towards Cstb as an important candidate for gaining further insight into the undestanding of the regulation of microglial phagocytosis efficiency and its relevance in pathology.

ID: P29

Title

Microglial phagocytosis is impaired in chronic mouse and human MTLE and correlates with inflammation

Authors

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In physiological conditions in the adult hippocampus, apoptotic cells are rapidly and efficiently phagocytosed by microglia. We have observed that during aging, inflammation, and excitotoxicity, microglia responded to the increase in apoptosis by adjusting proportionally their phagocytosis. Conversely, in a mouse model of mesial temporal lobe epilepsy (MTLE) by intrahippocampal administration of kainic acid (KA), microglial phagocytosis was reduced as early as 6 hours after injury, and continued to be impaired in the long term. Importantly, this phagocytic blockade led to the accumulation of non-phagocytosed apoptotic cells, and contributed to the development of an inflammatory response. Unexpectedly, in the subacute phase (3-7 days) of MTLE, microglia showed a hypertrophic, seemingly amoeboid morphology that was related to the cells becoming multinucleated. Further, we also detected some cases of phagoptosis or engulfment or non-apoptotic cells. In later stages (4 months) of MTLE, microglial phagocytosis remained impaired. Importantly, the microglial phagocytosis impairment was observed in human hippocampal tissue from MTLE patients. In the human tissue, we found the same kind of phagocytosis observed in the mouse brain by terminal or en passant branches of microglia. In addition, we observed a unique type of phagocytosis in which several microglial nuclei formed a surrounding the apoptotic cell, in an aster-like structure. These results demonstrate that the impairment of microglial phagocytosis is a novel mechanism contributing to the pathophysiology of epilepsy.

Abiega O, Beccari S, Diaz-Aparicio I, Nadjar A, Layé S, Leyrolle Q, Gomez-Nicola D, Domercq M, Pérez A, Sánchez-Zafra V, Paris I, Deudero JJP, Brewster AL, Anderson AE, Zaldumbide I, Galbarriaty L, Marinas A, Vivanco M, Matute C, Maletic-Savatic M, Encinas JM, Sierra A. 2016. Neuronal hyperactivity disturbs ATP microgradients, impairs microglial motility, and reduces phagocytic receptor expression triggering apoptosis/microglial phagocytosis uncoupling. PLos Biology.

ID: P30

Title

Regulation of neurogenesis by phagocytic microglia-derived factors

Authors

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During adult hippocampal neurogenesis, the majority of the newborn cells undergo apoptosis. To prevent disturbing the surrounding neurons, these apoptotic cells are quickly and efficiently removed by phagocytosis by resident microglia. Here we propose that phagocytosis is not merely a process to remove corpses but it has an active role in maintaining the homeostasis of the adult hippocampal neurogenic cascade by producing neurogenic regulators. To test this hypothesis, we first set up an in vitro model of phagocytosis in which primary cultures of postnatal microglia were fed with apoptotic SH-SY5Y (a human neuronal line) cells over a time course. Using this model, we performed a full genome-wide transcriptomic analysis of the phagocytic microglia using gene expression arrays comparing naïve vs phagocytic microglia. Gene ontology analysis revealed that, in addition to major changes in different cellular pathways, phagocytosis triggered a pro-neurogenic program in microglia. We found a significant upregulation of the neurogenesis function and we identified significant changes in 209 genes of genes involved in functions related to different stages of the neurogenic cascade. Afterwards, the expression of relevant genes with significant changes was validated by RTqPCR. To confirm the upregulation of the pro-neurogenic genes in phagocytic microglia *in vivo,* we resorted to utilize single-cell RNA sequencing comparing microglia isolated from dentate gyrus (DG; enriched in phagocytic cells) with that of CA (where there is no neurogenesis and therefore no apoptosis nor phagocytosis). Using bioinformatic tools we expect to discriminate changes in the profile associated to phagocytosis. In conclusion, these results give us a novel insight into the changes initiated by microglial phagocytosis in the neurogenic niche and strongly suggest the existence of a pro-neurogenic/repair program triggered by phagocytosis in microglia.

ID: P31

Title

Cystine/glutamate antiporter blockage induces myelin degeneration

Authors

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The cystine/glutamate antiporter is a membrane transport system responsible for the uptake of extracellular cystine and release of intracellular glutamate. It is the major source of cystine in most cells, and a key regulator of extrasynaptic glutamate in the CNS. Since cystine is the limiting factor in the biosynthesis of glutathione, and glutamate is the most abundant neurotransmitter, the cystine/glutamate antiporter is a central player both in antioxidant defense and glutamatergic signaling, two events critical to brain function. However, distribution of cystine/glutamate antiporter in CNS has not been well characterized. Here, we analyzed expression of the catalytic subunit of the cystine/glutamate antiporter, xCT, by immunohistochemistry in histological sections of the forebrain and spinal cord. We detected labeling in neurons, oligodendrocytes, microglia and oligodendrocyte precursor cells, but not in GFAP+ astrocytes. In addition, we examined xCT expression and function by qPCR and cystine uptake in primary rat cultures of CNS, detecting higher levels of antiporter expression in neurons and oligodendrocytes. Chronic inhibition of cystine/glutamate antiporter caused high toxicity to cultured oligodendrocytes. In accordance, chronic blockage of cystine/glutamate antiporter as well as glutathione depletion caused myelin disruption in organotypic cerebellar slices. Finally, mice chronically treated with sulfasalazine, a cystine/glutamate antiporter inhibitor, showed a reduction in the levels of myelin and an increase in the myelinated fiber g-ratio. Together, these results reveal that cystine/glutamate antiporter is expressed in oligodendrocytes, where it is a key factor to the maintenance of cell homeostasis.

ID: P32

Title

P2X4 receptor location and function in myelin phagocytosis

Authors

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Intra-endolysosomal Ca^{2+} release is required for endolysosomal membrane fusion with intracellular organelles. Previous data have demonstrated that P2X4 receptor (P2X4R) forms channels at endosomes that are activated by luminal ATP in a pH-dependent manner (Huang et al., 2014). Moreover, P2X4R activation promotes endolysosome fusion and vacuole enlargement (Cao et al., 2015). However, the role of P2X4R in phagocytosis has not been previously analyzed. We have checked the expression of P2X4R and its contribution to myelin phagocytosis during autoimmune inflammation. The expression of P2X4R increased in the acute as well as in the chronic phase of experimental autoimmune encephalomyelitis (EAE) mice. P2X4R expression correlated to the neurological score and perfectly matched to the expression of interferon regulatory factor (IRF) 5 and 8, key transcription factors for microglia M1 differentiation (Krausgruber et al., 2011). Accordingly, we showed that the expression and function of P2X4R were upregulated in M1 polarized microglia. At the subcellular level, P2X4R was located at both plasma-membrane and lysosomes. Next, we checked the role of P2X4R on myelin phagocytosis since it is well known that phagocytosis of myelin debris by microglia is essential for an efficient regenerative response after demyelination (Miron et al., 2013). As previously described, myelin phagocytosis and degradation was significantly increased in M2 polarized microglia. Interestingly, blockage of P2X4R with TNP-ATP significantly reduced myelin phagocytosis and degradation. On the contrary, treatment with ivermectin, an allosteric modulator of P2X4R channel, significantly increased myelin endocytosis and degradation. These results highlighted the role of P2X4R in microglia phagocytosis, and point to P2X4R as a new therapeutic target to promote myelin clearance and facilitate regenerative responses in demyelinating diseases.

ID: P33

Title

Palmitoylethanolamide increases CB2 receptor expression via PPAR-α and induces a reactive phenotype in microglial cells

Authors

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Palmitoylethanolamide (PEA) is an endocannabinoid-like compound, which belongs to a class of fatty acid ethanolamides, that has been shown to exert anti-inflammatory and analgesic effects mainly through inhibition of pro-inflammatory compound release from mast cells, macrophages, and microglia (Luongo et al., 2013). PEA is produced by neurons and glial cells in the CNS and is involved in the endogenous neuroprotective mechanisms that are activated following tissue damage or inflammation (Skaper et al., 2013; Mattace Raso et al., 2014). Although several mechanisms of action have been proposed, indirect activation of the cannabinoid (CB) system is thought to be responsible for the effects of PEA observed in several pain models. In the present study, using cultured rat microglia and human macrophages, we aimed to determine if PEA affects cannabinoid signaling, by focusing on CB2R expression, because of its wide expression on immune cells. We showed that PEA treatment increases CB2 mRNA and protein expression levels through peroxisome proliferator-activated receptor- (PPAR-) activation. The involvement of PPAR- was demonstrated through pharmacological PPAR- manipulation, PPAR- mRNA silencing, and molecular docking. Through indirect immunofluorescence analysis we showed that incubation of microglia with PEA also induced morphological changes (compared to the phenotype of untreated microglia) associated with a reactive phenotype. The same phenotype was found also in PPAR- agonist treated cells, confirming the involvement of this receptor in the mechanism of action of PEA. Linked to this reactive state, PEA treated microglia exhibited increased phagocytosis and migratory activity, which are both essential for exerting anti-inflammatory action. All together, these results suggest the indirect regulation of microglial CB2R expression as a new possible mechanism of action for PEA. Moreover, the reactive phenotype induced by PEA treatment suggests that this drug can be explored as a useful tool for counteracting the symptoms associated with neuroinflammation in CNS disorders.

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ID: P34

Title

Anatomical architecture of the type-1 cannabinoid receptor in hippocampal astrocytes of a new mutant mouse

Authors

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Astrocytes were only considered for a long time as energetics suppliers for neurons and protective elements against neuronal injury. Since the establishment of the "tripartite synapse" formed by the presynaptic and postsynaptic neuronal elements and by the surrounding astroglial processes, the role´s concept of the astrocytes in the brain has dramatically changed. Type-1 Cannabinoid (CB_1) receptor, which mediates the effects of the cannabinoids in the brain, has been shown to play a key role in the communication between neurons and astrocytes (Navarrete and Araque, 2008, 2010; Navarrete et al., 2013, 2014; Gómez-Gonzalo et al., 2014). However, no information is available on the anatomical architecture between the CB_1 receptor in astrocytes and the neuronal synapses.

In this study, the CB_1 receptor distribution in astrocytes was analysed by immunoelectron microscopy focusing on the expression of CB_1 in hippocampus of transgenic mice expressing hrGFP protein under the control of GFAP promoter in GFAPhrGFP-*CB_1*-WT and GFAPhrGFP-*CB_1*-KO mice. The results were compared with WT and *CB_1*-KO mouse hippocampi. Specific CB_1, GFAP and hrGFP antibodies combined with a preembedding immunogold and an immunoperoxidase method for electron microscopy were applied to hippocampal sections of the different mutant mice.

The percentage difference of CB_1 receptor immunopositive astrocytic processes in the CA1 radiatum layer was statistically significant between *CB_1*-WT (42.06% ± 3.56%) and GFAPhrGFP-*CB_1*-WT (59.91 % ± 3.29%) ($p < 0.001$ ***). Similarly, 44.67% ± 3.85% of the astrocytic profiles were immunopositive in the *CB_1*-WT dentate molecular layer whereas the proportion significantly increased in GFAPhrGFP-*CB_1*-WT (59.99% ± 3.37%; $p < 0.01$ **). Only scarce levels of CB_1 receptor immunolabelling were found in *CB_1*-KO and in GFAPhrGFP-*CB_1*-KO.

Since a better resolution was obtained in the GFAPhrGFP-*CB_1*-WT mouse, the distance between the CB_1 receptor in the astrocytic processes and the nearest synapse to them was assessed. In CA1, 51.07% ± 3.79% of the total synapses were at a distance between 400-800 nm from the astrocytic CB_1 receptor immunoparticle. Furthermore, 33% ± 1.01% of them were identified as excitatory synapses and only 9.91% ± 1.93% were inhibitory ones. In the dentate molecular layer, 57.25% ± 3.19% of the total of synapses surrounding were between 400-800 nm from the astrocytic CB_1 receptor gold particle, of them, 46.67% ± 2.17% were excitatory and 10.59% ± 2.18% were inhibitory synapses.

To sum up, more CB_1 positive astrocytic processes were observed in GFAP-hrGFP mouse hippocampus, suggesting that a better CB_1 detection in astrocytes can be achieved in this reporter mutant. Then, it is plausible that CB_1 receptors are at higher levels in astrocytes than previously described, from which CB_1 receptors regulate bidirectionally neuron-glia interactions.

ID: P35

Title

P2Y12 receptor regulates microglial phagocytosis in vivo

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Microglia is a key cell type in maintaining tissue homeostasis in the central nervous system by phagocytosing cellular debris and apoptotic cells, which is antiinflammatory in vitro and prevents the spillover of toxic intracellular contents. Microglia detect and recognize dying cells via several signaling molecules, such as ATP, recognized by receptors of the P2 family such as P2X7 and P2Y12, at least in vitro. To test their involvement in phagocytosis in vivo, we analyzed the efficiency of microglial phagocytosis in adult wild type (WT; C57BL/6), P2X7 and P2Y12 knock-out mice. In order to study phagocytosis we resorted to the adult hippocampal neurogenic niche, where a large percentage of newly generated cells undergo apoptosis and are efficiently cleared by "resting" microglia in physiological conditions. The proportion of non-phagocytosed apoptotic cells was increased in P2Y12 KO mice when compared to WT or P2X7 KO mice, suggesting that microglial phagocytosis is impaired in the absence of P2Y12. While similar numbers of microglia were found in WT and KO mice, P2Y12 KO animals showed a decreased number of phagocytic microglia leading to a decreased phagocytic capacity compared to WT mice. As a consequence, the coupling between apoptosis and phagocytosis that occurs in physiological conditions was lost. To further test the role of these receptors, we are currently using a pharmacological approach using specific P2X7 (brilliant blue G, BBG) and P2Y12 (PSB0739) inhibitors. Our data suggests that P2Y12, but not P2X7, is a regulator of microglial phagocytosis of apoptotic cells in vivo and suggest that P2Y12 could be used as a therapeutic target for diseases in which microglial phagocytosis is impaired.

ID: P36

Title

Role of Asrocytes in -synuclein mediated Neuronal Degeneration

Authors

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Parkinson´s Disease (PD) is a neurodegenerative disorder characterized anatomo-pathologically by the loss of dopaminergic neurons in the substantia nigra *pars compacta* and the presence of intraneuronal cytoplasmic inclusions called Lewy bodies (LB). The main protein component of the LB is the mysfolded - -synuclein (- -syn). Previous studies suggest that - - syn can self-propagate from cell to cell by a simple mechanism of endocytosis, suggesting a "prion-like" activity of - -syn. To validate the infectious nature of aggregated - -syn and to understand the role of astrocytes in the onset and propagation of - -syn-induced neuropathy, we have observed the bi-directional transmission of - -syn between neurons and astrocytes rat cultures on a microfluidic assay. Astrocytes and neurons are separated by microchannels and treated with purified human LB-derived - -syn assemblies from PD patients whereas transport of - -syn is validated by immunofluorescence.

Our results demonstrated that - -syn assemblies are uptaken by all cell type and transported directly through all directions (neurons to neuron, neurons to astrocytes, astrocytes to astrocytes and astrocytes to neurons). In addition, we then studied the mechanism by which the - -syn is uptaken by the cells and its localization once internalized. For that, we infected both astrocyte and neuron cultures with baculovirus which marked lysosomes or endosomes. These experiments showed the colocalization of the - -syn with the early endosomes and with the lysosomes in both neurons and astrocytes, suggesting the phagocytosis as the main mechanism of internalization.

Finally, we found that internalization of exogenous - -syn triggers an increase of endogenous - -syn, which is more significant in neurons than in astrocytes.

With these results we suggest that exogenous - -syn uptaken by astrocytes can be transported into the neurons where it triggers expression of toxic, endogenous - -syn.

By elucidating the role of the astrocytes and their properties could suppose the possibility to consider these cells as new therapeutic targets for PD.

ID: P37

Title

The role of P2Y-like receptor GPR34 on microglial phagocytosis

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Phagocytosis of cellular debris is an essential process in the response to damage, because it prevents the spillover of intracellular contents and is actively anti-inflammatory, at least in vitro. In the brain, is performed by microglia, the resident macrophages. Phagocytes detect and recognize dying cells via several signaling pathways. Among them, a novel player is the P2Y12-like receptor GPR34, which binds to lysophospatidilserine (lysoPS) released by apoptotic cells. GPR34 has been recently shown to regulate microglial phagocytosis of latex microbeads in vitro. Here, we show that lysoPS inhibits the phagocytosis of human apoptotic neurons (SH-SY5Y cell line) in primary cultures of microglia, possibly because its binding to the GRP34 receptor in microglia prevents the recognition of apoptotic cells. To test the GPR34 receptor involvement in phagocytosis in vivo, we analyzed the efficiency of microglial phagocytosis in adult wild type (WT; C57BL/6) and GRP34 knock-out mice. We focused on the adult hippocampal neurogenic cascade, located in the dentate gyrus, where the majority of newborn cells undergo apoptosis and are rapidly phagocytosed by "resting" microglia throughout adulthood in physiological conditions. We observed that the proportion of non-phagocytosed apoptotic cells in GPR34 KO mice was decreased compared to control mice, suggesting a partial impairment of microglial phagocytosis due to the absence of the receptor. While similar numbers of microglia were found in WT and KO mice, KO animals showed a decreased number of phagocytic microglia, leading to a decreased phagocytic capacity compared to WT mice. As a consequence of the phagocytosis impairment, the coupling or balance between apoptotic and phagocytic processes that occurs in physiological conditions was lost. These data indicate a role of GPR34 in the regulation of the phagocytic process in vivo. In the future, we will utilize these GPR34 KO mice to test the involvement of microglial phagocytosis in the regulation of inflammation, neurogenesis and other relevant processes.

ID: P38

Title

Myelinophagy: A Novel Mechanism for Schwann Cell Mediated Myelin Breakdown

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In peripheral nerves, myelin breakdown, or demyelination, is a universal outcome of a remarkably wide range of conditions that involve disturbance to Schwann cells or the nerve environment, whether due to genetic or acquired disease, toxicity, or nerve transection/crush. It has also become clear from studies on cut nerves that, perhaps surprisingly, Schwann cells themselves have the ability to turn against their own myelin and initiate myelin breakdown, destroying about 40–50% of the myelin during the first 5–7 d after injury. In spite of the central position of myelin breakdown in Schwann cell biology and pathology, the cellular and molecular mechanisms that make Schwann cell–mediated myelin digestion possible have not been established. In this talk, I will present evidence that autophagy, a mechanism by which many cells digest their intrinsic cellular components, plays a central role in Schwann cell myelin breakdown. We show that nerve injury triggers strong activation of Schwann cell autophagy, find myelin debris in autophagosomes, and demonstrate a strong requirement for autophagy in myelin digestion, revealing a novel form of selective autophagy of the myelin sheath, myelinophagy.

ID: P39

Title

P2X4 receptor switches microglia polarization and favours remyelination in EAE

Authors

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Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) characterized by an autoimmune inflammatory reaction to myelin components leading to oligodendrocyte death and demyelination and axonal damage. However, spontaneous yet transient myelin repair can occur during the course of the disease. A major component of this regenerative process is a robust innate immune response consisting of peripherally-derived macrophages and CNS-resident microglia. Microglia are the resident macrophages of the CNS involved in surveying the brain microenvironment for signals of injury or infection and they are essential for the initiation and resolution of pathogen- or tissue damage–induced inflammation. They demonstrate considerable plasticity that allows them to respond efficiently to environmental signals and change their phenotype and physiology in response to cytokines and microbial signals. Here we analyzed the role played by purinergic receptor P2X4 during autoimmune inflammation. Blockage of P2X4R signalling accelerated inflammation-induced demyelination and neurodegeneration during chronic experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. In vitro assays showed that blockage of P2X4R on lymphocytes did not alter T cell proliferation, viability or cytokine production, excluding any direct role on the immune reaction. On the other hand P2X4R blockage induced an increase in microglia cell number and an increase in M1 gene expression after EAE. Indeed, blockage of P2X4R in vitro demonstrated that this receptor modulated microglia polarization. Moreover, conditioned medium from microglia treated with P2X4R antagonists halted oligodendrocyte differentiation in vitro and remyelination after lysolecithin-induced demyelination in cerebellar slices cultures. On the contrary, potentiation of P2X4R signalling in microglia by the allosteric modulator ivermectin (IVM) favoured a switch to M2 phenotype, promoted the remyelination response and ameliorated EAE symptoms. Our results demonstrated that P2X4R modulate the microglia inflammatory response and identified IVM as candidate among currently used medications as potential therapies for the repair of myelin.

Cellular and molecular Neuroscience - Neurobiology of Disease

ID: P40

Title

MicroRNA-mRNA interaction network analysis in early retinal degeneration in the rd10 Mouse Model of Retinitis Pigmentosa

Authors

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Background: Retinitis Pigmentosa (RP) is a group of genetically determined retinal diseases characterized by progressive photoreceptor cells degeneration that leads to a consequent vision loss. RP is the most common form of hereditary retinal degeneration with a worldwide prevalence of about 1 in 3500–5000, and a total of more than 1 million affected individuals. Currently there is no standardized and effective treatment for this eye disease. MicroRNAs (miRNAs) are small non-coding RNAs involved in post-transcriptional modulation and have critical functions in virtually every physiological aspect of biology. They have been linked to several disease states such as cancer, heart disease, neurological diseases, or even hereditary diseases such as cystic fibrosis. With respect to retina, gene regulation via microRNA has been reported to participate in development, function and survival, and recently, several studies support the involvement of different miRNAs in the pathogenesis of retinal diseases such as macular degeneration, diabetic retinopathy and retinoblastoma. With respect to retinitis pigmentosa, a common miRNA signature of a group of differentially expressed miRNAs in four different mouse models of RP with genes involved in both autosomal dominant and autosomal recessive forms of the disease was reported.
Purpose: The aim of this study was to identify miRNAs differentially expressed that might play an important role in the pathophysiology of retinal degeneration using a well established mouse model of retinitis pigmentosa: rd10, at initial stages of retinal degeneration.
Methods: For that purpose, a detailed global expression analysis of miRNAs and the correlation with the transcriptome was performed using the GeneChip miR4.0 and GeneChip 1.0 MTA arrays (Affymetrix) and miSript miRNA PCR arrays (SaBiosciences). Out of ›1900 miRNAs analyzed, a total of 41 miRNAs were differentially expressed compared to WT mice (fold-change greater than +/- 2). Based on fold-change values and on prediction studies of their mRNAs targets, we selected 18 miRNAs. Their expression levels were subsequently validated using a second technique (RT-qPCR). MiRNA-mRNA interaction networks were generated using both miRNAs and transcriptome expression analysis data via Cytoscape open access software which permitted the identification of a list of genes with an inversely correlated expression to the candidate miRNAs. These genes were subsequently subjected to genetic ontology studies and analysis of biological pathways using DAVID and ClueGO databases. We selected those biological pathways involved in retinal degeneration such as apoptosis, inflammation and phototransduction.
Results: Using a different mouse model of RP, we have observed a group of miRNAs differentially expressed, some of which are common to those reported previously. Interestingly, we have identified 9 miRNAs that have not been assigned to any disease of the retina or other pathology. Therefore, modulation of those miRNAs differentially expressed will allow us to test novel therapeutic approaches for RP regardless the gene involved.
Conclusion: microRNA-mRNA expression profiling analysis provided valuable insights into molecular mechanisms underlying Retinitis Pigmentosa for better understand the biological processes implicated in the retinal degeneration of this disease and opens the way to future therapeutics in RP thought the modulation of those aberrantly expressed miRNAs.

ID: P41

Title

Myo-cytoblots: a method to quantify of dystrophin by in-cell western assay to accelerate preclinical assessment of DMD treatments

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An "in-cell western" (ICW), also known as a cytoblot, is a quantitative immunofluorescence assay performed in cell culture microplates that allows protein quantification directly in cultures. This method requires small amounts of cells and allows a higher number of experimental repeats and throughput.

Restoring dystrophin expression in Duchenne muscular Dystrophy (DMD) is the goal of many potential therapies in development. Due to the mutation-specific nature of many of these therapies, it is necessary to test them in patient's cell cultures. However, preclinical development of these therapies does not rely on accurate dystrophin quantification: western blotting, the current gold standard for protein quantification in these studies, requires a large amount of cultured cells and patients' cultures are scarce. This means that fewer compounds are routinely screened as thoroughly as would be advisable.

We have developed an in cell western (myo-cytoblot) to quantify dystrophin and other muscle associated proteins in control and DMD patients' cell cultures. After optimizing growth and differentiation rates of our cultures and selecting specific antibodies against our proteins of interests, we are able to accurately distinguish between the different sets of patients based on their dystrophin expression and detect dystrophin restoration after treatment with drugs. We are in the process of validating our cytoblot against standard methods.

ID: P42

Title

Antioxidants as a treatment for neonatal hypoxic-ischemic brain injury

Authors

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Hypoxic-ischemic brain injury continues representing a serious clinical problem despite the advances in neonatal care, and it is responsible for many cases of perinatal mortality, cerebral palsy, motor impairment and cognitive deficits. The high incidence of this lesion in newborns can be partly attributed to the fact that the developing brain is especially vulnerable to the oxidative stress. Since antioxidants can safely interact with free radicals and terminate that chain reaction before vital molecules are damaged, exogenous antioxidant therapy would be a powerful tool in order to diminish cellular damage caused by this pathology. Thus, the main goal of the present work was to evaluate the effects of resveratrol and docosahexaenoic acid (DHA) antioxidants against the hypoxic-ischemic brain injury in rats.

By permanent ligature of the left common carotid artery and then by asphyxia (135 minutes, 8% O_2) hypoxia-ischemia was provoked when rats were 7 days old. Treated rats received a single dose of resveratrol (20 mg/kg) or DHA (1 mg/kg) 10 minutes before hypoxia. So there were 4 experimental groups: control, hypoxic-ischemic group (HI), and the injured animals that received resveratrol (HI+RVT) and DHA (HI+DHA). The pathologic changes in brain tissues were observed by Nissl staining, and Glial Fibrilary Acid Protein (GFAP), Ionized calcium Binding Adaptor molecule 1 (IBA-1) and Myelin Basic Protein (MBP) immunostaining. The learning and memory function of rats were examined by T maze and novel object recognition test. One-way analysis of variance followed by Bonferroni-Dunn correction was performed.

Regarding the quantitative analysis of the infarct area, the HI group presented a high percentage of tissue loss in comparison with the control and the treated groups. Similarly, microscopic photographs and the semi-quantitative neuropathological scoring system demonstrated a damage located at the level of hippocampus and parietal cortex of the ipsilateral hemisphere in the HI group. This damage was reducted by the administration of the antioxidants. Concerning reactive astrocytes, there was a strongly increase in GFAP immunostaining in the HI group that was not so obvious in the pretreated animals. The HI group as well showed a significant decrease in MBP-immunostaining pattern in comparison with the control group that was avoided with the pretreatments. A substantial increase of ipsilateral IBA-1 immunostaining was presented in the hippocampus of animals that underwent hypoxia-ischemia respect to controls, while this increase was no so apparent with the antioxidant therapies. On the adulthood, animals pretreated with antioxidants demonstrated better long-term memory than the HI group, reaching similar values to the control one.

To sum up, our results suggest that resveratrol and docosahexaenoic acid antioxidants confer neuroprotection against hypoxic-ischemic brain injury in rats by decreasing the infarct area, ameliorating the neuronal damage, preserving myeling production and reducing the astroglial reactive response and microglial activity. Moreover, these antioxidants demonstrated long-term neuroprotective effects as they improve cognitive impairments assessed by behavioral tests.

ID: P43

Title

Axon-to-soma degeneration by local translation of transcription factors

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Alzheimer´s disease (AD) spreads through the brain in a non-random manner indicating propagation along connecting fiber tracts. The molecular mechanisms driving the spread of the pathology remain largely unknown. We have recently discovered an unexpected mode of long-range transmission of the pathological effects of A oligomers from axons to cell bodies that relies on intra-axonal translation of ATF4. These findings support a model in which retrograde transport of locally translated proteins leads to pathological, transcriptional changes in the neuronal cell bodies.

Local translation enables axons to react to extracellular stimuli in an acute manner. Intra-axonal protein synthesis is best understood in the context of neurodevelopment where it plays crucial roles in growth cone behavior, axon pathfinding and retrograde signaling. On the other hand adult axons have long been thought to be translationally inactive. However, high-throughput analyses have revealed that mature axons have a more complex and dynamic transcriptome than expected, especially under pathological conditions, and local translation is required for axonal regeneration upon nerve injury, it improves motor function in a mouse model of spinal muscular atrophy (SMA), and we have recently found that it mediates A-induced neurodegeneration in vitro and in vivo.

ATF4 mediates the cellular responses upon activation of the integrated stress response (ISR) by inducing the transcription of genes involved in cell death or survival, but it can also repress long-term potentiation under normal conditions acting as a CREB-1 antagonist. Our results establish that Aβ application selectively to axons triggers retrograde somatic degeneration through ATF4 axonal translation. Thus axonally-synthesized ATF4 could be targeted in order to prevent or slow down the spread of AD pathology throughout the brain. However, ATF4 is also translated in the neuronal soma of granule cells in the dentate gyrus (DG) following Aβ infusion in the mouse hippocampus in vivo. Atf4 knockdown increases Aβ-mediated neurodegeneration in the DG, suggesting that in this particular case ATF4 would rather be involved in a protective, adaptive response to Aβ exposure. This raises the intriguing possibility that ATF4 elicits distinct responses based on its translation at the subcellular level. Such differential responses could be explained by the availability of potential ATF4 binding partners in axons and cell bodies upon Aβ exposure. Here we characterize the role of ATF4-related transcription regulators in axons, whose interaction with ATF4 could be targeted in order to prevent axon-to-soma degeneration triggered by amyloid peptides.

ID: P44

Title

Adaptative changes in CA1 hippocampus ultrastructure after acute THC exposure in young-adult mouse

Authors

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Cannabis is the most widespread illicit drug in the world and its main psychotropic ingredient ^{9}-tetrahydrocannabinol (THC) exerts psychoactive effects through the activation of the neuronal cannabinoid receptor type 1 (CB_1), which is expressed by different neuronal subpopulations and glial cells in the central nervous system. Marijuana use often begins during adolescence, a highly susceptible period for environmental stimuli to alter functional and structural organization of the developing brain. However, not much information is available on the fine anatomical changes taking place in neurons after THC exposure in the adolescence.

In this study, an ultraestructural analysis was done in the CA1 hippocampus to determine the impact of acute ^{9}-tetrahydrocannabinol (THC) administration during adolescence on the size of dendrites and the distribution of neuronal mitochondria. Furthermore, as it was previously described cannabinoids treated animals show a decrease of CB_1 receptor. Therefore, in this study we have investigated the precise subcellular CB_1 receptor distribution in this region.

Young adult C57BL6 mice were subcutaneously injected with ^{9}-THC (5 mg/kg, only one injection) or vehicle. 30´ after injection, mice were sacrificed and perfusion-fixed through the heart. Then, a preembedding immunogold method for high resolution electron microscopy for the localization of CB_1 the receptor was applied to the hippocampus.

The average number of dendritic spines in 31.5 μm^2 of CA1 was 8 ± 0.25 in control versus 12 ± 0.30 in THC-treated, 5,100 μm^2 were analyzed in 3 mice per group. The difference observed between treated and untreated samples was statistically significant ($p < 0.001^{***}$, Mann Whitney test).

Of the total area analyzed, 49.57 % ± 1.39 corresponded to dendritic profiles in controls whereas 67.80 % ± 1.13 was in THC treated mice (difference statistically significant, $p < 0.001^{***}$, Mann Whitney test). Furthermore, a significant increase of dendritic mitochondria was observed after THC administration versus control, being this difference statistically significant ($p < 0.001^{***}$, Chi-square).

Finally, there was a significant decrease of CB_1 receptor immunopositive inhibitory terminals (47.74 ± 13.27%) in THC-treated versus control (78.40 ± 0.81%) ($p < 0.01^{**}$, Mann Whitney test). Interestingly, the percentage of CB_1 receptor immunopositive excitatory synaptic terminals was not significantly different between treated and untreated groups

In conclusion, the acute low-dose THC administration affects dendritic morphology and causes an increase of dendritic spines and mitochondria. Also, the CB_1 receptor distribution is drastically reduced in inhibitory synapses. The ultrastructure and receptor modifications observed in CA1 hippocampus after acute THC administration indicate the existence of fast brain adaptations that support morphologically the behavioural alterations provoked by cannabis intoxication.

ID: P45

Title

Clonal analysis of astrocytic response in a demyelinating disease model

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Multiple sclerosis (MS) is a chronic, neuroinflammatory demyelinating disease of the central nervous system (CNS). Astrocytes maintain homeostasis within the CNS but also can react toward brain injury by forming a glial scar and exert inflammatory responses in concert with microglia. They also play an active and dual role in MS, not just enhancing both immune response and myelin repair inhibition, but also they can protect and limit CNS inflammation, supporting oligodendrocyte and axonal regeneration. Our lab has recently developed a random retrospective clonal analysis approach, called StarTrack, that allows us to track the progeny of single glial cell progenitor. We determined that the positional and morphological identities of cortical astrocytes are specified during brain development, concluding that distinct astrocyte progenitors might give rise to diverse astrocyte subtypes and expand regional heterogeneity. With this tool we demonstrated that development has a fundamental role in determining astrocytic heterogeneity in mouse olfactory system and cerebral cortex. Furthermore, we described the different response of astrocyte clones to cortical injury. This revealed that some astrocytes clones exhibited either a strong morphological alteration or a high proliferative response to the injury. Other clones, located at similar distances to the lesion, were apparently unresponsive.

In this work we analysed the differential response of astrocyte clones to demyelinating lesions in the EAE mouse model using the StarTrack technique, to unravel a possible glial clonal response in the vicinity of the lesions. Positional identity and phenotypic characteristics of astrocyte clones in the injured brain, represents a new line to unravelling some relevant aspects of pathogenesis of MS.

ID: P46

Title

Blockade of CALHM1 channels attenuates ischemic brain damage

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Overactivation of purinergic receptors during cerebral ischemia results in a massive release of neurotransmitters, including ATP, to the extracellular space which leads to cell death. Some hypothetical pathways of ATP release are large ion channels, such as CALHM1, a membrane ion channel that can allow the flow of large molecules such as ATP. Therefore, and due to its location in brain cells, it is important to ascertain whether these channels are relevant therapeutic targets. Here, we analyzed their contribution to postanoxic depolarization after an ischemia model in cultured neurons and in brain slices. We observed that the blockade or silencing of CALHM1 channel, delayed the onset of postanoxic currents. These results were confirmed in acute cortical slices from CALHM1 knockout mice. Subsequently, we used transient middle cerebral artery occlusion (MCAO) to monitor the role of CALHM1 in this pathology, we found that ruthenium red, a blocker of CALHM1, substantially attenuated the motor symptoms and reduced the infarct volume to ~ 50% of that in vehicle-treated. These results show that CALHM1 channels mediate postanoxic depolarization in neurons and brain damage after ischemia. Therefore, targeting the CALHM1 may have a high therapeutic potential for treating brain damage after ischemia.

ID: P47

Title

Ultrastructural pattern of adaptative CB1 receptor changes in TRPV1-KO mouse

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We have recently demonstrated that the transient receptor potential vallinoid type 1 (TRPV1) localizes at both excitatory and inhibitory synapses in the dentate molecular layer of the hippocampus (Puente et al., 2014). It was shown an up-regulation of the receptor after epileptic seizures contributing to the altered activity of the brain circuitries taking place in this disease. On the other hand, within the endocannabinoid system, the endocannabinoid type 1 receptor (CB1) regulates glutamate release to control the excitatory synaptic transmission playing a protective role in the modulation of neuronal circuits affected by the epileptic seizures. We have observed that seizures assessed by behavioural score were milder in TRPV1-KO than in WT mice subjected to the kainate-induced status epilepticus model. Furthermore, the optical density analysis revealed a significant increase of CB1 immunorectivity in the dentate gyrus of TRPV1-KO versus WT mice, suggesting a compensation mechanism in which the lack of TRPV1 triggers some adaptive changes of the CB1 expression in the dentate gyrus that might be beneficial to control the epileptic seizures.

The aim of this present study was to investigate the ultrastructural CB1 receptor pattern in the TRPV1-KO mouse hippocampus. For this purpose, we used specific CB1 antibodies combined with a very sensitive pre-embedding immunogold method for electron microscopy. In WT, 36% of the synaptic terminals were CB1 immunopositive in the innermost zone of the dentate molecular layer (ML). In TRPV1-KO however, about 54% of the synaptic terminals were CB1 immunopositive in the same dentate zone. Moreover, 47.51% and 30.43% of the excitatory synaptic terminals in TRPV1-KO and WT, respectively, were CB1 immunopositive in the innermost zone of the ML from TRPV1-KO mice.

The CB1 distribution pattern in the TRPV1-KO mouse shows an increase of CB1 immunopositive excitatory synaptic terminals in the innermost dentate ML, the termination zone of the mossy cell axon buttons. This CB1 increase may participate in the mechanisms behind the milder seizures observed in the TRPV1-KO mouse. Current experiments are investigating this hypothesis.

ID: P48

Title

Monoacylglycerol lipase expression in Alzheimer's disease patients and in a rat model of cholinergic basal forebrain lesion

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Alzheimer's disease (AD) is the most common type of dementia. AD is characterized by a selective cholinergic damage, responsible for most of the clinical symptoms. The endocannabinoid system is regulating the cholinergic neurotransmission and CB_1 cannabinoid receptors are regulated in AD. The model of basal forebrain cholinergic lesion induced by 192IgG-saporin (SAP) is used to mimic the cholinergic basal forebrain vulnerability in AD. Therefore, in the present study we analyze the expression of the monoacylglycerol lipase (MAGL) enzyme, as the main responsible of the metabolism of the endocannabinoid 2-araquidonilglicerol (2-AG) in the frontal cortex (Brodmann area 8) of AD patients. In addition, the cellular localization of MAGL was studied by immunohistochemistry in the SAP rat model.

The results do not show any statistically significant differences in the expression of MAGL between control and AD patients (Control: 116 ± 17 vs. AD: 99 ± 24, $p > 0.05$. Data expressed in optical densities normalized with the actin protein expression). However, following the Consortium to Establish a Registry for Alzheimer's disease (CERAD) criteria, allowed us to classify the patients by likelihood of AD as "low" with CERAD sparse and Braak stage I-II, "intermediate" with CERAD moderate and Braak III-IV, and "high" with CERAD frequent and Braak V–VI. The results showed a slight decrease of MAGL during early stages of AD and a slight increase to the control levels in the late stages (Control: 116 ± 17; AD I-II: 100 ± 26; AD III-IV: 90 ± 19; AD V-VI: 104 ± 27, $p > 0.05$). In the animal model, an increase of MAGL expression was found in the damaged frontal cortex, at the level of the lesion area.

In conclusion, the absence of regulation in the expression of MAGL with the progress of the AD indicates the preservation of this component of the endocannabinoid system that seems to be activated in the lesion model. Further studies analyzing the MAGL activity in AD will indicate the usefulness of the MAGL as a therapeutic target for the AD treatment.

ID: P49

Title

RNAseq study in smooth muscle cells isolated from carotid atherosclerotic plaque for identification of markers involved in plaque instability

Authors

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There are two types of carotid artery disease that could provoke stroke. One is unstable form of atheroma plaque and likely to produce symptoms such us transient ischemic attack, amaurosis fugax or stroke because of embolization or carotid occlusion, and second is stable plaque with low risk of producing symptoms. Smooth muscle cells are one of the main cell types in human atherosclerotic plaque and they can migrate and proliferate occluding the artery and/or can secrete molecules, which degrade plaque and provoke plaque rupture, leading to plaque instability. Because it is not known when and why a stable plaque becomes unstable and breaks, transcriptomic analysis of vascular smooth muscle cells based on Next Generation Sequencing methods are essential to give us clues to understand the mechanism/s by which an atheroma plaque becomes prone to rupture causing cerebrovascular events. In this study have participated 14 patients with carotid atherosclerotic disease, 7 patients were classified as symptomatic and 7 as asymptomatic. Atherosclerotic plaques were obtained by carotid endarterectomy by surgery in Basurto University Hospital. Smooth muscle cells were isolated from the collected plaques following total RNA extraction and purification. Quality control of RNA was performed with the Bioanalyzer equipment (RIN ›8). Complete transcriptome sequencing was done using the platform Illumina HiSeq 2000 with TruSeq RNA library preparation Kit, which drives from sample/library preparation to sequencing and data analysis. Bioinformatic analysis was based on gene and isoform identification, transcript quantification, gene annotation, functional enrichment and differential gene expression analysis between asymptomatic and symptomatic patients using specific software packages, such us FastQC quality control tool, TopHat sequence analysis package, Cufflinks software and The Program to Assemble Spliced Alignment software (PASA). Complete transcriptomic analysis has shown differential gene expression patterns between smooth muscle cells from asymptomatic and symptomatic patients, revealing novel data which can help us to understand the mechanism/s involved in atherosclerotic plaque development and disruption. The acquired knowledge could be applied in the near future for the development of new therapeutic targets to prevent plaque rupture.

ID: P50

Title

Retinal transcriptome profiling analysis in rd10 mouse model of retinitis pigmentosa

Authors

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Background: Retinitis pigmentosa (RP) is a heterogeneous collection of inherited forms of retinal degeneration that is currently untreatable and leads to vision impairment. It is characterized by a progressive death of retinal photoreceptor cells. The primary defect underlying RP affects the function of photoreceptors where molecular and cellular mechanisms trigger the apoptotic degeneration of rods and in many cases cones. However detailed mechanisms involved in photoreceptor death remains poorly characterized.
Purpose: To study the gene expression profiling in the degenerating retina in order to advance in the understanding of the biological pathways involved in this disease.
Methods: We used the retinal degeneration 10 (rd10) mice, a model of autosomal recessive retinitis pigmentosa (RP). Rod apoptosis is due to a spontaneous point mutation in the rod-specific phosphodiesterase (PDE6) gene. Retinal degeneration starts around postnatal day 16 (P16) due to calcium overload and calpain activation. Retinas from rd10 mouse and wild-type (WT) with the same genetic background were used at different time points: P15 and P17, just before and after the photoreceptor degeneration onset, respectively. Altered mRNA expression of degenerating retinas was detected by Genechip Mouse Transcriptome Array of Affymetrix, and results were validated by Real-time quantitative PCR.
Results: We have identified a group of approximately 55 genes significantly regulated with greater than +/- 1.5 fold-change, 30 of them were up-regulated and 25 down-regulated. DAVID and KEGG pathways analysis revealed that these genes are involved in different pathways that could play an important role in the retinal degeneration process such as apoptosis, regulation of calcium, visual perception, inflammation and immune response.
Conclusions: This study provides information about alteration of gene expression during the retinal degeneration and hints on molecular pathways involved in this process. This could help to advance in the knowledge of mechanisms involved in retinitis pigmentosa and might help discover new therapeutic targets.

ID: P51

Title

Calcium dysregulation in human in vitro models of LGMD2A muscular dystrophy

Authors

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Limb girdle muscular dystrophy type 2A (LGMD2A) is one of the most frequent forms of recessive muscular dystrophies and it is characterized by primary wasting of scapular and pelvic muscles. LGMD2A is caused by deficiency in Calpain 3, a non-lysosomal Ca^{2+}-dependent cysteine protease that is necessary for normal muscle function. Previous studies suggest that dysregulation of Ca^{2+} homeostasis is involved in the pathogenic mechanisms of LGMD2A. The aim of this work is to analyze in more detail the effect of Calpain 3 deficiency on Ca^{2+} homeostasis, and on the proteins involved in this process using human cellular models of LGMD2A. We found abnormally increased cytosolic resting Ca^{2+} levels in Calpain 3-deficient myotubes when compared to control ones. We also observed reduced expression and function of sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) proteins, which represent the major mechanism of Ca^{2+} reuptake from the cytoplasm to the sarcoplasmic reticulum. Additionally, evidence of sarcoplasmic reticulum stress was detected in Calpain 3-deficient myotubes as shown by gene expression analysis. In conclusion, our findings provide new evidence of the impact of Calpain 3 deficiency on LGMD2A physiopathology through destabilization of SERCA proteins, compromising Ca^{2+} homeostasis.

ID: P52

Title

Effect of a novel modulator of ryanodine receptors in mouse and human models of Duchenne muscular dystrophy

Authors

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In the mdx mouse model of Duchenne muscular dystrophy (DMD), the sarcoplasmic reticulum ryanodine receptor (RyR1) is abnormally nitrosylated and this leads to calstabin1 depletion from the protein complex and subsequent calcium leak through the channel. RyR modulators enhance RyR1-Calstabin1 binding preventing calcium leak, reducing biochemical and histological evidence of muscle damage and improving muscle function. In this work we have analysed the effect of A6, a novel RyR1 calcium release channel stabilizer, in the mdx mouse model and in human myotubes. Treatment of 1 month-old mdx mice with A6 during 5 weeks reduced histological evidence of muscle damage, improved muscle function and reduced basal cytosolic calcium concentration and serum CK levels. Additionally, we have studied the effect of A6 in primary immortalized human control and dystrophic myotubes in vitro. Dystrophic myotubes show lower resistance to hypo-osmotic shock, which increases membrane tension due to cell swelling. We found that pre-treatment with A6 prevented myotube bursting under hypo-osmotic shock. In conclusion, our results show that A6 is effective in improving dystrophic phenotype in cellular and animal models of Duchenne muscular dystrophy. In addition, they consolidate RyR1-calstabin complex as a useful therapeutic target for drug development against muscular dystrophies.

ID: P53

Title

Ketamine promotes electrophysiological and biochemical alterations in the glutamatergic transmission in the dorsal raphe

Authors

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Evidence demonstrates that administration of glutamatergic antagonists, and particularly ketamine, exerts rapid antidepressant effects. The tight interactions between glutamatergic and monoaminergic systems and the shared actions between the classical and the novel antidepressants have been well-described. This gives rise to the possibility that ketamine could exert its antidepressant effects by acting on monoaminergic areas, such as the dorsal raphe (DR). Here, we used electrophysiological and biochemical approaches to determine whether ketamine is altering the glutamatergic transmission and the mTOR pathway in the mouse DR, besides promoting rapid antidepressant-like behaviors. In the tail suspension test we replicated the preclinical findings showing that ketamine (30 mg/kg, i.p.) has acute (30 min) and sustained (24h) antidepressant-like effects in mice. Whole-cell patch-clamp recordings revealed that bath perfusion of ketamine (50 µM, 10 min) increased the frequency of sEPSCs in DR neurons. In addition, the AMPA receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX, 10 µM) blocked this effect, and the NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5, 50 µM) failed to increase the frequency of sEPSC, indicating that AMPA but not NMDA receptors are involved in the observed ketamine-induced glutamate release. Furthermore, pre-incubation of the slices with the mTOR inhibitor PP242 (2.5 µM, 40 min) attenuated the effect of ketamine on sEPSC. However, when ketamine was administered 24 hours prior to the recordings, it produced no effects either in sEPSCs or eEPSCs, pointing out a functional difference between the acute and sustained effects of this drug in the DR. Finally, western blot experiments revealed that ketamine (30 mg/kg, i.p.) did not induce acute (30 min) phosphorylation of mTOR in the DR, whereas 24 hours after the injection ketamine increased the levels of the phosphorylated-active form of mTOR. Collectively, these results identify functional actions of ketamine on the glutamatergic transmission in the DR, and also reveal that the AMPA receptor-mediated electrophysiological "rapid onset" effects, which may trigger other functional/cellular effects, are not maintained in the DR 24 hours after the administration of ketamine.

ID: P54

Title

Is the blockade of the 2-AG hydrolase ABHD6 a novel therapeutic strategy in demyelination?

Authors

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Activation of cannabinoid CB_1/CB_2 receptors is considered a useful therapeutic strategy for the treatment of demyelinating inflammatory disorders such as multiple sclerosis (MS) based on the beneficial effects of cannabinoid agonists in experimental models of the disease. Nevertheless, the clinical utility of exogenous cannabinoids is limited by the appearance of unwanted responses related to memory and learning impairment. Recent evidence indicates that blocking the enzymatic metabolism of the main endocannabinoid 2-arachidonoylglycerol (2-AG) may engage therapeutic benefits against neuroinflammation whilst limiting adverse effects. We have shown that pharmacological inhibition of the major 2-AG degrading enzyme monoacylglycerol lipase (MAGL) elicits myelin protective and anti-inflammatory effects *in vivo* and protects oligodendrocytes *in vitro* from excitoxicity (Bernal-Chico et al., 2015). Nevertheless, chronic administration of MAGL inhibitors induces desensitization of brain CB_1 receptors, an undesirable effect that may attenuate the clinical efficacy of these compounds. Here we have evaluated the potential of targeting / hydrolase domain containing 6 (ABHD6), responsible of approximately 4% of 2-AG hydrolysis as novel strategy to treat demyelination. Mice treated with the ABHD6 selective inhibitor KT182 showed less severe symptomatology during the acute phase of experimental autoimmune encephalomyelitis (EAE). Nevertheless, the extent of neurological disability and inflammation at the end of disease course was similar between vehicle and KT182 treated mice. On the other hand, administration of KT182 significantly attenuated myelin loss and inflammation in the cuprizone model of primary demyelination. Experiments in cultured oligodendrocytes and neurons suggest that protection from excitotoxicity is not a relevant mechanism underlying the beneficial efficacy of ABHD6 inhibitors *in vivo*. Finally, chronic administration of KT182 did not elicit widespread downregulation or desensitization of brain CB_1 receptors. Collectively, these results provide evidence supporting the potential use of ABHD6 inhibitors for therapeutic intervention in white matter injury.

ID: P55

Title

Regenerating axons of RGCs regrow and the intracellular cAMP increase despite the presence of Nogo-A and the upregulation of the NgR in the Lizard *Gallotia galloti*

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The myelin-associated protein Nogo-A contributes to the failure of axon regeneration in the mammalian CNS. Inhibition of axon growth by Nogo-A is mediated by the Nogo-66 receptor (NgR) which activates RhoA GTPase and reduces cAMP/pkA activity, preventing the dynamic remodeling of the growth cone cytoskeleton required for axon growth. However, the lizard *G. galloti*, are capable of spontaneous CNS axon regeneration which raises the question whether the expression pattern on Nogo-A and NgR in the lizard differs to that in mammals. Thus, we analysed Nogo-A, NgR immunostainings during the ontogeny and after unilateral optic nerve transection, between 2 months (when RGC axons begin to cross the lesion site) and 6-12 months (when the regrowing axons reach the optic tectum). Moreover, cAMP labeling in axotomized lizard and rat RGCs at the height of their regenerative response (3 months and 10 days post optic nerve lesion, respectively) were compared.
In the developing lizard visual pathway Nogo-A was detected in RGC somata and processes along the visual pathway. In the adult, a shift of Nogo-A expression to oligodendrocyte cell bodies and myelin was evident, shown by colocalization with the CNS myelin protein, PLP. During RGCs regeneration Nogo-A immunostaining persisted and NgR was up-regulated in regenerating RGC axons. Lizard RGCs from the lesioned side showed a significant increase of cAMP immunofluorescence signal compared to the intact side, or to rat RGCs after optic nerve lesion. We conclude that 1) Nogo-A and NgR are expressed in a mammalian-like pattern in the developing and adult lizard visual system. 2) The presence of Nogo-A and upregulation of NgR do not inhibit lizard RGC axon regeneration. 3) lesion-induced increase of cAMP levels, and hence increased pkA activation, are likely to facilitate axon regeneration of lizard RGCs.

ID: P56

Title

Models of cognitive impairment for the study of Alzheimer's disease

Authors

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The selective vulnerability of the basal forebrain cholinergic neurons (BFCN) pathway is responsible for most alterations in learning and memory processes that are characteristic of the Alzheimer's disease (AD). The aim of the present study was to validate the lesion induced by immunotoxin 192 IgG-saporin in rat, to investigate the dementia associated to diseases such as AD, using behavioural memory tests. Barnes Maze (BM) is used to evaluate spatial memory, and passive avoidance test (PA) to evaluate fear conditional memory.

The pharmacological model of antagonism of MR (SCOP) was compared with the model of BFCN depletion. Furthermore, the learning and memory processes were evaluated throughout 7 months without reinforcement of the training. The neurochemical status of BFCN was analysed by immunohistochemistry studies using specific markers for cholinergic enzymes (ChAT, AChE) and microglia (IBA-1).

The memory impairment was similar in both cholinergic depletion models. The time spent in the target quadrant was measured using the BM test (SCOP: 45.6 ± 3 sec vs SAP: 50 ± 5 sec, $p>0.05$) and the PA test (SCOP: 0 % positive responses vs SAP: 0.25 % positive responses, $p>0.05$). The time necessary to forget the BM trainings was up to 6 months in control rats (Initial: 90.27 ± 8 sec vs 6 months: 51.32 ± 5 sec, $^*p<0.05$), and 7 months to forget the aversive stimulus in PA (Initial: 0.75 % positive responses vs 7 month: 0.27 % positive responses, $^*p<0.05$). BFCN depletion (80%) was found in lesion model accompanied by a high proliferation of activated microglia.

Both BFCN lesion and MR antagonism models show a similar memory and/or learning impairment and are useful as animal experimental models for the study of AD.

ID: P57

Title

Amyloid β peptide activates transiently glycogen phosphorylase via integrin β in astrocytes

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Although Alzheimer's disease (AD) is considered an illness that mainly affects neurons, recent studies have found molecular and functional abnormalities in astrocytes at brain from both animal models and brain of patients suffering from this pathology. These cells are responsible for maintaining neurotransmitter levels, the extracellular ion balance and are also responsible for metabolic and nutrient contribution to the neurons. In addition, astrocytes actively participate in synaptic transmission and plasticity of several brain areas. In a recent study from our laboratory, we have described the effects of amyloid beta (A) peptide in the biology of astrocytes. A peptide is a key molecule involved in the development of AD. However, the mechanism by which A peptide activates the neurodegenerative program is still poorly understood. Here, we have examined the effects produced by A peptide on the glycogen metabolism into the cultured primary astrocytes. After 8 DIVs, we have observed that A peptide produces a dramatic and transient reduction of intracellular glycogen stores, and this decrease in intracellular glycogen levels is previously associated to a robust activation of the glycogen phosphorylase (PYG), an enzyme responsible for the glucose-1-phosphate release from the glycogen stores. Furthermore, pharmacological inhibition of integrin prevents PYG activation and glycogen degradation induced by A peptide. In addition, A peptide-treated astrocytes reduce significantly the maximal respiratory rate and the respiratory capacity while pharmacological inhibition both integrin 1 and PYG rescue either the maximal respiratory rate or the respiratory capacity. Finally, we have deciphered where A peptide binds to integrin 1 in the extracellular region. This binding site is located at the first 20 a.a. at integrin β 1 N-terminal. Our results provide insights into an unsuspected connection between A peptide, Integrin 1 N-terminal and glycogen metabolism in the same signal-transduction pathway and points out integrin 1 and glycogen phosphorylase as potential targets in order to block the toxic effects of A peptide in astrocytes.

ID: P58

Title

Regulation of NMDA receptor activity by amyloid beta oligomers in neurons

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Alzheimer disease is the most frequent cause of progressive cognitive decline in the aged population. Several studies have demonstrated that amyloid beta peptides (Aβ) have a causal role in its pathogenesis promoting disturbances in glutamatergic neurotransmission. NMDA receptors (NMDARs) are glutamate-gated ion channels able to control synaptic plasticity and homeostasis by regulating calcium influx into the synapse. Using a combination of pharmacological, immunocytochemical and calcium imaging approaches, we have investigated the regulation of NMDARs by Aβ oligomers. First, we analyzed the effect of Aβ on NMDA receptor expression on neuronal cell surface. Cultured cortical neurons were treated with Aβ oligomers (1uM, 30min and 24h) and cell surface receptors were quantified by biotinylation assays. Here, we identified that incubation of cells with Aβ for 30 min increased the surface expression of the NMDA receptor subunit proteins NR2B compared to untreated cells, however the chronic treatment for 24h reduced NR2B surface expression on neurons; no change was observed in the total amount of NR2B. Next, we used fluorescent calcium imaging to confirm that Aβ could modulate the density of NMDA receptors on neuronal cell surfaces. Accordingly, we observed that NMDA-induced Ca^{2+} influx was increased by the short pretreatment with Aβ oligomers, but chronic preexposure reduced the Ca^{2+} influx into neurons. Modulating the density of NMDA receptors at the cell surface is expected to modulate NMDA receptor–mediated excitotoxicity as well. Unexpectedly, pretreatment of Aβ for 30 min provided neuroprotection against NMDA-induced excitotoxicity, whereas the long-term exposure of 24 h exacerbated the NMDA-induced neuronal cell death. Overall, these results suggest that Aβ oligomers may differentially regulate the NMDA receptor expression and function of cortical primary cultured neurons.

ID: P59

Title

Dexamethasone protects cultured RGCs against hyperglycemia

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Diabetic retinopathy (DR) is a common complication of diabetes in which hyperglycemia plays a major role. Previous studies have shown that diabetic patients have abnormal retinal glial cells (Müller cells) and retinal ganglion cells (RGCs), suggesting that DR involves neural degeneration. Dexamethasone is a glucocorticoid used to treat many inflammatory and autoimmune conditions, and it is commonly used in several eye diseases. Our goal was to study the effect of Dexamethasone on the survival of RGCs and Müller cells *in vitro* under conditions of high glucose. Primary cell cultures have been widely used and they have proven useful to study events that occur in the retina. Primary cell cultures of RGCs, and co-cultures of RGCs and Müller cells from rat retinas were grown on poly-L-Lysine and laminin coated coverslips. The cells were cultured at 37ºC and in 5% CO2 for 6 days under different conditions: (1) Control; (2) 1 µM Dexamethasone; (3) 30mM Glucose (hyperglycemia); and (4) 30mM Glucose + 1 µM Dexamethasone. At the end of this period, the RGCs and Müller cells were quantified using antibodies against Beta III tubulin or Vimentin to label RGCs or Müller cells, respectively.

RGC death was evident under high glucose conditions both when RGCs were growing alone (46%) and when they were co-cultured with glia (67%). In terms of Müller cells, their survival in high glucose conditions decreased in co-culture (RGCs-Müller cells) with a loss of up to 80% of the cells found in control conditions. Significantly, the presence of dexamethasone RGC cell death was reduce to 20% in high glucose conditions, both in co-culture and when cultured alone. However, dexamethasone had no significant effect on the survival of Müller cells in high glucose media.

This study shows that dexamethasone can protect RGCs against apoptosis in high glucose conditions *in vitro,* although its possible effect on Müller cells should be investigated further.

ID: P60

Title

Nanodelivery of cerebrolysin and rearing in enriched environment induce neuroprotective effects in an early stage of an experimental rat model of Parkinson's disease

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Rearing in enriched environment (EE) is beneficial for recuperation in animal models of Parkinson's disease (PD). Administration of TiO2-nanowired cerebrolysin, could represent an additional interesting strategy to protect or repair the nigrostriatal system. Thus, the aim of this study was to test morphofunctional and biochemical changes in a preclinical stage of PD after housing rats in an EE and after the intraperitoneal nanodelivery of Cerebrolysin in order to elucidate the synergistic efficiency combining both strategies.
31 Sprague Dowley male rats (250-300 gr) receiving three injections of 6-OHDA into striatum with a short time of evolution (2 weeks) were segregated into 4 groups: rats receiving saline, rats received nanowired cerebrolysin, rats housed in an EE and rats housed in an EE and receiving nanowired cerebrolysin (ip 5 mg/kg bw/week).
The prodromic stage was characterized by a mild presence of motor symptoms (1-3 round/min in the amphetamine induced rotational behavioral test). After survival period, rats were sacrificed and brains were removed. Morphological analysis was carried out by immunohistochemistry against tyrosine hydroxylase (TH) in striatum and SN. Quantitative analysis was developed by stereology. Biochemical analysis was performed by western blot to elucidate the molecular pathways involved in this process measuring the protein expression of the P-Akt/Akt as survival marker and caspase-3 as apoptotic marker in striatum and SN. Actin was used as loading control.
Results from motor test showed that CBL group (-42.9±12.14 turns) and CBL+EE group (-8.53±5.58 turns) decreased the number of rotations. However, only CBL group showed statistically significant differences respect to control group (*$p<0.05$). Monitoring by the EE cages revealed that rats belonging to CBL+EE group showed more number of turns in the wheel than EE group (252.63 vs. 112.5). Changes in TH immunopositivity were observed, showing all the groups the most loss of TH expression in striatum in the middle sections comparing to rostral and caudal ones. CBL+EE group promoted neurorestorative effects in the SN, showing statistically the highest density of dopaminergic neurons regarding the control-group (51.55±4.55% *vs.* 28.72±3.17%) (***$p<0.001$). Molecular mechanisms were activated leading to assess molecular changes after the treatments. pAkt/Akt ratio was significant higher and casapase-3 expression was lower in CBL+EE group respect to 6-OHDA group (*$p<0.05$).
Concluding, the combination of Cerebrolysin and EE provided evidence of neuoprotective-neurorestorative mechanisms by which this combined strategy promoted functional and morphological improvement by activation of survival signaling pathways after dopamine depletion in a preclinical rat model of PD.

ID: P61

Title

Gene expression alteration after perinatal hypoxic ischemic injury in rat brainstem and the effect of several antioxidant agents

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The perinatal brainstem is known to be very vulnerable to hypoxic–ischemic events which can lead to deafness, swallowing dysfunction, and defective respiratory control.
The aim of the present work was to evaluate the potential neuroprotective effects of nicotine, melatonin, resveratrol, and docosahexaenoic acid on the expression of a panel of genes in the brainstem following hypoxic–ischemic damage.
Quantitative PCR was used to examine gene expression 3 and 12 h after the damage, and immunohistochemistry was employed to evaluate neurons, astrocytes, and synaptic vesicles 24 h post insult.
We found that the expression of some immediate-early genes, as well as that of inflammatory genes TNF– , COX2, and caspase 3, was upregulated in response to the insult. Twenty-four hours after the damage, the percentage of NeuN and synaptophysin immunolabeled cells was found to be reduced while GFAP expression was upregulated. No differences were observed in ROS gene expression following treatments.
In conclusion, the present work supports the hypothesis that the brainstem is also affected by hypoxic–ischemic injury with alterations in the expression of several key genes and in the activation of some cellular responses. Loss of neuronal and synaptic vesicles ensues and astrocyte activation occurs, leading to the infarct area days after the damage. The studied antioxidant treatments do seem to confer neuroprotection to the brainstemafter the injury, but the underlying mechanisms not well understood. However, the present findings indicate that their neuroprotective effect is not due to their antioxidant properties, at least in the brainstem. It can be that depending on the area the protective mechanism is different.

M. Revuelta, O. Arteaga, A. Alvarez, A. Martinez-Ibargüen, E. Hilario.Characterization of gene expression in the rat brainstem after neonatal hypoxic-ischemic injury and antioxidant treatment. Molecular Neurobiology. (Accepted: 04/01/2016). DOI:10.1007/s12035-016-9724-6.

ID: P62

Title

Rat DRG neurons and lizard retinal explants show divergent responses to the presence of soluble recombinant Nogo-A-Fc and the protein kinase A inhibitor, KT5720, in the culture media

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Regrowth of retinal ganglion cells (RGCs) occurs in fish, amphibians and certain reptiles as G. galloti, but not in mammals. Nogo-A and its receptor show a mammalian-like staining pattern in G. galloti. However, Nogo-A contributes to the failure of CNS axon regeneration in mammals but not in G. galloti. Inhibition of axon growth by Nogo-A is mediated by the Nogo-66 receptor (NgR). Stimulation of the NgR complex activates RhoA GTPase and reduces cAMP levels and thus pkA activity, preventing the dynamic remodeling of the growth cone cytoskeleton required for axon growth or even causing growth cone collapse. Our purpose is to compare Lizard CNS and mammalian PNS as models of successful neurite outgrowth. Thus, for comparative functional studies, neonate rat DRG and lizard retinal explant cultures were evaluated by *in vitro* time-lapse analysis in presence of soluble Nogo-A-Fc protein. In addition, the role of the cAMP/pkA signaling pathway was studied by adding the pkA inhibitor, KT5720, to the culture media.
We observed that Nogo-A-Fc elicited a robust growth cone collapse and neurite retraction in rat DRG whereas it was a permissive substrate for the regrowth of lizard RGC axons. KT5720 blocked growth of lizard RGC axons on substrates of Nogo-A-Fc,but not laminin. On patterned substrates of Nogo-A-Fc, KT5720 caused restriction of axon growth to areas devoid of Nogo-A-Fc. We conclude that outgrowth-promoting substrates and activation of the cAMP/pkA signaling pathway play a key role in spontaneous lizard retinal axon regeneration in the presence of Nogo-A. Accordingly, cAMP levels were elevated in lizard RGC growth cones. Restriction of axon growth by patterned Nogo-A-Fc substrates suggests that Nogo-A may contribute to axon guidance in the lizard visual system.

ID: P63

Title

Analysis of the Retina of Osteopontin-Deficient Mice

Authors

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Osteopontin (OPN) is a secreted glycosylated phosphoprotein that influences cell survival and apoptosis, inflammation, migration, chemotaxis or homeostasis after injury. As the role of OPN in the retina remains unclear, the goal of this study was to gain insight into this by investigating the effect of the absence of OPN in the retina in an OPN knock-out mouse model. The study focused on two cell types: 1) retinal ganglion cells (RGCs), the neurons that relay visual signals to the brain; and 2) macroglia, the astrocytes and Müller cells that are involved in structural support, metabolism and the maintenance of homeostasis within the retina.
These two cell types were analyzed in the retinas of 3 month old and of 20 month old mice, which were used to evaluate the effect of ageing. RGCs were quantified using RBPMS, a specific marker of these cells, and the density of astrocytes labelled with GFAP was also assessed. In addition, the morphology of Müller cells was examined in retinal sections using glutamine synthetase as a specific marker.
The deficiency in OPN induces a 25.09% reduction in RGC density relative to the control mice at 3 months of age, which increased to 60.37% at 20 months of age. A reduction of astrocyte density of 51.01% was also detected in 3 month old mice and of 57.84% at 20 month old mice, although no differences in Müller cell structure was observed.
This study demonstrates that OPN influences astrocytes and RGCs. It seems that at early stages of retinal development, astrocytes are more susceptible to a deficit in OPN, and that its effects on neurons may be secondary to the reduction in astrocyte density. However, Müller glia cells seem not to be affected by the lack of OPN. Thus, OPN could be a candidate molecule to develop treatments in neurodegenerative disease, and astrocytes may also represent a target of interest.

ID: P64

Title

Analysis of the chr5q11 autoimmune risk locus points to a role for CD4+ T lymphocyte-expressed ANKRD55 in multiple sclerosis and neuroinflammation

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An intronic variant in *ANKRD55*, rs6859219, is a genetic risk factor for multiple sclerosis (MS) but the biological reasons underlying this association are unknown. This chr5q11 region contains various plausible risk genes for MS including *IL6ST, IL31RA, DDX4, SLC38A9* and *ANKRD55*. We characterized the expression of these genes in human PBMCs and cell lines, and related their expression levels with genotype. For ANKRD55, three transcript variants (Ensembl isoforms 001, 005 and 007) could be detected in PBMCs and $CD4^+$ T cells, but were practically absent in $CD8^+$, $CD14^+$, $CD19^+$ and $CD56^+$ cells. Rs6859219 was significantly associated with ANKRD55 transcript levels in both PBMCs and $CD4^+$ T cells (*cis*-eQTL). The processed noncoding transcript 007 was the most highly expressed variant in $CD4^+$ T cells, followed by 001 and 005, respectively, but was not detected in Jurkat, U937 and SH-SY5Y cell lines. Homozygotes for the risk allele produced over 4 times more transcript copies than those for the protective allele. ANKRD55 protein isoforms 005 and 001 were predominantly located in the nucleus of $CD4^+$ T cells, Jurkat and U937 cells. ANKRD55 was induced in $CD14^+$-monocyte-derived dendritic cells and regulated by tolerogenic/inflammatory stimuli. ANKRD55 was produced by primary cultures of murine hippocampal neurons and microglia and by the murine microglial cell line BV2, and was induced by inflammatory stimuli. ANKRD55 protein was increased in the murine mouse model of experimental autoimmune encephalomyelitis (EAE). Flow cytometric analysis of CNS-infiltrating mononuclear cells showed that $CD4^+$ T cells and monocytes expressed ANKRD55 in EAE mice with the higher fluorescence intensity found in $CD4^+$ cells. A low percentage of microglia also expressed ANKRD55. Together, these data support an important role for ANKRD55 in MS and neuroinflammation.

ID: P65

Title

The effect of Buspirone on the activity of the Basal Ganglia output nuclei in rats

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The basal ganglia (BG) nuclei form an integrative subcortical network directly related with the motor performance. In Parkinson's disease (PD), the loss of dopamine induces changes in the oscillatory activity and synchronization of the cortical-BG-thalamus loop leading to the motor symptoms that characterize the disease. Most BG nuclei receive important serotonergic innervation and in the last years several serotonin-based therapies, such 5-HT_{1A} agonists, have shown promising results in PD.

The aim of the present study was to investigate the effect induced by a 5-HT_{1A} receptor partial agonist, buspirone, on the neuronal activity of the BG output nuclei, the *substantia nigra pars reticula* (SNpr) and *entopeduncular nucleus* (EP). To that end, electrophysiological recordings were carried out in urethane-anesthetized rats. Neuron basal parameters, oscillatory activity and synchrony were studied.

Buspirone (0.0625-1 mg/kg, i.v.) failed to modify the basal properties, firing frequency or pattern, in SNpr neurons. The low-frequency oscillatory activity in the nucleus or in the cortex remained unaltered after the drug application. In the same line, synchronization in the SNpr and the cortex was unchanged. In contrast, preliminary results suggest that buspirone modulates EP neuron activity. Buspirone administration dose-dependently decreased the firing rate while firing pattern became more irregular. In the synchronization and oscillatory activity no significant modifications were observed.

The present results suggest that this serotonergic drug unevenly modulates the BG output nuclei in control animals, uniquely affecting the EP activity. Taking into account that the serotonergic system is especially relevant in PD, when the BG functionality is highly disrupted, it would be necessary to explore the effect of this drug in dopamine-depleted conditions.

ID: P66

Title

Moderate recovery of visual functions after unilateral optic nerve transection in the lizard Gallotia galloti

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Significant regrowth of retinal ganglion cell axons occurs after optic nerve transection through a permissive glial scar in Gallotia galloti. Although several of the cellular and molecular events underlying this process have been studied by our group, the functionality of the system has not been tested until now. The pupillary light reflex, accommodation and head orienting have been also used in other reptiles to test visual function (Dunlop et al., 2004). We examined 18 lizards at 3, 6, 9 and 12 months after transection. Our results revealed a tendency of eyelid closing within the first months after operation. Interestingly, by 6 months we detected a significant recovery of pupillary light reflex in two thirds of specimens including a robust response in 17 of them. However, visually guided behaviour recovery was observed only in 2 specimens, yet when presenting a prey (mealworm) in the right, affected eye, most lizards (89%) did not constrict the pupil to focus nor did they follow it as it moved, a behaviour which was detected in the unlesioned side. We conclude that a partial recovery of the visual pathway functionality takes place spontaneously in adult G. galloti, which could be enhanced by training or pharmacologically.

ID: P67

Title

Microglia exacerbates synaptic dysfunction induced by amyloid peptide in Alzheimer´s disease

Authors

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Alzheimer's disease (AD) is a degenerative disorder and the most common cause of progressive cognitive decline in the aged population. The progressive and anatomically selective accumulation of - amyloid (A) peptide and the synaptic dysfunction are the main hallmarks of AD neuropathology. Synaptic dysfunction is increasingly viewed as an early manifestation of AD, but the cellular mechanism by which A may affect synapses remains unclear. Some studies have reported that the cultured APP mutant neurons have selective alterations in pre- and post-synaptic compartments compared to wild-type neurons. Loss of synapses, as seen by loss of synaptophysin immunoreactive pre-synaptic terminals, occurs early in AD and is considered the best pathological correlate of cognitive decline.

Microglia are innate immune cells of the brain that mediate responses to pathogens and injury. Recent discoveries pointing to the key role of microglia on synapses in healthy brains opens new research routes in neurodegeration research field. In AD, microglia is not able to remove A plaques, which could contribute to AD pathology. A recent publication also shows that microglia mediates early synapse loss in AD models. Therefore, the role of microglia at synapses in AD is becoming more and more relevant and further study is needed.

To study the role of microglia in the synapsis in the presence and absence of A we have performed immunofluorescence to measure the levels of synaptophysin in neuronal and microglia-neuron primary cocultures with or without A containing condition media (CM) from Swedish N2A cell line. First, we found that the presence of A in neuronal primary culture reduces 46% the synaptophysin labeling compared to controls. We also found that after adding primary microglía to the primary neuron culture, the synaptophysin labeling increases 60% comparing with the control. However, adding A containing CM to primary cocultures of microglia and neurons, the synaptophysin labeling decreases 69% comparing with the control.

Overall, these results indicate that the presence of microglia in the coculture favors the formation of synapses in normal conditions. Nevertheless, the addition of A to microglia-neuronal cocultures abolishes the synaptogenic potential of microglia and leads to a further synaptic dysfunction. These results indicate that microglia could exacerbate the synaptic dysfunction induced by A in AD.

Cellular and molecular Neuroscience - Neurogenesis

ID: P68

Title

Extracellular adenosine modulates postnatal neurogenesis

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Toxicity of extracellular purines is among the factors inhibiting adult neurogenesis during neurodegenerative diseases. After neurodegeneration extracellular ATP and adenosine are released at high concentrations and alter the homeostasis and survival of glia and neurons. In this study we examined the effects of adenosine in modulating the cellular fate of post-natal neural stem cells from the rat subventricular zone in an *in vitro* model of purinergic cyto-toxicity and *in vivo* after MCAO ischemia. We observed that high concentrations of adenosine (100μM) promote astrogliogenesis at the expense of neurogenesis. Although all adenosine receptors (A1, A2a, A2b and A3) are expressed in these cells, we found that only A1 is involved in the inhibition of neuronal differentiation, as demonstrated by qRT-PCR, Western blot and specific gene silencing. In turn, we found that the mechanisms by which adenosine inhibits neuronal differentiation and sustains astrogliogenesis involves the release of IL10 and further activation of the Bmp2/SMAD pathway. *In vitro* data were confirmed in *in vivo* experiments using intracerebroventricular infusion of the A1 agonist CPA that showed a drastic reduction of neurogenesis and a parallel increase of astrogliogenesis in the olfactory bulb of adult rats.
In contrast, blocking the A1r with the specific antagonist DPCPX during focal ischemia in adult mice, we observed a rescue of neurogenesis in the infarcted area accompanied by a reduction of newly generated astrocytes.
These data further supports the idea that purinergic signaling contributes to the regulation of adult neurogenesis, especially in pathological conditions when purines are present at high concentrations in the extracellular space.

ID: P69

Title

A study on the neurogenic potential of FTY720/fingolimod

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FTY720 (fingolimod, Gilenya), a structural analog of sphingosine-1-phosphate (S1P), is the first oral drug approved for treatment the relapsing-remitting form of the multiple sclerosis, based on its ability to induced-lymphopenia and immunosuppression via modulation of S1P1 receptors. Recently, direct effects on central nervous system, whereby S1P receptors are widely expressed by neural cells, have been demonstrated. S1P-S1PRs signalling has been correlated with different aspects of neurogenesis, but the role of FTY720 in modulating neurogenic niches is poorly known. We previously reported the effectiveness of FTY720 as a neuroprotective and anti-inflammatory agent in an in vivo model of excitotoxicity induced by intracerebroventricular (i.c.v.) administration of kainic acid (KA). In the present study, we investigated the neurogenic potential of FTY720. For this aim, we used multipotent stem cells (MSCs) derived from the young rat subventricular zone (SVZ) and cultured them in vitro as neurospheres. We stimulated neurospheres at different concentrations of FTY720 and we tested its ability to promote MSCs differentiation using immunofluorescence for markers of mature neurons or oligodendrocytes. We found that FTY720 significantly increased MSCs differentiation both to neurons and oligodendrocytes. We then tested the effect of FTY720 in vivo on the hippocampal neurogenic niche after neuronal injury, caused by i.c.v. injection of KA (0.5µg/2µl) on adult rats. FTY720 (1µg/2µl), alone or together with KA was i.c.v. acutely applied, and also i.p. injected (1 mg/kg) 24 h before and thereafter daily until sacrifice at 7 days after i.c.v. surgery. Rats also received 5-bromo-2-deoxyuridine (BrdU) 100mg/kg, i.p. every two days to label proliferating cells. Immunohistochemical studies were performed in coronal brain slices (40µm thickness) at dorsal hippocampal level. Quantitative analysis of number of BrdU positive cells in the subgranular zone (SGZ) and granular cell layer (GCL) of the dentate gyrus revealed that KA induced a significant increase of proliferation both in ipsilateral and contralateral hippocampus, as reported previously. In addition, we observed that FTY720, alone or in combination with KA, slightly enhanced the number of positive cells, but the increase was not statistically significant. However, colocalization analysis of BrdU and doublecortin (DCX) positive cells showed the ability of FTY720 to rise the percentage of BrdU cells coexpressing DCX marker, both in basal (FTY720 alone) and after injury (FTY720 + KA) conditions, indicating a potential role of FTY720 in promoting new neuroblasts formation in the hippocampus.

We also analyzed FTY720 effects on oligodendrocyte progenitor cells (OPCs) population after KA-induced injury. FTY720 did not modify proliferation and differentiation of OPCs population in vivo, neither in basal (FTY720 alone) nor in neurodegenerative (FTY720 + KA) conditions, as revealed by double immunofluorescence for BrdU and NG2, a marker for OPCs.

We conclude that, at least at this time point, FTY720 promotes neurogenesis after KA-induced brain injury while it does not exert any significant effect on oligodendrogenesis.

ID: P70

Title

Single activation versus activation/quiescence cycling in adult hippocampal neural stem cells

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Neurogenesis (the generation of new neurons), is maintained throughout adulthood in the hippocampus of most mammals including humans, although it decreases markedly with age. The neural stem cells (NSCs) of the hippocampus are mostly quiescent but get eventually activated to give rise to neurons. Then, NSCs differentiate into astrocytes, losing their NSC capabilities and as a consequence the pool of NSCs gets depleted overtime.
Former experiments strongly suggest that most NSCs activate only once and divide several times consecutively before their terminal differentiation into astrocytes. However, highlighting NSCs heterogeneity, it has been hypothesized that NSCs can also switch back and forth between periods of activation and quiescence, which in turn would have an impact into the dynamics of depletion and overall neurogenic output.
To distinguish between these two possibilities we have administrated bromodeoxyuridine (BrdU), a thymidine analog that gets incorporated into DNA during the S phase of the cell cycle and can be later visualizes by immunostaining, for one week in drinking water. Thus we assure continuous and long-term labeling of dividing cells. BrdU retention by cell types was assessed immediately after the one-week period of BrdU administration, or one month later.
Our results show that most of the newly generated cells become neurons or astrocytes, and that the proportion of NSCs that remain labeled with BrdU, representing NSCs that switch between activation and quiescence, is extremely low. These results support the "single-activation" model. The almost absolute lack of colocalization of BrdU with the cell-cycle marker Ki67 in NSCs, one month after the administration of BrdU, further support this model.

ID: P71

Title

Reactive Neural Stem Cells in the Adult Brain

Authors

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In the adult hippocampus of most mammals, including humans, a mostly quiescent population of neural stem cells (NSCs) generates neurons and astrocytes throughout adulthood. New neurons integrate into the hippocampal circuitry and play a role in spatial-related memory and learning, as well as in pattern separation, and responses to fear and stress. We have recently discovered that after epileptic seizures NSCs become reactive (RNSCs), as they get activated massively and transform into reactive astrocytes (RAs) abandoning their neurogenic potential. This seizure-induced impairment of neurogenesis might explain some of the cognitive deficits and comorbidities associated with mesial temporal lobe epilepsy (MTLE). MTLE specifically affects the hippocampus and related structures and is characterized by poor prognosis and being resistant to drug treatment. We have found that the induction of RNSCs in a rodent model of MTLE is a process regulated by the epidermal growth factor receptor (EGFR) signalling pathway. Antagonizing EGFR ameliorates the induction of RNSCs and partially preserves neurogenesis. In addition, we have identified the lysophosphatidic acid receptor 1 (LPAR1) as a potential specific marker for RNSC-derived RAs in the epileptic hippocampus using the LPAR1-eGFP transgenic mice, suggesting differences between this cell type and parenchymal astrocytes-derived RAs. Finally, we have found that with aging, NSCs also acquire a reactive-like phenotype that associates with impaired functionality. Together, our results provide new insight into the plasticity and heterogeneity of hippocampal NSCs and highlight their potential as therapeutic targets in epilepsy and aging.

ID: P72

Title

Role of Phospholipase C 1 in differentiation of Human Postmitotic Neurons Derived from NTERA2/D1 Cells

Authors

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Phospholipase Cβ1 (PLC- β1) is the predominant PLCβ in human cerebral cortex. The analysis of its expression (mRNA and protein) and its functional activity in the cortex of adult and fetal human brain indicate a key role for PLC during CNS ontogeny (1, 2). Moreover, it has been shown using PLC 1 knockout mice (-/-), that PLC 1 is required for the correct development of the barrel cortex, indicating that this isozyme is involved in neuronal differentiation (3). In this regard, the pluripotent NTera2/DI (NT2) cell line, which can be induced to differentiation into postmitotic NT2N neurons, represents a valuable model to study the role of PLC 1 in neuronal differentiation (4). We have recently developed a new differentiation procedure of NT2 cells with a short exposure of cytosine- - D-arabinofuranoside (AraC), improving differentiation efficiency of the traditional retinoic acid treatment. As we have published, NT2 precursor cells treated during 6 days with AraC acquire a post mitotic neuronal phenotype, expressing numerous neuron-specific cytosqueletal proteins, as neurofilament 200kDa (NF200) or - III-tubulin and losing the ability to incorporate BrdU (5,6).
To evaluate PLCβ1 involvement in this process, we first determined the expression level of PLCβ1 protein, by immunofluorescence and western-blot analysis, along the NT2 differentiation process induced by AraC. Also, using PLC 1 siRNAs we analyzed the effect of knocking down of PLC1 gene in NT2 cells, on AraC induced neuronal differentiation. To do that, NT2 cells were transfected, either with non-targeting or PLC 1 gene targeting siRNAs, and treated with AraC for 72 h, a time point at which neuron-specific markers are already expressed.
Results showed that AraC treatment produced differentiation process, was accompanied by a significant increase in PLC 1 protein expression. More importantly, PLC 1 silencing prevented cell acquisition of the typical neuronal polarity, cell growth arrest, and the increase in NF200 and -III-tubulin protein expression levels, induced by AraC treatment.
These results demonstrate that PLC 1 is necessary for AraC-induced neuronal differentiation of NT2 cells.

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ID: P73

Title

Morphological and Functional Heterogeneity of Neural Stem Cells in the Aged Hippocampus

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Neural stem cells (NSCs) located in the subgranular zone (SGZ) of the dentate gyrus (DG) give rise to neurons throughout adulthood in the hippocampus. New neurons integrate into the hippocampal circuitry and participate in spatial-related memory and learning, as well as in pattern separation, and responses to fear and stress. Hippocampal neurogenesis, however, declines sharply with age. As the proportion of activated versus quiescent NSCs remains constant over time, the progressive depletion of NSCs is the main force driving the age-related decline of hippocampal neurogenesis. We have now discovered that in addition to overall NSC depletion, there is an age-associated relative increase of NSCs with reactive-like morphology and impaired function.

Using Nestin-GFP transgenic mice in which NSCs can be readily visualized and analyzed, we have observed that NSCs switch from a typical radial glia-like morphology (–NSCs) to a multibranched reactive-like morphology (Ω-NSCs), with a marked net increase in cell complexity as measured by 3D-Sholl analysis. Furthermore Ω-NSCs increase their number of primary and secondary, processes; increase their volume, and translocate their soma from the SGZ.

Importantly the proportion of Ω-NSCs increases with age, accounting for most of the NSCs population in the aged hippocampus. Furthermore, Ω-NSCs became functional impaired, as they divide with lower probability compared to –NSCs, as measured by bromodeoxyuridine incorporation. After an intrahippocampal injection of kainate in a dose known to recruit an activate NSCs in higher numbers without altering other parameters (inflammation or NSCs phenotype), we observed that Ω-NSCs still maintained their mostly non-proliferative profile and that most of the NSCs activation was accounted for –NSCs.

These results shed light into the process of aging in the hippocampus and provide novel insight on adult NSC heterogeneity.

ID: P74

Title

LPAR1-eGFP Expression Marks the Transition from Developmental to Adult Neural Stem Cells in the Hippocampus

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In the adult mammal brain, the hippocampus is one of the only two structures where new neurons are born and integrate functionally into the existent circuitry. This phenomenon is known as adult hippocampal neurogenesis and it occurs due to the existence of glia-like cells that act as neural stem cells (NSCs). They give rise to neurons that integrate into the granule cell layer (GCL) of the dentate gyrus (DG) and play a role in memory, learning and responses to fear, stress and antidepressants.

The population of hippocampal NSCs decreases markedly with age, with small proportion of self-renewal. As the relative proportion of activated NSCs remains constant over time, the neurogenic output throughout adulthood is mainly determined by the size of the initial pool of NSCs. How and when the NSC population gets established in the SGZ of the DG remains unknown.

Using Nestin-GFP transgenic mice in which NSCs can be readily visualized and analyzed, we observed that NSCs acquire their typical radial morphology around postnatal day 7 (P7) and maintain this phenotype through the later postnatal period and adulthood. Furthermore, we evaluated NSC proliferation (by expression of the cell cycle marker Ki67) at different time points from P4 to P21. The number of cell-cycling NSCs dropped markedly from P7 to P10, when the NSCs showed a low activation rate that remained constant through adulthood.

Moreover, we resorted to Lysophosphatidic Acid Receptor 1-enhanced Green Fluorescent Protein (LPAR1-eGFP) transgenic mice, recently shown to selectively label NSCs in the hippocampus, to study the development of the DG. In contrast to nestin and Nestin-GFP expression, we found that the expression of LPAR1-eGFP starts in the NSC population by P7, suggesting a possible functional role of LPAR1 in the development of the DG that we are currently investigating *in vitro* using agonists and antagonists of LPAR1.

According to our results, we can conclude that P7-P10 is an important period for the establishment of the adult hippocampal NSC population and predict that any alterations taking place during that period will have a profound effect on the adult neurogenic output.

ID: P75

Title

EGFR inhibition reduces aberrant cell proliferation in hyperexcitatory kainate model of epilepsy

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Epidermal growth factor receptor (EGFR) expression is upregulated in activated neural stem and progenitors cells (NSPCs) in the neurogenic niche of the subventricular zone, and its stimulation induces cell proliferation[1,2,3]. In addition EGFR inhibition impairs astrocytic differentiation.[4]. In the present work we demonstrate the presence of EGFR receptor in both *in vitro* and *in vivo* in hippocampal neural stem cells and progenitors. When EGFR signalling is blocked for 48h it reduces around 70% of cell proliferation *in vitro* even in presence of fibroblast growth factor 2 (FGF2).

Kainic acid (KA) infusion into the hippocampus trigger seizures mimicking mesial temporal lobe epilepsy (MTLE) and has been recently shown to induce hyperactivation and exhaustion of hippocampal (NSCs) by differentiation into reactive astrocytes.[5]. In the present work we block EGFR signalling in the KA-MTLE model using Gefitinib, a reversible inhibitor of the EGFR in clinical phase II for cancer treatment. When administered at 10mg/Kg twice a day for consecutive 3 days after induction of MTLE, Gefitinib reduces NSC hyperactivation, as measured by BrdU incorporation and KI67 expression, and ameliorates NSC-derived astrogliosis. At 14 days after the induction of MTLE the inhibition of the EGFR by Gefitinib rescues neurogenesis, increasing the numbers of doublecortin and NeuN-positive cells. However, these newborn immature neurons, mostly mislocalized to the hilar region.

Our results demonstrate that EGFR pathway is involved in the massive activation of hippocampal NSCs induced by seizures, and that the inhibition of the EGFR pathway is a good candidate to preserve neurogenesis and suggest that the preservation of the niche is crucial for the final settlement of newborn neurons.

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Cellular and molecular Neuroscience - Neurotransmitter receptors

ID: P76

Title

Role of $GABA_A$ receptor in regulating the proliferation and differentiation of oligodendrocyte precursor cells

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Myelination is the result of complex neuron-glia interactions that is necessary for the efficient and rapid transmission of vertebrate actions potentials. This process is affected in multiple sclerosis (MS), a chronic and neurodegenerative disease characterized by focal lesions with inflammation, infiltration of immune cells, oligodendroglial death and axonal degeneration. Remyelination after demyelination promotes tissue repair and functional recovery. The source of remyelinating oligodendrocytes in MS remains unclear although studies in rodents increasingly implicate oligodendrocyte precursor cells (OPCs), either surviving or locally recruited from adjacent unaffected areas, which proliferate and differentiate into myelinating OLGs. This process involves several molecules such as neurotransmitters and growth factors that are transmitted between glial cells and neurons. In this line, we have recently shown that the expression and function of $GABA_A$ receptors in cultured oligodendrocytes are regulated by axon-to-glia interactions (Arellano et al., 2016). Based on it, here we have evaluated whether GABA signaling is involved in OPC proliferation and/or differentiation into mature myelinating OLGs and this neurotransmitter could regulate axon-glia recognition processes and myelination.
First, in a purified rat OPC culture system, we have observed that the exposure to GABA increases the number of Olig2+ cells, as well as the proportion of $PDGFR\alpha^+/Olig2^+$ cells. The presence of bicuculline, a $GABA_A$ receptor antagonist, prevents this effect induced by GABA. In addition, we have observed that the viability of both cultured OPCs and OLGs derived from rat optic nerve is increased when cultures were maintained in the presence of GABA from plating and during, at least, 72 hours. Furthermore, we have also checked myelin basic protein (MBP) expression by immunohistochemistry and western blot analysis. So, we have observed that the addition of GABA promotes a significant increment of MBP in OLGs derived from cortical OPCs, both alone or cocultured with DRGs, which indicates that GABA may regulate the expression of myelin proteins and the myelination process of oligodendrocytes when they are in contact with axons. Altogether, these results strongly suggest that GABA and the regulation of their receptors may be relevant to OLG maturation and could be a potential therapeutic approach for promoting remyelination following white matter injury.

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ID: P77

Title

Cannabinoids modulate information transmission through the limbic circuit of the basal ganglia

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The limbic circuit of the basal ganglia arises from different areas of the medial prefrontal cortex (mPFC) and is known for participating in motivational behavioural aspects. Also, the CB1 receptor is densely located in both the basal ganglia, and mPFC synaptic terminals. Thus, the aim of the present study was to determine the effect of the cannabinoid WIN 55,212-2 on spontaneous and cortically evoked activity in the substantia nigra *pars reticulata* (SNpr) neurons. To that purpose, extracellular recording techniques in SNpr neurons along with simultaneous electrical stimulation of the mPFC were conducted in anaesthetized animals.

WIN 55,212-2 (125 µg/Kg, i.v.) administration increased the firing rate of SNpr neurons by 24,56 ± 9,68 % (n=7). This agonist also increased the regularity of these neurons as shown by a reduction in their variation coefficient (-22,95 ± 5,85 %, n=5), and a decrease in bursty events displayed by SNpr neurons.

The cortically evoked activity registered in SNpr neurons consisted of an early excitation (activation of the hyperdirect cortico-subthalamic circuit), an inhibition (activation of the direct striatonigral circuit), and a late excitation (activation of the indirect striato-pallido-subthalamo-nigral circuit). The administration of WIN 55,212-2 showed to modulate all these cortically evoked responses on SNpr neurons. WIN 55,212-2 (125 µg/Kg, i.v.) administration caused a reduction in both the early excitation and the inhibition in almost all the neurons studied. Whilst such effect on the early excitation and the inhibitory component is clear, this cannabinoid induced a mixed pattern of response in the late excitation; it consisted in a reduction of the said response in 4 out of 7 neurons, and an increase in the 3 neurons remaining.

These results suggest that WIN 55,212-2 modulates not only the spontaneous activity of SNpr neurons, but also the cortico-nigral transmission through the limbic circuit of the basal ganglia. This modulation may be important in the understanding of involvement of the cannabinoid system in motivational behaviours.

ID: P78

Title

Characterization of cannabidiol effect on the firing rate of dorsal raphe nucleus neurons in rat brain slices

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Cannabidiol (CBD) is the most abundant non-psychoactive cannabinoid present in the *Cannabis sativa* plant. There is increasing evidence that this cannabinoid could be an effective drug for the treatment of neuropsychiatric diseases. The lack of psychoactive activity of CBD makes it a suitable candidate for clinical use. It has been suggested that serotonergic 5-HT_{1A} receptor plays an important role in eliciting CBD effects, but the interaction between them remains to be studied. Therefore, the present work was carried out to characterize the effect of CBD on the neuronal activity of dorsal raphe nucleus (DRN), which is the main serotonergic nucleus in the brain that expresses somatodendritic 5-HT_{1A} receptors. Thus, we recorded the firing rate of DRN 5-HT cells from rat brain by single-unit extracellular electrophysiological technique *in vitro*. Direct perfusion with CBD (30 μM) did not modify the firing rate of DRN 5-HT cells. Furthermore, administration of CBD (30 μM) failed to alter the inhibition of 5-HT cells produced by 5-HT (50-100 μM). However, CBD (30 μM) significantly blocked the inhibitory effect of the more selective 5-HT_{1A} agonists ipsapirone (100 nM) and 8-OH-DPAT (10 nM). Thus, in the presence of CBD (30 μM) the inhibitory effects of ipsapirone and 8-OH-DPAT were reduced by 42% and 66%, respectively. These results suggest that CBD does not directly bind to the 5-HT_{1A} receptor, but it seems to regulate the activity of the receptor in an indirect manner.

ID: P79

Title

Regulation of 2-arachidonoylglycerol (2-AG) signalling at neuronal nuclei: study of nuclear expression of proteins regulating 2-AG production and degradation

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The phospholipase C- (PLC) 1 / diacylglycerol lipase- (DAGL) signalling cascade has been described to be located near the post-synaptic density of excitatory synapses, where it plays an important role in the control of neuronal excitability of glutamatergic neurons through the synthesis of 2-arachidonoilglycerol (2-AG), which is the main endocannabinoid in the central nervous system **(1)**. Recently, we have described the presence of a PLC /DAGL -dependent mechanism for 2-AG production in isolated nuclei. We reported that phosphatidylinositol-phospholipase C (PtdIns-PLC) and diacylglycerol lipase (DAGL) activities are co-compartmentalized in isolated adult cortical neuronal nuclei, leading to a nuclear-localized production of the endocannabinoid 2-AG **(2)**. These results suggest a role for 2-AG locally produced within the neuronal nucleus. The magnitude and time course of 2-AG signalling are thought to depend on the balance between the production and degradation of 2-AG. In this context, the analysis of the expression of different proteins and/or enzymes involved in the modulation of 2-AG production and degradation in neuronal nuclei could give us some information about how this signalling works at nuclear location.
Therefore, the purpose of the present study was to evaluate the neuronal nuclei expression of 2-AG metabolizing enzymes either by hydrolytic pathway, monoacylglycerol lipase (MAGL) and monoacylglycerol lipase containing Alpha/beta-hydrolase domain 12 (ABHD12), (as) or by the oxidative pathway, cyclooxygenase-2 (COX-2). Also, we wanted to evaluate the expression at nuclear location of calcium/calmodulin dependent protein kinase II (CaMKII), and its phosphorylated form (pCaMKII), because it has been described as a potential modulator of DAGL activity **(3)**.
To this aim, Double-inmunofluorescence and Western Blot techniques were used.
In order to compare the subcellular distribution of these proteins Western Blot analyses were performed in different cellular fractions such as intact nuclei (N), microsomal fraction (P3), cytosol (S3) and cell membrane (P2).
Inmunofluorescence assays revealed that apparently all these proteins are present in neuronal nuclei. However, the different immunofluorescence profiles suggest that these proteins are distributed in different nuclear compartments. Moreover, double immunofluorescence assays resulted in different colocalization degrees of these proteins with DAGL .
Western Blot analysis confirmed the presence of all these proteins at neuronal nuclei. In addition, the analysis of subcellular distribution of these proteins suggest and enrichment of their expression in nuclear fractions regarding to other cellular compartments.
Our results show that some of the main proteins involved in the regulation of 2-AG levels could be operating at nuclear location. However, the distribution profile in different nuclear compartments, and their different colocalization degrees with DAGL , could condition their role in regulating 2-AG levels at this location. Future functional studies aimed at evaluating the contribution of each of these proteins to the regulation of 2-AG production and/or degradation will be necessary in order to understand 2-AG signalling at neuronal nuclei.

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ID: P80

Title

Protein kinase CK2 downregulation activity modulates MAPKs signaling and ER stress and contributes to oligodendrocyte protection against excitotoxicity

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Protein kinase CK2 (Casein kinase 2) is a serine/threonine kinase involved in the regulation of cell proliferation, survival and/or cell death, and its activity has been found to be highly enhanced in some human and experimental diseases. CK2 mediates its activity by modulating diverse molecules and pathways and it has been suggested to be implicated in pro-apoptotic signaling via interacting with the ASK1/JNK pathway. However, and despite the numerous studies about CK2, little progress has been made in understanding its function in death cellular processes. In this work, we have investigated a possible linkage between CK2 and the excitotoxic death induced by activation of AMPA receptors in rat primary cultured oligodendrocytes.
First, we observed that brief and moderate AMPA receptor activation in oligodendrocytes triggered CK2 activity, analyzed by *in vitro* kinase assay. In addition, we deduced that CK2 activity was involved in the subsequent AMPA-induced excitotoxic damage because it was abolished by TBB, a CK2-specific inhibitor. Additionally, these excitotoxic insults caused an early and potent activation of ASK1/JNK/p38 signaling axis, associated with the implementation of the apoptotic cascade, and this robust activation of JNK was significantly attenuated in the presence of TBB and when endogenous CK2 expression was knocked-down by shRNA. These results suggest that modulation of this pro-apoptotic JNK signaling could be responsible, at least in part, of the protective effect that CK2 downregulation exerts on AMPA-mediated damage in oligodendrocytes. In other hand, there are evidences that CK2 and some of its substrates are located to the endoplasmic reticulum (ER) where CK2 can phosphorylate, regulate and modify the role of ER resident proteins in response to stress. We have observed that CK2 was expressed at the ER of cultured oligodendrocytes. In addition, AMPA exposure triggered ER stress and activated the PERK/eIF2 and IRE1 /XBP1 arms of UPR, without affecting the chaperon protein GRP78/94 levels, and pharmacological inhibition and/or gene silencing of CK2 by shRNA regulated these pathways. Together, these results show that CK2 modulates both ASK1/JNK/p38 pro-apoptotic axis and ER stress-induced signals and, specifically, we assume that the regulation CK2-dependent of IRE1 /XBP1 could represent the linkage between MAPKs signaling and ER stress in the excitotoxic context. These evidences suggest that modulation of CK2 activity may be relevant for developing novel oligoprotective drugs with therapeutic potential for the treatment of demyelinating diseases.

ID: P81

Title

Identification of a new partner and regulator of the dopamine transporter: snapin

Authors

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Dopamine (DA) is a major regulator of sensorimotor and cognitive functions. The importance of DA neurotransmission is emphasized by its direct implication in devastating neurological and psychiatric disorders. The DA transporter (DAT) is the key protein that regulates the spatial and temporal activity of DA release into the synaptic cleft via the rapid reuptake of DA into presynaptic terminals. There is strong evidence suggesting that DAT-interacting proteins may play a role in its function and regulation.
Methods: We used yeast-2-hybrid screening, first, and then different molecular, cellular and in vivo approaches to identify new partners of DAT and understand the role of these interactions.
Results: We have identified snapin, a SNARE-associated protein implicated in synaptic transmission, as a new binding partner of the carboxylic terminal of DAT, and determined the domains required for this interaction in both proteins. We also characterized the DAT-snapin interface by the generation of a 3D modelleing. In situ hybridization assays showed that snapin is expressed in vivo in dopaminergic neurons along with DAT. By different approaches we demonstrated that both proteins colocalise in cultured cells and brain, and are present in the same protein complex. With functional studies we have shown that snapin produces an important decrease in DAT activity. Finally, using a shRNA lentivirus directed against snapin, we have demonstrated that the downregulation of snapin produces an increase in DAT levels and activity in vivo, which is accompanied by a higher locomotor behavioural response to amphetamine and higher DA levels.
Conclusions: Our data show that snapin is a new direct partner and regulator of DAT, and we provide evidence for the relevance of this regulation in vivo.

ID: P82

Title

Evaluation of the functional coupling of cannabinoid receptors in mouse brain

Authors

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For most of G protein-coupled receptors (GPCRs) distinct agonists can differentially regulate several signaling pathways through the same receptor by a selective activation of different intracellular effectors. Both the CB_1 and CB_2 cannabinoid receptors have been shown to preferentially couple to the $G_{i/o}$ family of heterotrimeric G-proteins. Furthermore, CB_1 receptor has been demonstrated to be capable of coupling to different families of G-proteins when activated by an agonist drug suggesting that different intracellular responses may be activated by the CB_1 receptor depending on the ligand.

The aim of the present study was to evaluate the functional coupling of both CB_1 and CB_2 receptors to different subtypes of G proteins (G_{i1}, G_{i3}, G_s, G_z, $G_{12/13}$ and $G_{q/11}$) in mouse brain cortex membrane homogenates.

Methods: Stimulation of the [^{35}S]GTPγS binding by the CB_1/CB_2 cannabinoid agonists WIN55,212-2 and ^{9}THC (10^{-5} M) was determined by Scintillation Proximity Assay (SPA) technique.

Results: WIN55,212-2 stimulated [^{35}S]GTPγS binding to all the G proteins evaluated, except G_s (E_{max} range 120±2% to 137±3%). On the other hand, ^{9}THC induced a stimulation of the [^{35}S] GTPγS binding to G_{i1} and $G_{q/11}$ (E_{max} range 118±2% to 131±3%) but not to G_{i3}, G_s, G_z and $G_{12/13}$ proteins. In all cases, activation of the G proteins by WIN55, 212-2 and ^{9}THC was blocked by the antagonist O 2050 (10^{-5} M). To elucidate the role of each cannabinoid receptor in this G protein activation, the same experiments were carried out with brain membranes from CB_1ko, CB_2ko and CB_1/CB_2 double ko mice. Results suggest that the stimulation of $G_{q/11}$ protein subtype by WIN55, 212-2 and ^{9}THC is CB_1 receptor-mediated. However, WIN55, 212-2 seems to stimulate the coupling to $G_{12/13}$ protein through a CB_2 receptor-dependant mechanism.

Conclusions: Our results demonstrate that, in mice brain tissue, different exogenous cannabinoid ligands are able to selectively activate different G protein subtypes, inhibitory and non-inhibitory G protein subtypes, through the activation of both CB_1 and CB_2 receptors.

ID: P83

Title

EP_3 receptor characterization in locus coeruleus neurons by electrophysiological recordings

Authors

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Prostanoids are known to regulate several brain functions and to play an important role in pathophysiological situations including pain, fever and inflammation. Prostaglandin E_2 (PGE_2) is the most abundant prostaglandin in the body. PGE_2 receptors (EP) are members of the G protein-coupled receptor superfamily and comprise four subtypes: EP_1 (coupled to G_q proteins), EP_2 and EP_4 (coupled to G_s proteins) and EP_3 (coupled to $G_{i/o}$ proteins). To date, the function of the prostanoid system in the brain has not been well characterized. The locus coeruleus (LC), the main noradrenergic nucleus in the brain, has been described to express EP_2, EP_3 and EP_4 receptors. The aim of this study was to characterize pharmacologically the EP_3 receptors in the LC by single-unit extracellular electrophysiological recordings in rat brain slices. We performed concentration-effect curves for selective EP_3 receptor agonists, such as the synthetic drugs sulprostone and misoprostol or the endogenous compound PGE_2. Thus, increasing concentrations of the EP_3/EP_1 agonist sulprostone (0.3-80 nM) fully inhibited the neuronal activity of LC cells with an EC_{50} value (concentration needed to obtain the 50% of the maximal response) of 15 nM. The selective EP_3 receptor antagonist L-798106 (10 µM) caused a rightward shift in the concentration-effect curve for sulprostone, but the EP_2 receptor antagonist PF-04418948 (10 µM) or the EP_4 receptor antagonist L-161982 (10 µM) failed to shift sulprostone concentration-effect curve. This highlights the involvement of EP_3 receptors in sulprostone effect. On the other hand, perfusion with the endogenous prostanoid PGE_2 (0.3 nM-1.28 µM) or the PGE_1 analogue drug misoprostol (0.3-320 nM) inhibited the firing activity of LC cells in a concentration-dependent manner, with EC_{50} values being 51 nM and 112 nM, respectively. Likewise, only the EP_3 receptor antagonist L-798106 (10 µM) caused a rightward shift in the concentration-effect curves for PGE_2 and misoprostol. Finally, blocking the $G_{i/o}$ protein by overnight perfusion with pertussis toxin caused a significant shift to the right in the concentration-effect curves for sulprostone (EC_{50} = 91 nM). In conclusion, LC neurons may be regulated by the prostanoid PGE_2 system in an inhibitory manner through somatodendritic $G_{i/o}$ protein-coupled EP_3 receptors.

ID: P84

Title

Role of neuronal nitric oxide synthase and reactive oxygen species in the development of cellular tolerance to different opioid agonists in the rat locus coeruleus

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Nitric oxide (NO) is involved in the neuroadaptations observed following chronic opioid use, such as tolerance and -opioid receptor (MOR) desensitization in the locus coeruleus (LC). However, the role of NO and NO-derived reactive oxygen species (ROS) in the development of cellular tolerance to different opioids in the LC, the main noradrenergic nucleus in the brain, remains to be studied. Herein, we examined the effect of the selective neuronal nitric oxide synthase inhibitor 7-nitroindazole (7-NI) and the antioxidants Trolox + ascorbic acid (TX +AA) and U-74389G on the development of cellular tolerance induced by morphine, methadone and fentanyl in rat brain slices containing the LC. For induction of morphine tolerance, animals were treated with a slow release emulsion containing free base morphine (200 mg/kg, 3 days, s.c). Methadone (60 mg/kg/day, 6 days) and fentanyl (0.2 mg/kg/day, 7 days) tolerance was induced by subcutaneous implantation of osmotic pumps. Sham animals were implanted with the vehicle of the opioid. To study the cellular tolerance, we performed concentration-effect curves for the inhibitory effect of Met5-enkephalin (ME; 0.05-12.8 M, 2x, 1 min) on the neuronal activity of LC cells. Morphine, methadone and fentanyl treatments shifted to the right concentration-effect curves for ME and increased the EC50 (concentration needed to obtain the 50% of the maximal response) by 4, 2 and 3 folds, respectively. Co-administration of TX+AA (40 and 100 mg/kg/day, respectively, i.p.) or U-74389G (10 mg/kg/day, i.p.) in morphine-treated animals prevented the development of cellular tolerance. Conversely, co-treatments with U-74389G or 7-NI (30 mg/kg/12 h, i.p.), failed to affect the induction of cellular tolerance after methadone or fentanyl treatments. Our results suggest that MOR agonists with different intrinsic efficacies cause variable degrees of cellular tolerance in LC cells. Moreover, NO/ROS pathways are differentially involved in opioid tolerance after prolonged treatments with morphine, methadone and fentanyl.

ID: P85

Title

Serotonin 5-HT_3 receptor antagonism potentiates the antidepressant activity of citalopram

Authors

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Selective serotonin reuptake inhibitors (SSRIs), in addition to enhance serotonergic neurotransmission, are able to regulate neurotransmission activity of other brain pathways involved in depression such as the noradrenergic system. In fact, citalopram administration increases serotonin (5-HT) release in the locus coeruleus (LC) area, contributing to the modulation of this noradrenergic nucleus. It has been shown that local serotonin 5-HT_3 receptor activation in the LC decreases synaptic noradrenaline (NA) neurotransmission in prefrontal cortex (PFC). In this context, the blockade of 5-HT_3 receptors in coadministration with SSRIs has been proposed as a potential strategy for avoiding countertherapeutic effects of SSRIs in the early stages of treatment.

In this study, dual probe microdialysis in freely awake rats (n=4-9 per group) was used to evaluate the involvement of serotonin 5-HT_3 receptors located in somatodendritic and terminal noradrenergic areas in the effect exerted by citalopram on NA release. Extracellular NA concentrations in the LC and PFC were measured. Besides, forced swimming test (FST) and open field test (OFT) were carried out in mice to evaluate the antidepressant-like effect of citalopram in combination with a 5-HT_3 receptor antagonist.

Systemic administration of the 5-HT_3 agonist SR57227 (10 mg/kg i.p.) increased NA in LC (Emax=200±27%) and in PFC (Emax=133±2%) while saline (1 ml/kg i.p.) did not induce NA changes. The increase in PFC was higher in the local presence in the LC of the 5-HT_3 antagonist Y25130 (50 µM) (Emax=296±41%; F_i[7,77]=6,101; p<0.0001; n=13), suggesting a tonic inhibition of LC activity by 5-HT release in the area. In addition, local administration in PFC of SR57227 (1-100 µM) increased NA in the area (Emax=815±148%). This effect was attenuated by the local presence in PFC of Y25130 (Emax=366±58%), which is compatible with the presence of cortical 5-HT_3 receptors involved in NA release.

Systemic administration of citalopram (10 mg/kg i.p.) increased NA in LC (Emax=185±11%) and decreased it in PFC (Emax= -35±7%). In the local presence in LC of the 5-HT_3 antagonist Y25130 both effects of citalopram on NA release were blocked. By contrast, when Y25130 was pre-administered systemically (10 mg/kg i.p.) with citalopram, the effect on NA in LC was blocked, whereas in PFC, NA concentrations switched from an inhibition (Emax= -40±5%) to an increase (Emax=117±8%). In addition, systemic Y25130 (10 mg/kg i.p.) + saline (1 ml/kg i.p.) coadministration did not modify NA concentrations neither in LC nor in PFC.

In the FST, systemic coadministration of subeffective citalopram (2.5 mg/kg i.p.) and Y25130 (10 mg/kg i.p.) doses was able to decrease the immobility time whereas no change of locomotor activity was observed in OFT.

These results show that (1) NA in the PFC increases by local 5-HT_3 receptor activation whereas decreases by somatodendritic 5-HT_3 receptor activation; and (2) the addition of a 5-HT_3 receptor antagonist to citalopram is able to reverse the inhibition of NA release in PFC exerted by systemic citalopram and to induce antidepressant-like effects in animal models. Therefore, the addition of a 5-HT_3 receptor antagonist to SSRIs could represent a strategy to improve antidepressant response.

Cellular and molecular Neuroscience - Plasticity and learning

ID: P86

Title

The effects of - 9-tetrahydrocannabinol in dorsal striatum structural plasticity

Authors

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Derivatives from the *Cannabis* plant are the most commonly abused illegal substances in the world. Its main psychoactive component -9-tetrahydrocannabinol (THC) exerts its effects through the specific activation of CB1 cannabinoid receptors. These receptors show a distinct lateral-medial gradient expression in the dorsal striatum, which is involved in the anatomical circuits that support goal-oriented behavior and habit formation. It is known that dendritic spines exhibit important synaptic functional attributes and a potential for plasticity which is thought to mediate long lasting changes in behavior. In the present study, adult, male C57BL6/J mice were intraperitoneally injected with THC or vehicle for 15 days. Using single cell intracellular injections and confocal microscopy 3D reconstruction of labeled neurons, we studied the effects of chronic treatment with THC in dendritic and spine morphology in medium spiny neurons (MSNs) of the anterior dorsolateral (aDLS) and posterior dorsomedial (pDMS) areas of the striatum. Data analysis showed that THC treatment is related to a slight increase in dendritic spine density in the distal part of the dendrites of the posterior dorsomedial striatum, but no changes were found in the rest of the parameters analyzed in either region studied. We also observed a difference in spine density, area and volume between the aDLS and the pDMS in the THC group which was not present in the control group. Considering that the distal part of MSNs dendrites show regenerative activity that produces somatic potential changes resembling sustained depolarization, our results seem to indicate that the effects of THC in the transition from goal directed behavior to habitual learning might be due to alterations of structural plasticity in the circuits involving the posterior dorsomedial striatum MSNs.
Key words: -9-tetrahydrocanabinol, CB1 receptors, anterior dorsolateral striatum, posterior dorsomedial striatum, spine morphology.

ID: P87

Title

Effects of intermittent alcohol exposure during adolescence on behaviour and cannabinoid CB1 receptor dependent long-term excitatory synaptic plasticity in adult mouse brain

Authors

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Alcohol drinking, especially among adolescents and young adults, is a serious public health concern. Ethanol interacts with the endocannabinoid system (ECS) whose function isaltered in ethanol dependence. Here, we investigated the effect of ethanol consumption during adolescence on excitatory synaptic transmission and plasticity mediated by the cannabinoid CB_1 receptor in adult hippocampal dentate gyrus (DG). Also, behavioural tests were applied during the withdrawal period to evaluate recognition memory, motor coordination and balance.

Male C57BL6 mice were exposed to intermittent ethanol intake (20% (v/v) in tap water) using a 4 days drinking-in-the-dark procedure during adolescence (PD 30 to 54). Animals were given access to ethanol (or water) for 2h sessions during 3 days, and 4h session on the 4th day. Ten days after withdrawal, the animals were subjected to behavioural tests (novel object recognition test, rotarod and beam walking balance test). 18-21 days after ethanol withdrawal, adult mice were sacrificed and electrophysiological, immunohistochemical, and molecular techniques were applied.

Excitatory postsynaptic potentials (fEPSPs) were evoked after stimulation of the medial perforant path and recorded in the supragranular zone of the dentate molecular layer (ML) in the presence of the $GABA_A$ receptor antagonist picrotoxin. CB_1 activation by CP55,940 (10 M) inhibited fEPSPs in controls (26.43 ± 2.77% of baseline) as already shown. However, this effect was not observed in ethanol-exposed mice (4.9 ± 7.47% of baseline). Furthermore, ML synaptic stimulation (10 min, 10 Hz) triggered a long term depression (LTD) of the excitatory transmission that was absent in adult mice after ethanol consumption during adolescence (2.7 ± 3.12% of inhibition). This plasticity was CB_1 dependent as the AM251 antagonist (4 µM) abolished LTD (8 ± 6.6% of inhibition). CB_1 immunoreactivity decreased in ML of ethanol-exposed (87.47 ± 0.58%) vs control (100 ± 0.77%) mice. Also, the relative mRNA and CB_1 protein significantly decreased, while a significant increase in MAGL (mRNA and protein) was detected in adult hipocampus. Finally, we observed a significant lower recognition memory ($P<0.001$***), motor coordination ($P<0.05$*) and balance ($P<0.05$*) in adult mice ethanol-exposed during adolescence.

In conclusion, repetitive exposure to ethanol during adolescence leads to a deficit of endocannabinoid-dependent LTD in adult DG excitatory synapses, probably due to a down-regulation of CB_1 receptors and a reduction of the endocannabinoid tone by an increase of MAGL. On the other hand, alcohol intake during adolescence leads to long-term deficits in recognition memory, motor coordination and balance.

ID: P88

Title

Environmental enrichment reverses cognitive impairment, motor coordination and balance disturbance associated with alteration of excitatory CB_1-dependent LTD and transmission after ethanol consumption during adolescence

Authors

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Alcohol consumption, especially during adolescence, is one of the main problems that concern our society. Studies in the last decade indicate that the endocannabinoid system (ECS) function is affected in ethanol dependence. An enriched environment (EE) has been shown to significantly facilitate recovery from brain injury due to important anatomical, molecular and functional changes that occur along brain development (Faherty et al. 2003; Pham et al. 2002). However, it is not known whether an EE has any positive effect on cognitive impairment, motor coordination and balance associated with ethanol consumption. Our aim is to investigate this during the adolescence period and the role of an enriched environment to counteract ethanol administration effects on plasticity and transmission mediated by cannabinoid CB_1 receptors in the hippocampal dentate gyrus (DG).

Male C57BL6 mice were exposed to a 4 days drinking-in-the-dark (DID) procedure during adolescence (PD 30 ± 2 to 54 ± 2). The animals were given free access to ethanol (20% (v/v) in tap water) or water for 2-h sessions during three consecutive days, and a 4-h session on the 4th day. Then, from postnatal 56 to 74 withdrawal days, the animals were reared under two different conditions: standard laboratory condition (SC) and enriched environment. In the last four days of the withdrawal period (from p70 to p73) recognition memory, motor coordination and balance were assessed by the novel object recognition test (NORT), rotarod and beam walking balance test (BWBT) respectively. Then, from p74 to p78 mice were sacrificed and electrophysiological and molecular techniques were applied.

Synaptic stimulation (10min, 10Hz) of the medial perforant path triggered a CB_1-dependent long term depression (LTD) of the excitatory transmission that was absent in adult mice after ethanol consumption during adolescence ($p<0.001^{***}$). Furthermore, a significant lower recognition memory ($p<0.001^{***}$), motor coordination ($p<0.05^{*}$) and balance ($p<0.05^{*}$) was observed in the alcohol treated standard group (SC-OH) compared to the untreated control group (SC-H_2O). Interestingly, enriched EE-OH group significantly recovered CB_1-dependent synaptic plasticity ($p<0.01^{**}$) and transmission ($p<0.05^{*}$) in adult hippocampal DG and significantly improved recognition memory ($p<0.01^{**}$), motor coordination ($p<0.01^{**}$), and balance ($p<0.05^{*}$) compared to SC-OH mice.

These results suggest that an environmental enrichment may have potential benefits for the recovery in adult of the brain impairment caused by binge-drinking alcohol during adolescence.

ID: P89

Title

Spine neck morphology shapes postsynaptic potentials in hippocampal neurons

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It has been a long-standing fundamental question whether the dendritic spine neck influences excitatory postsynaptic potentials and their somatic integration. While the importance of the spine neck for biochemical compartmentalization is well established, its role in electrical signaling remains highly controversial. Two main obstacles have hindered progress. 1) The average spine neck diameter is around 150 nm, which is too small to be resolved by conventional light microscopy; 2) The inaccessibility of the spine head to direct electrophysiological recordings.
We have developed a new approach to address this controversy and test the hypothesis that spine necks can provide a sufficient electrical resistance to affect the EPSPs measured at the soma. It is based on a combination of STED microscopy, two-photon glutamate uncaging and patch-clamp electrophysiology in organotypic hippocampal slice cultures. To discriminate between the effects of synaptic conductance and synaptic morphology on measured voltages, we obtained matched electrophysiological recordings of synaptic conductance (g_{syn}) and voltage (V) for the same spine by performing the 2-photon glutamate uncaging both in voltage-clamp and current-clamp. In addition, we acquired super-resolved STED microscopy images to correlate the electrophysiological data to spine morphology. This strategy allowed us to normalize the synaptic EPSP measured at the soma to the strength of the synapse in terms of conductance. The V/g_{syn} ratio (or 'synaptic gain') for a given spine was compared to spine geometry obtained from the corresponding STED image.
We identify a correlation between neck geometry and synaptic gain. Specifically, the longer and thinner the neck is, the lower the synaptic gain. We further find that spines with high neck resistances show a more pronounced sub-linear response to repetitive stimulation than neighboring spines with lower neck resistances. Finally, we present numerical simulations to model and explore these relationships in more detail.
Our results provide strong new evidence for an influential role of spine geometry in electrical signaling, which is consistent with the idea that the neck resistance boosts the spine head voltage, leading to gradual saturation of the synaptic potential. Effectively, this means that spines with shorter and wider necks can convey larger synaptic currents for a given conductance.

ID: P90

Title

Identifying the substrates of Ube3a the enzyme involved in Angelman Syndrome

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Angelman Syndrome (AS) is a severe neurodevelopmental disorder most frequently caused by a maternal deletion of the 15q11-q13 chromosomal region. Even though this deleted region contains other genes, it has been shown that mutations affecting the gene *UBE3A,* and more specifically those affecting the activity of its product, an ubiquitin E3 ligase enzyme, are the primary cause of the syndrome. Some proteins have been identified as UBE3A ubiquitin substrates; in most cases, without any *in vivo* evidence. Using available AS *Drosophila* models in combination with an efficient approach to isolate ubiquitin conjugates, we have identified by mass-spectrometry several proteins whose ubiquitination levels increase when the *Drosophila* UBE3A ortholog (Ube3a) is overexpressed, and we have further validated their direct *in vivo* ubiquitination by Ube3a. Among the proteins identified to be substrates of Ube3a we found four proteasome-related proteins, as well as the ribosomal protein RpS10b. Our results place Ube3a as a regulator of protein homeostasis by regulating both protein degradation and protein synthesis.