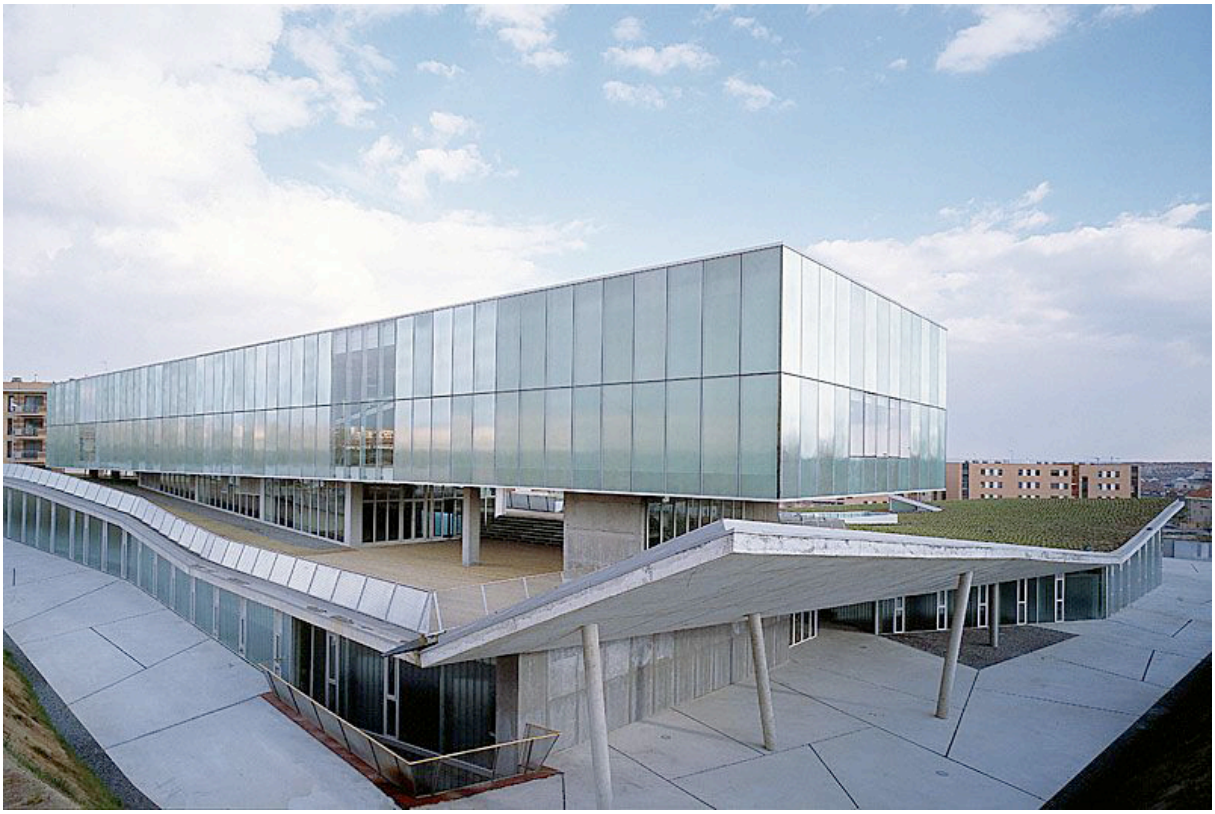
INSTITUTO DE NEUROCIENCIAS DE CASTILLA Y LEÓN (INCYL)
SESIÓN CIENTÍFICA- EXHIBICIÓN DE *PÓSTERES*



21 de noviembre de 2019

INSTITUTO DE NEUROCIENCIAS DE CASTILLA Y LEÓN. UNIVERSIDAD DE SALAMANCA.

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1. - SESIÓN CIENTÍFICA

Salón de Actos, Instituto de Neurociencias de Castilla y León
Moderadores: Dra. Aranzazu Tabernero Urbieta y Dr. Manuel Sánchez Malmierca

9:00 - 9:10 h. Dra. Almudena Velasco.
"Degeneración y regeneración del sistema visual."

9:10 - 9:20 h. Dra. Dolores E. López.
"Presente y futuro del Laboratorio 12: TAM (trastornos audiomotores)."

9:20 - 9:30 h. Dr. Enrique Saldaña.
"Laboratorio de Neurohistología: Conexiones entre núcleos auditivos del cerebro de los mamíferos."

9:30 - 9:40 h. Dr. Enrique A. López Poveda.
"El desafío de mejorar la comunicación verbal de las personas sordas e hipoacúsicas en entornos ruidosos."

9:40 -10:00 h. Descanso

10:00 - 10:10 h. Dr. Manuel Sánchez Malmierca.
"Predecir el futuro es fantástico: mecanismos neurofisiológicos de adaptación neuronal."

10:10 - 10:20. Dra. María del Carmen Tarbenero Urbieta.
"Regulación emocional ante situaciones estresantes con registros de biofeedback y neuroimagen funcional."

10:20 - 10:30 h. Dr. Juan Carlos Arévalo.
"Mecanismos reguladores de las neurotrofinas y sus receptores en condiciones fisiológicas y patológicas."

10:30 - 10:50 h. Descanso

10:50 - 11:00 h. Dra. Arantxa Tabernero.
"Posibilidades terapéuticas de péptidos basados en la conexina-43 en gliomas."

11:00 - 11:10 h. Dra. Arantxa Tabernero.
Sesión informativa sobre el doctorado en neurociencias.

11:10 - 11:20 h. Dr. Enrique Saldaña.
Taller de Escritura Científica.

11:20 - 11:50 h. Descanso

12:00 - 12:45 h. Ponente invitado: Dr. Juan del Río-Hortega. (Médico y Profesor Asociado de Historia de la Medicina de la Universidad de Valladolid).

"*Aspectos personales de la vida de Pío del Río-Hortega en el centenario del descubrimiento de la microglia (1919-2019).*"

12:45 h. Acto conmemorativo del centenario del descubrimiento de la microglía presidido por la Vicerrectora de Investigación y Transferencia, Dª. Susana Pérez Santos.

13:00 - 13:45 h. Ponente invitado: Dr. Jorge Valero (ACHUCARRO-Basque Center for Neuroscience).

"*Fagocitosis microglial: desde la neurogénesis hasta la neurodegeneración.*"

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2.- EXHIBICIÓN DE PÓSTERES 2019

Vestíbulo del Instituto de Neurociencias de Castilla y León

16:30 - 19:30 h

PÓSTERES SESIÓN CIENTÍFICA 2019

1. Cholinergic Modulation of deviant detection in rat auditory cortex.

Cristian Aedo Sánchez[1,2,4], David Pérez Gonzalez[1,2], Manuel Sánchez Malmierca[1,2,3]

1. Auditory Neuroscience Laboratory, Institute of Neuroscience of Castilla y León (INCYL), Salamanca, 37007 Castilla y León, Spain.
2. The Salamanca Institute for Biomedical Research (IBSAL), Salamanca, 37007 Castilla y León, Spain.
3. Department of Cell Biology and Pathology, Faculty of Medicine, University of Salamanca, Salamanca, 37007 Castilla y León, Spain.
4. Departament of Medical Technology, Faculty of Medicine, University of Chile.

In the auditory cortex, there are neurons that specifically decrease their response to repetitive sounds (standard tones) but that increase their firing rate against novel stimuli (deviant tone), and the difference of both responses is known as stimulus specific adaptation or SSA. This study describes the cholinergic modulation of stimulus-specific adaptation (SSA) in rat auditory cortical areas. Here, we measured SSA levels in single neurons of lemniscal and non-lemniscal auditory cortical areas, in response to oddball and many standard/cascade auditory paradigms, before, during and after the microiontophoretic application of acetylcholine (Ach). As a control, in a subset of neurons we also applied cholinergic antagonists of muscarinic (scopolamine) and nicotinic (mecamylamine) receptors. We observed that the SSA levels (CSI) increase the values for most of the neurons recorded (65/80) on average after the injection of Ach. These changes were mediated mainly by an increment in the neuronal firing rate in response to the deviant tones (0.91 Spikes/s control vs 1.51 spikes/s Ach) on average. On the other hand, the cholinergic antagonists decreased the SSA index, mainly the muscarinic type (0.62 control CSI levels vs 0.55 scopolamine CSI levels). In contrast, the effect of blocking the nicotinic receptors was not statistically significant. All these effects have a duration between 60-90 minutes approximately. We concluded that cholinergic modulation in the auditory cortex increases SSA values mainly by increasing the neuronal firing rate in response to the deviant tones and this modulation would be mediated mainly by muscarinic receptors.

2. Prediction errors explain mismatch signals of neurons in the medial prefrontal cortex.

Lorena Casado-Román[a,b], David Pérez-González[a,b] & Manuel S. Malmierca[a,b,c,*]
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[b]The Salamanca Institute for Biomedical Research (IBSAL), Salamanca 37007, Castilla y León, Spain
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According to predictive coding theory, perception emerges through the interplay of neural circuits that generate top-down predictions about environmental statistical regularities and those that generate bottom-up error signals to sensory deviations. Prediction error signals are hierarchically organized from subcortical structures to the auditory cortex. Beyond the auditory cortex, the prefrontal cortices integrate error signals to update prediction models. Here, we recorded neuronal activity in the medial prefrontal cortex of the anesthetized rat while presenting oddball and control stimulus sequences, designed to separate prediction errors from repetition suppression effects of mismatch responses. Robust mismatch signals were mostly due to prediction errors. The encoding of a regularity representation and the repetition suppression effect over the course of repeated stimuli were fast. Medial prefrontal cells encode stronger prediction errors than lower levels in theauditory hierarchy. These neurons may, therefore, represent the neuronal basis of a fundamental mechanism of hierarchical inference.

3. Colaboración científica del BTN-INCYL en el diagnóstico y un mayor conocimiento de las enfermedades raras pediátricas.

Herrero Turrión, M.J.[1,2,3,4]; Blázquez Hidalgo, E.[1,3,4]; Sánchez Sánchez, J.[2,3,4]; Ruiz Ayúcar, I.[5]; Hernández Fabian, A.[4,5]; Marcos Vadillo, E.[6]; García Berrocal, B.[6]; Isidoro García, M.[6]; Prieto Matos, P.[4,5]; Rábano Gutiérrez, A.[7]; Sánchez Malmierca, M.[1,2,3,4]

1 Banco de Tejidos Neurológicos del Instituto de Neurociencias de Castilla y León (BTN-INCYL). 2 Instituto de Neurociencias de Castilla y León (INCYL). 3 Instituto de Investigación Biomédica de Salamanca. 4 Universidad de Salamanca, Salamanca. 5 Servicio de Pediatría. Complejo Asistencial Universitario de Salamanca (CAUSA). 6 Unidad de Referencia Regional de Diagnóstico Avanzado de Enfermedades Raras de Castilla y León (DiERCyL), Servicio de Bioquímica (CAUSA). 7 Dpto. Neuropatología y Banco de Tejidos, Fundación CIEN, Instituto de Salud Carlos III.

INTRODUCCIÓN El Banco de Tejidos Neurológicos del Instituto de Neurociencias de Castilla y León (BTN-INCYL) oferta una amplia gama de servicios de apoyo técnico y científico a cualquier tipo de investigación que pudiera derivarse de sus muestras obtenidas. Esta prestación diferenciada nos permite estar en disposición de participar de forma activa en algunos proyectos de investigación a propuesta de los propios investigadores solicitantes o por iniciativa propia de nuestro biobanco. Desde el año 2017, el Servicio de Pediatría del Complejo Asistencial Universitario

de Salamanca (CAUSA) nos ha facilitado 4 donaciones de cerebro. En 2 casos, la Unidad de Referencia Regional de Diagnóstico Avanzado de Enfermedades Raras de Castilla y León (DiERCyL) participó junto con este servicio en el diagnóstico de la patología que tenía cada uno de los posteriores donantes de cerebro. En aras a lograr un mayor conocimiento científico de este tipo de casos, el BTN-INCYL ha iniciado una colaboración científica junto con estos ambos servicios para la realización de proyectos de investigación que ayuden a profundizar en el conocimiento de las enfermedades raras pediátricas.
OBJETIVOS Describir el proyecto de colaboración del BTN-INCYL con el Servicio de Pediatría y la Unidad DiERCyL del CAUSA. Potenciar la investigación básica y traslacional de las enfermedades raras pediátricas.
MATERIAL Y MÉTODOS Muestras de donantes y distintas técnicas de microscopia y biología molecular.
RESULTADOS Se han producido un total de 4 donaciones pediátricas de cerebro y en dos de ellas se postuló en vida el diagnóstico de dos tipos de enfermedades raras, pudiéndose confirmar tales diagnósticos en los estudios realizados con las muestras de tejido nervioso obtenidas post-mortem.
CONCLUSIONES El modelo de colaboración científica entre el BTN-INCYL, el Servicio de Pediatría y la Unidad DiERCyL puede ser un buen modelo del valor que tienen los biobancos como parte activa de las investigaciones.

4. Target detection Task in an Oddball Context: A New Training Protocol for rats.

Camilo J. Morado-Díaz, Gonzalo Terreros, Cristian Aedo-Sánchez, Daniel Duque, Manuel S. Malmierca

The oddball paradigm has been widely exploited to study deviance detection. It serves to evaluate expectation, as it provokes an enhancement of the representation of a low-probability stimulus randomly intermingled in a sequence of frequent stimuli. We have previously used this paradigm tostudy stimulus-specific adaptation (SSA) in single unit recordings, in rodents. However, how the brain deals with the conflict between what is likely to happen (SSA) and what is behaviorally relevant (attention) remains unknown. Thus, in order to evaluate SSA modulation during an attentional task, we have trained rats to discriminate deviant tones as in an oddball paradigm. For that, we have designed and progressively implemented three levels of complexity with foodrewards where the animals learn first to respond to a sound activating a nose-poke in an operational chamber, then they learnt to recognize a deviant tone presented in between periods of silence, and finally to distinguish the deviant tone embedded in an oddball paradigm. This trainingprotocol took around 30 days, and once it was completed, data were

collected. The rat discrimination ability was quantified by calculating the so-called *d'* index. Results so far (n=19) show that trained animals were able to complete this target-detection tasks with 70.8±2.1% of correct detections, 8.6±0.7% of false alarms, and a *d'* value of 2.05±0.09 (mean ± sem). Thus, our results demonstrate that rats can successfully discriminate the salience of deviant, low probability sounds embedded in a sequence of repeating, high probability sounds. This behavioral study will be useful in future neurophysiological studies in the behaving animal, as it will be the key resource to understand the neuronal link between expectation and attention.

Supported by the Spanish MINECO (SAF2016-75803-P to MSM, CJM-D, FJCI-2016-27897; DD: IJCI-2016-29358) Fondo de Movilidad U. de O'higgins, and Beca Iberoamericana Santander Universidades to GT.

5. LATERALIZATION OF VIRTUAL SOUND SOURCES WITH A BINAURAL COCHLEAR-IMPLANT SOUND CODING STRATEGY INSPIRED BY THE MEDIAL OLIVOCOCHLEAR REFLEX

Enrique A. Lopez-Poveda, Almudena Eustaquio-Martín, Milagros J. Fumero, Josh S. Stohl, Reinhold Schatzer, Peter Nopp, Robert D. Wolford, José M. Gorospe, Rubén Polo, Auxiliadora Gutiérrez Revilla, Blake S. Wilson

Many users of bilateral cochlear implants (BiCIs) localize sound sources less accurately than do people with normal hearing. This may be partly due to using two independently functioning CIs with fixed compression, which distorts and/or reduces interaural level differences (ILDs). Here, we investigate the potential benefits of using binaurally coupled, dynamic compression inspired by the medial olivocochlear reflex; an approach termed "the MOC strategy" (Lopez-Poveda et al., 2016, Ear Hear 37:e138-e148). Twelve BiCI users were asked to localize wideband (125-6000 Hz) noise tokens in a virtual horizontal plane. Stimuli were processed through a standard (STD) sound processing strategy (i.e., involving two independently functioning sound processors) and three different implementations of the MOC strategy: one with fast (MOC1) and one with slow (MOC2) contralateral control of compression but effectively greater contralateral inhibition in the higher than in the lower frequency channels, and one with slower control of contralateral inhibition and slightly greater inhibition in the lower than in the high frequency channels (MOC3). Localization was most accurate with the MOC1 strategy, presumably because it provided ILDs that were closer to the natural head-shadow ILDs. The angle error improved slightly from 25.3 degrees with the STD strategy to 22.7 degrees with this MOC1 strategy. The improvement disappeared when the contralateral control of compression was made slower and/or greater in the lower frequency channels. Results suggest that some MOC implementations hold promise

for improving not only speech-in-noise intelligibility, as shown elsewhere, but also sound source lateralization. [Work supported by MED-EL GmbH, the Spanish Ministry of Economy and Competitiveness (grant BFU2015-65376-P), and the European Regional Development Fund.]

6. Speech Predictability Hinders Sentence Recognition in Difficult Listening Conditions

Miriam I. Marrufo-Pérez, Almudena Eustaquio-Martín, Enrique A. Lopez-Poveda
University of Salamanca

The auditory system adapts progressively to the background noise, and this adaptation improves the recognition of isolated words embedded in noise over time. On the other hand, listeners are continually making predictions during language comprehension, and correct predictions can facilitate the recognition of upcoming words in running speech. These two mechanisms should make listeners more likely to recognize the later than the earlier words in congruent, spoken sentences. We tested this hypothesis by presenting normal-hearing listeners (N = 100) with sentences in quiet or in noise at individualized levels where they had 50% probability of recognizing a full sentence. Word recognition was measured as a function of word position in the sentence. Contrary to expectations, recognition gradually deteriorated with increasing word position along the sentence. The worsening in recognition was unlikely due to differences in word audibility or word type, and was not correlated with age or working memory capacity. In fact, results revealed that the recognition of a word was significantly biased by the recognition of the preceding word. We developed a probabilistic model of word recognition based on the assumption that our perception of a word in a sentence can bias our perception of the upcoming word. Using the model, we show that the worsening in word recognition along a sentence occurs because misunderstandings generate inaccurate predictions that outweigh the benefits from accurate predictions. Analyses also revealed that noise adaptation was insufficient to compensate for this harmful effect. We conclude that although speech predictability can facilitate sentence recognition, it also causes word recognition to decrease along a sentence. [Work supported by the University of Salamanca, Banco Santander, and MINECO (BFU2015-65376-P)].

7. TAT-CX43$_{266-283}$ PEPTIDE IMPAIRS MALIGNANT GROWTH IN MOUSE MODELS OF GLIOMA IN VIVO

R. Talaverón, M. Jaraíz- Rodríguez, L. García –Vicente, S. G. Pelaz , J.M. Medina. A. Tabernero
Instituto de Neurociencias de Castilla y León (INCYL), Universidad de Salamanca,Salamanca, Spain.

Malignant gliomas are the most frequent primary brain tumors and remain among the most incurable cancers. Connexin43 (Cx43) is an integral membrane protein that forms gap junctions and is widely expressed in astrocytes. This protein is down-regulated in high-grade gliomas and, particularly, in glioma stem cells (GSCs). When Cx43 is restored, the stem cell phenotype of GSCs is reversed and their tumorigenicity is reduced. We previously reported that TAT-$Cx43_{266-283}$, a cell-penetrating peptide based on Cx43, retains the ability to recruit Src together with its endogenous inhibitors PTEN and CSK, causing c-Src inhibition and exerts potent anti-tumor effects in different types of glioma cells in vitro. TAT-$Cx43_{266-283}$, by inhibiting c-Src, also reduces the expression of Sox-2, the formation of neurospheres, the rate of proliferation, migration and invasion in different types of GSCs, including primary GSCs derived from patients. Here, we studied the effect of TAT-$Cx43_{266-283}$ when human glioma stem cells were intracranially injected into NOD/SCID mice. We analyzed human nestin, stem 121 and Sox-2 by immunohistochemistry at different days post-implantation. We observed that TAT-$Cx43_{266-283}$ reduced the expression of the stemness markers nestin and Sox-2 in GSCs at 7 days post-implantation. Consistent with the role of Sox-2 as a transcription factor required for GSC tumorigenicity, TAT-$Cx43_{266-283}$ strongly reduced the number and stemness of human glioma cells at 30 days post-implantation in NOD/SCID mice. Taken together, our results confirm that TAT-$Cx43_{266-283}$ reduces the growth, invasion, and progression of malignant gliomas, which highlights the importance of this compound for the design of new therapies against malignant gliomas.

8. Effect of connexin43 cell-penetrating peptides on astrocyte activation in glioma models

Laura García-Vicente, Myriam Jaraíz-Rodríguez, Sara G. Pelaz, Jose M. Medina, Arantxa Tabernero

Departamento de Bioquímica y Biología Molecular, Instituto de Neurociencias de Castilla y León, Universidad de Salamanca, Salamanca, Spain

Gliomas are among the most aggressive cancers, with a median survival of approximately 15 months. One of the hallmarks of brain cancers is the formation of a layer of reactive astrocytes surrounding and infiltrating the tumor. Astrocytes in the tumor microenvironment communicate with tumor cells through gap junctions and secreted growth factors and inflammatory cytokines, which promote tumor growth by enhancing proliferation and invasion of cancer cells, as well as protection from chemotherapy and the immune system. In this way, the modulation of astrocyte activity emerges as an essential element to control glioma progression. A cell-penetrating peptide based on connexin43 (Tat-$Cx43_{266-283}$) exerts an antitumorigenic effect by inhibiting c-Src activity in glioma stem cells, appearing as a promising therapeutic tool for the treatment of gliomas. In this

work, we study the effect of this peptide in the reactive astrocytes that conform the tumor microenvironment. One of the main players in astrocyte reactivity is signal transducer and activator of transcription 3 (STAT3), which has been shown to regulate astrocyte activation in a variety of neurodegenerative diseases and models of acute injury and is known to play a key role in malignant transformation. Nevertheless, its function in the reactive astrocytes of the tumor microenvironment is not so well known. Here, we show an increase in the nuclear localization of Tyr705-phosphorylated STAT3 in astrocytes exposed to G166 glioma stem cells (GSCs) and examine the outcomes of this activation. Interestingly, we observe a reduction in STAT3 phosphorylation upon Tat-Cx43$_{266-283}$ treatment, suggesting a role for this peptide in the control of astrocyte activation.

9. Towards a metric to quantify information transmission by cochlear-implant coding strategies

Thibaud Leclère, Aswin Wijetillake, Manuel Segovia-Martinez, Enrique A. Lopez-Poveda

Processing strategies in cochlear implants (CI) aim to transmit information from the acoustic input signal to the auditory nerve via electrical pulsatile sequences with the most fidelity. For hardware, surgical, or physiological reasons, important acoustic cues may not be well encoded and transmitted to auditory nerve fibers, resulting in an important behavioral gap between CI and normal hearing listeners observed in many aspects (speech understanding in noise, localization, pitch perception, etc…). Instead of running psychophysical tests on CI users to determine the potential benefit of a strategy, the efforts in improving CI-user performance could be aided by computational models that could predict outcomes of CI users. While many models already exist for normal hearing listeners to predict performance from the acoustic stimulus (e.g. speech intelligibility, binaural hearing, pitch and loudness discrimination …), equivalent models dedicated to CI listeners are not as advanced and need to be further developed. As a first step to predict psychophysical data for CI listeners, the present study intends to develop a method to objectively quantify how much information is present in the auditory nerve of an individual CI user for a given sound processing strategy. Models of electrode-nerve interface were combined with adapted-version of existing phenomenological models of auditory nerve in order to generate the output spike trains evoked by a population of auditory nerve fibers when receiving an input electric current from the electrode array. Several models (e.g. Biphasic Leaky Integrate and Fire, Point-process) and metrics (e.g. mutual information, cross-correlation) are presented and discussed as potential candidates to describe the

information transmission between acoustic and neural representations. This research project is funded by Oticon Medical and Oticon Foundation.

10. A c-Src inhibiting peptide based on Connexin43 regulates glucose metabolism in human glioma stem cells

Sara G. Pelaz[1,2,3], Myriam Jaraíz-Rodríguez[1,2,3], Rocío Talaverón[1,2,3], Laura García-Vicente[1,2,3], Marta Gómez de Cedrón[4], María Tabernero[4], Ana Ramírez de Molina[4], Concepción Lillo[1,3], José M. Medina[1,2,3] and Arantxa Tabernero[1,2,3*]

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[2]Departamento de Bioquímica y Biología Celular, Universidad de Salamanca, Salamanca, Spain
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Connexin43 (Cx43) is the main gap junction channel-forming protein in astrocytes. In addition, the carboxy-tail of Cx43 contains several motifs that can regulate signaling proteins such as the oncoprotein c-Src. Astrocytes are characterized by high Cx43 expression and low c-Src activity, whereas glioma stem cells (GSCs), the cells responsible for the resistance of this type of brain cancer to radiotherapy and chemotherapy, show decreased Cx43 expression and increased c-Src activity. It is well known that GSCs reprogram their glucose metabolism through increased expression of glucose transporters GLUT-1 and GLUT-3 and hexokinases HK-1 and HK-2, all of which can be in turn regulated by c-Src directly, or indirectly throughHIF1-α.

Interestingly, restoring Cx43 in GSCs reverses their phenotype and tumorigenicity through c-Src inhibition. We have developed a cell-penetrating peptide (TAT-$Cx43_{266-283}$) containing the region of Cx43 that interacts with c-Src that mimics the antitumorigenic effect of Cx43 on GSCs. Because TAT-$Cx43_{266-283}$ inhibits c-Src activity in GSCs, it is possible that this peptide could also modulate glucose metabolism through c-Src inhibition. Indeed, we found decreased 2-NBDG uptake when GSCs were treated with TAT-$Cx43_{266-283}$, both in vitro and in ex vivo organotypic brain slice-GSCs co-cultures, yet neuron and astrocyte metabolism was unaffected by the same treatment. We also found decreased mitochondrial metabolism and hexokinase activity, and a change in spatial distribution of the mitochondrial network, HK-1 and HK-2 in GSCs upon TAT-$Cx43_{266-283}$ treatment. Moreover, preliminary results showed that TAT-$Cx43_{266-283}$ reduced GLUT-3 and HK2 expression in GSCs in an in vivo model of glioma. In conclusion, in vitro, ex vivo and in vivo experiments revealed that TAT-$Cx43_{266-283}$ impairs glucose metabolism selectively in human GSCs.

11. Cochlear synaptopathy uncorrelated with age-related auditory temporal processing deficits in humans

Enrique A. Lopez-Poveda, Peter T. Johannesen, Byanka Cagnacci
University of Salamanca, Salamanca, Spain

In humans, ageing is associated with auditory temporal processing deficits as well as with cochlear synaptopathy, and several studies suggest that synaptopathy degrades the neural coding of temporal sound features. Here, we investigate if synaptopathy may underlie age-related temporal processing deficits. For each of 62 listeners (aged 12 to 68 years) with normal hearing (thresholds < 20 dBHL), we measured the slope of threshold-duration functions (TDSs), gap detection thresholds (GDTs), frequency modulation detection thresholds (FMDTs), life-span noise exposure, and the slope of the ABR wave-I magnitude-level function (in units of microVolts/dB). TDSs were inferred from absolute detection thresholds for pure tones (0.5- to 8 kHz) with durations from 2 to 20 ms and TDSs were corrected for the effects of long tone (500ms) threshold. GDTs were measured as the shortest detectable silent gap between two marker tones (2 kHz, 80 dB SPL) with 5 or 50 ms durations. FMDTs were measured for 30 and 60 dB SL carrier tones at 1.5 kHz modulated at 2 Hz. ABR wave I slope decreased with increasing age but was not correlated with noise exposure, consistent with age-related but not noise-related synaptopathy. TDSs, GDTs and FMTDs were not correlated with noise exposure. GDTs and FMDTs increased significantly with increasing age (consistent with previous studies) but TDSs were not correlated with age. TDS and FMDT were not correlated with wave I slope. GDTs increased with decreasing wave I slope, but the correlation became not significant when the GDTs and the slope were corrected for the effect of absolute threshold. Assuming that shallower wave I slope is a good indicator of synaptopathy, the data suggest that synaptopathy is not the cause of age-related deficits in gap and frequency modulation detection. In addition, the data dispute the notion that synaptopathy impairs the detection of brief sounds (Marmel et al., 2014, Front. Aging Neurosci. 7:63). [We thank James M. Harte, Niels H. Pontoppidan and Filip Rønne for useful discussions. Work supported by the Oticon Foundation (ref. 15-3571), Junta de Castilla y León (SAP023P17), MINECO (BFU2015-65376-P), and the European Regional Development Fund].

12. OLEOYLETHANOLAMINE RESTORES THE NEUROBEHAVIORAL DEFICITS OBSERVED IN A MODEL OF CEREBELLAR DEGENERATION

E. Pérez-Martin [1,2], D. Díaz López [1,2], C. del Pilar Rodríguez [1,2], M.J. Sánchez-Domínguez [1], J.R. Alonso Peña [1,2,3], E. Weruaga Prieto [1,2]
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The *Purkinje Cell Degeneration* (PCD) mouse is a genetic model characterized by the selective and progressive death of Purkinje cells (PCs), which causes not only motor but also gradual cognitive and social impairments. The cerebellar degeneration is a complex process divided into two different stages: a pre-neurodegenerative one -from postnatal day 15 (P15) to P18- characterized by cytological and nuclear alterations in PCs, and a degeneration *per se* in which the PCs' death takes place –from P18 to onwards-. Otherwise, the endocannabinoid *n*-oleoylethanolamide (OEA) has been reported to prevent neuronal death in different animal models of neurodegeneration, including PCD mice, when it is administered prior to the beginning of cerebellar alterations.
The main objective of this study is to refine the temporal window of OEA treatment and to assess its neuroprotective effect both by histological and behavioral analyses. OEA was intraperitoneally administered at a dose of 10 mg/kg at P14, P12 or P10. Behavioral tests were performed at different ages throughout the entire neurodegenerative process (P15, P17, P22, P30 and P40) and immunohistochemistry analyses were carried out at the end of the experiment (P30 or P40).
Our results showed that the OEA administration prior to the pre-neurodegenerative process in the PCD mutant mouse is able to deal with PCs degeneration. This neuroprotective effect follows an inverted U-shaped time-response curve where the acute administration of OEA at P12 is the most effective treatment. According with these findings, results from behavioral tests showed that the acute administration at P12 prevents partially the neurobehavioral deficits observed in this mouse model until P30. In view of these results, OEA stands out as a promising drug to fight against both the cerebellar neurodegeneration and the behavioral impairments.
Supported by MINECO (SAF2016-79668-R) and Junta de Castilla y León (SA030P17).

13. GENETICALLY IMPROVED BONE MARROW TRANSPLANTATION STOPS THE NEURONAL LOSS IN A MURINE MODEL OF NEURODEGENERATION

A. de la Mata Sampedro [1], J.F. Zapata Acebedo [1], A.N. Mota Rodríguez [1], M. Nieto Sobrino [1], J.R. Alonso Peña [1,2,3], E. Weruaga Prieto [1,2], D. Díaz López [1,2]
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The PCD (*Purkinje Cell Degeneration*) mutant mouse suffers a postnatal neurodegeneration of both Purkinje and mitral cells in the cerebellum and the olfactory bulb, respectively. Previous findings of our laboratory demonstrated that a bone marrow transplant from wild-type (healthy) donors can slow down the bulbar degenerative process but not the cerebellar one. Since PCD mice are defective for the insulin growth factor-1 (IGF-1), and this molecule also exerts neuroprotective effects, our work was aimed at enhancing genetically the bone marrow cells to be transplanted, either the hematopoietic or the mesenchymal fraction.

We have employed lentiviral particles to induce the expression of the *Igf-1* gene under a constitutive promoter in both bone marrow cell populations harvested from healthy donors. Then, we have employed these genetically enhanced cells for enriching a whole bone marrow suspension, also obtained from healthy animals. The cellular mixture was intravenously transplanted at postnatal day 20 (P20) in PCD mice that have been previously subjected to bone marrow ablation. Recipients were sacrificed at P150 and their cerebellum and olfactory bulbs analyzed to determine the effect of the transplant.

In the case of the cerebellum, transplants did not exert a neuroprotective effect on Purkinje cells, but enhanced the survival of oligodendrocytes. By contrast, in the olfactory bulb, our results showed a virtual stop of the mitral cell loss, whose density was similar to wild-type animals. The transplantation seemed not to have a strong effect on the glial reaction of PCD mice, but the IGF-1 expression reduced radically the DNA damage in PCD mice, which can be the basis of neuronal survival. Our findings clearly support the benefits of a combined gene and cell therapy.

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14. Plasticity of nitric oxide synthases in the mouse olfactory bulb

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The sense of smell is critical for the survival of most mammals. In the sensory pathway of this sense, the main olfactory bulb (MOB) rises as the first structure for the integration of the olfactory information. Within the wide variety of neurotransmitters appearing in this structure, the nitric oxide (NO) is especially relevant. NO is a small gaseous molecule that triggers many biological functions throughout the body. In the MOB, NO is produced mainly by the neuronal nitric oxide synthase (nNOS), but also by two other isoforms: the inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS). Both the MOB and the NOS exhibit a noticeable plasticity, mainly aimed at the compensation of pathological and functional alterations. Therefore, the objective of this work is to

study the expression of the different types of NOS, as well as the production of NO in the mouse MOB in the absence of the nNOS isoform. For this purpose, two experimental models were used: control and nNOS KO mice. The expression of all NOS isoforms was analyzed by immunofluorescence and Western Blot in the MOB of both experimental groups. Moreover, the NO expression was estimated in this region by the Griess method. The results obtained show that the absence of nNOS increases the expression of the eNOS isoforms, without apparent changes in the global synthesis of NO. For all this, we can conclude that plasticity of NOS in the MOB is engaged to maintain normal levels of NO in this structure, which suggests that these levels are fundamental for a correct processing of the olfactory information.

15. THE PURKINJE CELL THAT SAID NO TO DEATH

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The PCD (Purkinje Cell Degeneration) mouse is a model of neurodegeneration that suffers ataxia due to a Purkinje Cell (PC) loss. This cell death is caused by a mutation in the *Ccp1* gene, which codifies for a cytosolic carboxypeptidase that deglutamilates the α and ß tubulins. The absence of this protein causes a hyperglutamilation of tubulins, which destabilises the cytoskeleton causing the PC death. Interestingly, the cerebellar lobule X presents a noticeable neurorresistance to this all death, whose underlying factors are the objectives of this study. We studied by qPCR in both PCD and wild type mice the expression pattern of *Ccp1* and other genes with similar function. Additionally, we have studied the expression pattern of *Ttll1*, implicated in the glutamilation of tubulins (the opposite function of *Ccp1*). Finally, we have studied by immunohistochemistry the expression of HSP25; a Heat Shock Protein that is a proved neuroprotective factor in other ataxia models.

Our qPCR results suggest that *Ccp1* is lower expressed in the lobule X than in the other lobules at all ages studied in wild type mice. This fact suggests that the lobule X is less dependent of the expression of *Ccp1*, so under its absence in PCD mice, the damage caused is less severe. We have also seen a complementary function of the other *Ccp* proteins to *Ccp1*. The immunohistochemistry results show that HSP25 is higher expressed in the PCD lobule X than in the WT one at P30 and P35. Therefore, we can conclude that both genetic and neuroprotective factors could be the reason why the lobule X resists longer that the other cerebellar lobules in the PCD mouse.

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16. IMMUNE CELL MODULATION BY SELECTIVE NEURONAL DEATH

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Neurodegenerative diseases share associated inflammatory reactions, which can be associated with an altered immune cell infiltration into the brain parenchyma.

We wanted to know how selective neurodegeneration modulates immune cell infiltration into the brain parenchyma, and the phenotype of peripheral immune cells. Therefore, distinct neuroinflammatory states were evaluated: a) no damage, b) low gamma radiation (3 Gy), c) administration of lipopolysaccharide (LPS, 2 mg/kg) and d) Purkinje Cell Degeneration (PCD) mice, with two separated neurodegenerative scenarios(cerebellum and olfactory bulb).Animals were perfused intracardially at postnatal day 15 (P15), P20, P25, P30, P40 or P70, and their brains dissected. Both cerebellum and olfactory bulb were analyzed by immunohistochemistry to visualize the infiltrated cells. For the exploration of peripheral immune cells, myeloid and lymphoid populations from WT and PCD spleens were analyzed by flow cytometry.

The number of leukocytes (CD45-positive cells) in the cerebellum increased moderately in PCD mice and dramatically in LPS-treated animals, when compared with controls. By contrast, irradiated animals suffered a substantial decline of such cells. Interestingly, leukocytesin PCD mice tended to distribute in those layers of the cerebellar cortex mostly affected by Purkinje cell degeneration. This phenomenon was not observed in LPS-treated and irradiated mice, which held a general inflammation. No significant differences between PCD and control mice were found in the olfactory bulb, probably due to a milder neurodegeneration occurring in this region. Concerning the analysis of spleens, we found a lower expression of several myeloid cell subsets, together with a less apparent mean fluorescence intensity in markers related to antigen presentation in PCD animals than WT mice.

These findings suggest that the selective death of Purkinje cells induces an attractive effect on immune cells. On the other hand, the *pcd* mutation seems to be responsible for a blockage in leukocyte maturation.

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17. SECRETAGOGIN EXPRESSION IN THE MOUSE OLFACTORY BULB UNDER DIFFERENT SENSORIAL IMPAIRMENTS

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Secretagogin (SCGN) is a calcium binding protein of the "EF-hand" family that is widely expressed in the olfactory bulb. In the present work, we have studied such expression in different experimental models with olfactory alterations: PCD (Purkinje Cell Degeneration) and olfactory-deprived –with occlusion of one nostril– mice. PCD mutants suffer the degeneration of the mitral cells, the main projecting neurons of the olfactory bulb. Besides, olfactory-deprived mice do not receive sensory information in one of their olfactory bulbs. The migration and differentiation of new SCGN-positive neurons was also analyzed.

Unilateral olfactory deprivation was performed by occluding surgically the right nostril of mice at P70 (coinciding with the beginning of the PCD bulbar degeneration). Animals were perfused at P110 (when mitral cell degeneration of PCD mice has already ended), and the tissue was studied by immunohistochemistry, with specific antibodies against SCGN, also combined with other neuronal and proliferating markers.

Our results confirmed that the cells expressing SCGN are mainly confined to glomerular and granular layers. Besides, PCD mice did not show differences in the expression of SCGN respect to wild-type animals. However, olfactory-deprived mice showed a significant increase in the number of SCGN-positive cells mainly in the granule cell layer and in the medial region of the glomerular layer.

Our results demonstrate that the SCGN expression increases due to the loss of olfactory inputs, caused by the closure of a nostril, but not in the PCD model, where the arrival of olfactory inputs is normal. Therefore, SCGN distribution depends on olfactory stimuli but not of bulbar efferences.

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18. A NEW PROFILE OF BULLYING VICTIMS: PREMATURE INFANTS WHO REPORT COMMUNICATION DISORDERS

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Numerous campaigns against bullying are currently carried out, but the research on this phenomenon in Primary School Grades is very limited. Although there are several investigations aimed at studying the profile of bullying victims, the analyses of the causes are scarce. Therefore, in the present investigation, a possible new profile of bullying victims is analyzed; it is focused on premature infants who report Communication Disorders, more specifically speech disorders (F315. 39).
More precisely, we have analyzed whether the causes of the bullying-suffering infants can be derived from their premature birth or its fallout (mainly, because of a Phonological and Fluency Disorder). For this purpose the Test *Acoso y Violencia Escolar*; AVE (Piñuel y Oñate, 2006) has been applied to students from Primary School grades, in order to determine the susceptibility for bullying and its type. Moreover, other factors such as prematurity, speech pathologies, gender, age, school grade, etc., have been also taken into account for the establishment of putative relationships.
The results show that premature children report a high correlation coefficient (0.334) of bullying suffering ($p = 0.009$). This risk is even bigger when prematurity is accompanied by any other factor (dyslalia and/or other speech pathologies) and linked to potential characteristics of bullying victimology (uncommon ethnic group, physical features, eating disorders…; $p = 0.023$). This confirms a high correlation coefficient (0.295) between prematurity and any type of comorbidity fallout.
As a conclusion, the present work suggests that premature children with speech disorders should be included as a possible bullying victim profile. This measure should be implemented at newborn facilities of hospitals, to follow up this risk population for preventing and avoiding future problems with their schoolmates.
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19. A binaural hearing aid inspired by the contralateral medial olivocochlear reflex

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Activation of the medial olivocochlear reflex (MOCR) reduces cochlear mechanical compression, restores the dynamic range of auditory nerve fibers for tones in noise to values observed in quiet, and presumably facilitates the recognition of speech in noise. The MOCR operates via the outer hair cells (OHCs) in the cochlea. Sensorineural hearing loss is often associated with total or partial loss or

dysfunction of the OHCs and with reduced or absent cochlear compression. Hearing-impaired listeners show greater difficulties understanding speech in noise than normal-hearing listeners, even when using hearing aids. We hypothesize that this may be partly due to their having reduced or absent MOCR antimasking effects. Indeed, cochlear-implant (CI) users can show better speech-in-noise intelligibility with a binaural sound processing strategy inspired by the contralateral MOCR (the MOC strategy) than with two independently functioning audio processors. Here, we report the first steps towards implementing the MOC strategy in combination with 'conventional' hearing aids (MOC-HA). Our approach involves two time-domain, multi-channel, non-instantaneous compressors (one per ear) with bilateral and dynamic control of compression. We discuss to what extent the functioning of the MOC-HA resembles that of the natural MOCR.

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20. ESTUDIO DESCRIPTIVO Y DE INTERVENCIÓN DIETÉTICA EN LA ESCLEROSIS MÚLTIPLE: REPERCUSIONES CLÍNICAS Y ANALÍTICAS.

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La esclerosis múltiple (EM) es la enfermedad autoinmune inflamatoria y degenerativa del sistema nervioso central más prevalente en nuestro medio, probablemente desencadenada por un agente ambiental en un individuo susceptible genéticamente.

Nuestro objetivo principal es conocer los hábitos dietéticos de los pacientes, cómo comen, y si el cambio de esos hábitos de forma individualizada y con objetivos concretos y predeterminados tiene repercusiones clínicas y/o analíticas.

Se han analizado los hábitos nutricionales de 41 voluntarios diagnosticados de EM y se han registrado variables clínicas (edad, sexo, EDSS, peso, IMC, complexión) y analíticas (hemoglobina, colesterol, glucosa, urato, creatinina, calcio, triglicéridos, proteínas totales, albúmina, hierro, ferritina, vitamina D, transferrina, homocisteína (Hcy), TSH, vitamina B12 y ácido fólico).

Se establecieron pautas dietéticas para conseguir un IMC óptimo en aquellos casos en que era necesario. Se realizó un seguimiento en consulta de nutrición semanal/quincenal y en consulta de EM cada 3-6 meses con control analítico. Los resultados obtenidos nos indican que la intervención dietética en pacientes con EM reduce los niveles plasmáticos de Hcy y esta reducción es mayor en aquellos

pacientes que disminuyen su IMC. Asimismo, se observa un aumento en los niveles plasmáticos de ácido fólico. El resto de resultados analíticos está en fase de estudio estadístico. También se analizarán las variaciones en la EDSS de los pacientes durante los meses de intervención dietética, aunque queda fuera del alcance de este estudio establecer una relación entre disminución de niveles de Hcy mediante la dieta y el pronóstico de la enfermedad, pero de sus conclusiones tal vez se pueda señalar el camino para futuros estudios.

21. Analysis of the Neurobiological effects of drugs of abuse and of analgesic agents. An *in vivo* approach

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At present, drugs of abuse are considered a serious public health problem due to their addictive properties. The recreational use of new compounds, either obtained from natural products or of synthetic origin, with potential to develop tolerance and dependence is rapidly rising up. Drugs of abuse elicit their actions by stimulating the reward pathway and by increasing mesencephalic dopamine release, but there is a lack of knowledge regarding their side effects, putative targets and signalling pathways, as well as their long-term consequences. Zebrafish (*Danio rerio*) is an advantageous tool to evaluate the *in vivo* effects of pharmacological agents. Zebrafish neurotransmitter systems share similar molecular, pharmacological and biochemical profiles with their human homologues, so that the obtained results can be easily extrapolated to higher vertebrates.

Our research interests are focused on the effects of several agents in the development of the Central Nervous System using the zebrafish as an experimental model and their effect on several behavioural responses. Morphine and other opiate analgesics are the drugs of election to treat moderate-to-severe pain and they elicit their actions by binding to the opioid receptors. The activation of this neurotransmitter system is also responsible for the undesirable side-effects of opiates, such as tolerance and dependence, which lead to the instatement of addiction and produce long-term dysfunctions in the Central Nervous System.

Zebrafish embryos were exposed to morphine and cocaine from 5 hpf (hours post-fertilization) to 3-6 dpf (days post-fertilization) and then we have used different experimental approaches to analyze the biochemical effects of these drugs of abuse. The amount of drug absorbed by the embryos was assessed by Mass Spectrometry techniques, transcriptomic changes have been determined by RNAseq

followed by qPCR validation, Western Blot has been used to determine the effect of these treatments in the protein levels, global methylation assays have shown epigenetic modification elicited by morphine and cocaine and finally we have measured the levels of several low-weight metabolites. Our results indicate that all levels of biological information can be modified by drugs of abuse: epigenetics, transcriptome, proteome and metabolome, and that the elicited changes depend on the drug used, the length of the treatment and the developmental stage, which can be related to the maturation of the Central Nervous System.
Our results indicate that we have validated an experimental model to *in vivo* test the biological effects of drugs of abuse, and the outcome can be easily extrapolated to higher vertebrates (i.e. mammals), as the observed effect is similar to those found in mammalian models and in human subjects.
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22. Expression of Sox2 in the Visual System of Fish

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The visual system of teleost fish has growth and regeneration capacities during the entire animal's life. Retinal neurogenesis continues after embryonic development by the preservation of a peripheral germinal zone (PGZ) in the circumferential edge of retinal tissue. Thus, the pre-encephalic visual system [retina, optic nerve head (ONH), and optic nerve (ON)] of adult fish serves as a model for studying neurogenesis and regeneration of the vertebrate central nervous system (CNS).
Our study focused on the particular expression pattern of Sox2 in fish. Sox2 is a transcription factor known for its function in keeping stem cell properties, and as a regulator of cell fate during development, especially in the visual system.
It is not yet clear why Sox2 expression is maintained in mature cells, but it is known that down-regulation of Sox2 expression during development affect the expression of others transcription factors.
We used two different fish species each with a particular advantage: *Astatotilapia burtoni* (cichlid fish) and *Danio rerio* (zebrafish). Cichlid fish is a model organism for behavior and visual system growth adding substantial retinal tissue and ON fibers throughout life, and zebrafish is an established teleost model organism.
Staining retina, ONH and ON cryosections, with antibodies for Sox2 we found many positive cells in several retinal layers and in the ON. Surprisingly, we did not find any Sox2 expression in the ONH. For cell identification, we used co-localization with known antibodies for cell proliferation, cells of the astroglial family and

neuronal cells. We showed co-localization of Sox2 and the different used antibodies, i.e. in proliferating and differentiated cells. Results are summarized and generalized for both fish in the schematic diagram.
To determine which cell types occur in the entire extent of the pre-encephalic visual system of fish, and to discover their functions will be essential to understand the high plasticity that the fish show. The variety of glial cells is remarkable, and the unknown mechanisms of cell interaction suggest a role in the normal growth and regenerating processes of the vertebrate CNS.

23. Optic Nerve Glial Cell Culture of Three Fish Species

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Growth and regeneration processes directly involve the participation of at least two types of glial cells, astrocytes and oligodendrocytes to support, guide, nourish, and myelinate the elongating axons in both processes. The optic nerve (ON) is a central nervous system structure formed by axons from retinal ganglion cells, and glial cells surrounded by meninges.
To study the biological and physiological properties of these glial cells, cell culture tools provide several advantages. In addition, this approach reduces the use of animals. Currently, a specific protocol for glial cells from ON of fish does not exist.
Our goal was to establish an appropriate and effective protocol to culture glial cells from fish ON. We describe the design and refinement of such a protocol and prove the efficacy in three fish species, *A. burtoni*, *C. auratus*, and *D. rerio*.
The cichlid fish is a model organism for behavior and visual system growth, *C. auratus* is a cyprinid with an evident bigger ON than the cyprinid zebrafish, and *D. rerio*, is an established teleost model organism with available genetic tools.
We used adult fish, wild type and the double transgenic Tg(sox10:TagRFPt)(gfap:EGFP) in the case of zebrafish. The animals were deeply anaesthetized and killed by decapitation. We removed the extraocular part of ON in PBS (Saline Phosphate Buffer) supplemented with antibiotics. For the enzyme treatment, we used trypsin with EDTA, and to the mechanical dissociation, we used double pipette tips. After stopping the trypsin reaction by adding FBS (Fetal Bovine Serum), we centrifuged the cell suspension for 5 min at 1400 rpm. The pellet was re-suspended in Leibovitz's (L15) medium supplemented with 20% of FBS and antibiotics. Cells were seeded and cultured in an incubator at 28±1°C. During the first three days in culture, the flasks were untouched in the incubator. Afterwards, the medium was changed every 5–7 days.

The established protocol allows us to culture cells of the ON which were identified as glial cells using different known antibody markers and expressing fluorescent protein in case of transgenic zebrafish. This protocol was essentially feasible for all three species, however due to the ON size, the success of cultures and gain of cells was much higher in cichlid fish and goldfish compared to the zebrafish.

24. Attentional modulation of the middle ear muscles: neural circuits and functional implications.

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One of the most striking abilities of the brain is to selectively attend to a specific auditory stimulus in a complex multisensory environment. The underlying mechanisms and neuronal circuitries involved in this attentional gain control have been hotly debated, but they still remain unclear. Here, we carried out functional studies in humans and neuroanatomical experiments in rats to investigate the role of the middle-ear muscles in the auditory selective attention. In the functional study, we assessed the middle-ear admittance and frequency resonance of normal hearing participants that were asked to pay attention to a specific conversation in a noisy environment. Thus, tympanometry was tested using a white noise of 70 dB accompanied by a recorded low- and high-pitch conversations of 82 dB. Our results indicated that acoustic impedance of the middle-ear changed depending upon whether the subject focuses attention on the noise or a specific conversation. To determine the neuronal circuits underlying the modulation of the middle-ear muscles, we injected the retrograde tracer Fluorogold® into the tensor tympani and stapedius muscles to visualize their corresponding motoneurons. Subsequently, we carried out immunohistochemistry to determine putative afferent neurotransmitters on these Fluorogold-labeled motoneurons. To provide more insight into this modulatory network circuitry, we performed double tract-tracing experiments that combined Fluorogold® injections into the middle-ear muscles with injections of the anterograde dextran tracer into different brain nuclei. In sum, our study opens a new perspective in the functional role of the middle-ear muscles, acting as a frequency-specific selective filter that optimizes sound information previous to the cochlear mechanotransduction. Our results also provide neuroanatomical data that explains how the central nervous system exerts influence of auditory selective attention on the peripheral auditory system.

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25. Efectos de la administración de cannabidiol para el tratamiento de la epilepsia en el modelo GASH/Sal

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Introducción. En los últimos años, los cannabinoides se están empleando para el tratamiento de la epilepsia. El cannabidiol (CBD) es el principal compuesto no psicoactivo de la planta *Cannabis sativa*. Está descrito como un excelente anticonvulsivo, pero aún existe controversia en cuanto a su posología y posibles efectos secundarios. El modelo GASH/Sal, gracias a las crisis audiógenas convulsivas que padece, supone una herramienta para el desarrollo de nuevas estrategias terapéuticas contra la epilepsia. Objetivos. Determinar la eficacia del CBD como fármaco antiepiléptico y evaluar posibles efectos secundarios tras su administración semicrónica. Material y métodos. Mediante el programa ethomatic se evaluó la presencia y severidad de las crisis convulsivas en el GASH/Sal antes, durante y después del tratamiento con CBD. Para determinar los posibles efectos derivados de la administración prolongada de la droga y su interrupción, se realizaron estudios de campo abierto en hámsters control y GASH/Sal con y sin tratamiento, empleando pare ello el software ANY-maze. Resultados. La administración del CBD elimina las crisis epilépticas entre la hora y las 4 horas tras su administración única y este efecto se vuelve permanente a los pocos días de tratamiento repetido. No se observaron diferencias en el comportamiento de los animales con y sin tratamiento, ni durante el mismo, ni en los días posteriores a su interrupción. Conclusiones. La administración semicrónica de CBD elimina la susceptibilidad a sufrir crisis convulsivas del modelo GASH/Sal y no provoca cambios de comportamiento observables ni durante el tratamiento, ni tras la suspensión del mismo.

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26. Enhanced Thermal and Inflammatory Pain on an ARMS/Kiddins220 Knock-Down mouse model

J. Sánchez-Sánchez , S. López-Benito , C. Vicente-García , J.C. Arévalo

Pain is defined as “an unpleasant sensory and emotional experience that is associated with actual or potential tissue damage or described in such terms” and compasses a wide spectrum of aetiologies. Nerve Growth Factor (NGF) and its receptor TrkA, are directly involved in nociception and have been implicated in chronic pain as interfering in NGF/TrkA axis ameliorates the symptoms of osteoarthritic patients. Among other downstream proteins, the scaffold protein ARMS/Kidins220 participates on NGF/TrkA signalling. However, no relationship between ARMS/Kidins220 and nociception has been established yet. To assess this matter, we generated a transgenic mouse model in which ARMS/Kidins220 expression has been reduced exclusively in TrkA-positive neurons. During the behavioural tests, ARMS/Kidins220 knock-down mice showed altered thermal and inflammatory pain perception compared to their control littermates. Nevertheless, no differences were found under cold or mechanical stimuli. These results suggest a specific role of ARMS/Kidins220 in heat sensitivity and inflammatory pain. Further experiments are being performed to decipher the molecular mechanisms by which this scaffold protein is acting on the sensory pathways.

27. CRB2 IS INVOLVED IN THE APICOBASAL POLARIZATION OF RPE CELLS BY PARTICIPATING IN TIGHT JUNCTION MAINTENANCE AND CELL CYCLE ARREST

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Apicobasal polarity is essential for the precise performance of epithelial cell’s functions. It is determined by the expression of three polarity protein complexes named Scribble, Par and Crumbs. While Scribble complex promotes the differentiation of the basolateral domain, both, the Par and the Crumbs complexes, promote the apical side identity. The Crumbs complex is composed by PALS1, PATJ and the CRB proteins. We have described the expression of one of the CRB proteins in retinal pigment epithelial (RPE) cells, the CRB2 protein. To better understand the highly regulated process of polarization, we have studied the role of the polarity protein CRB2 in human RPE cells during differentiation in vitro and in mature murine RPE cells in vivo. To do this, we have analyzed the sequential expression and localization of the polarity proteins during human RPE cells differentiation. We have knocked down CRB2 in cultured RPE cells and analyzed their proliferation rate, expression and localization of proteins related with the establishment of junctional complexes and those involved in polarization. We have

also measured the transepithelial electrical resistance, a direct measure of the strength of the cell-cell junctions, during ordinary differentiation and after a severe disruption of junctional complexes with a calcium switch assay. Finally, we have analyzed the role of CRB2 in adult mouse RPE cells in vivo. The results showed that CRB2 is the latest of the whole pull of polarity proteins to be positioned at the cell membrane. Subsequently, the absence of the CRB2 protein from these cells results in a delay in the formation of cell-cell junctions and in an increase in cell proliferation in human RPE cells. In addition, our studies in vivo show that knocking down CRB2 in RPE cells affects the distribution of different apical polarity proteins and perturbed the retinal homeostasis. Together our results demonstrate that CRB2 is a key protein for the development and maintenance of a polarized epithelium.

28. Relationship between NGF/TrkA axis and ADRA2b in osteoarthritic knee pain.

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Acute pain is an unpleasant but necessary sensation for survival, but when it becomes chronic is a major social, health and economical burden. Osteoarthritis (OA), a degenerative joint disease, is nowadays responsible for a large percentage of chronic pain observed in humans. Nerve growth factor (NGF) and its receptor, TrkA, play an important role in the generation of acute and chronic pain and blocking NGF alleviates pain symptoms of knee OA patients. To identify genes downstream of NGF/TrkA axis responsible for the enhanced mechanical sensitivity in OA, we have used a mouse expressing a mutant TrkA that develops mechanical hypersensitivity compared to wild type (WT) mice in the monoiodoacetate (MIA) model of OA. We have carried out gene expression analysis from WT and KI ipsilateral L3-5 DRGs collected at early pain phase, 5 days post-saline or MIA injection. One of the most significant genes altered in the microarrays and qPCR analysis was ADRA2B, which belongs to the α2 adrenergic receptor family, whereas ADRA2A and ADRA2C, the other two members of the family, showed no differences in gene expression. At the protein level, ADRA2B was upregulated exclusevely in the ipsilateral DRGs of mice in response to MIA. In addition, blocking ADRA2B, with the specific inhibitor Imiloxan, enhanced the mechanical sensitivity in MIA-injected

mice in the ipsilateral paw. Finally, in vitro studies showed that ADRA2B expression regulates specifically TrkA expression. Altogether these results support the idea that ADRA2B participates together with NGF/TrkA axis in a functional network that may play a relevant role in pain modulation in osteoarthritis.

29. Neurons along the auditory pathway exhibit a hierarchical organization of prediction error

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According to the predictive coding framework, our perceptions emerge from the reciprocal exchange of predictions and prediction error signals between hierarchically organized neural regions. This interaction has been identified in macroscopic brain activity (e.g. MMN), but hierarchical predictive processing at the neuronal level remains unrevealed in the auditory pathway. In this study, anaesthetized rats and awake mice were played sound sequences designed to separate the neuronal responses due to prediction errors from those due to adaptation, while single-neuron and local-field activity was recorded from different subdivisions of auditory midbrain, thalamus and cortex. Predictive activity was indeed detected already at the level of inferior colliculus, increasing in proportion from subcortical structures towards cortical ones, and from lemniscal to nonlemniscal divisions. Greater predictive activity was found for low intensity stimulation, maybe acting as a gain system for challenging stimular contexts. Local-field recordings confirmed the time frame of this neuronal prediction error signals was compatible with that of the MMN. The generation of prediction errors was consistent between species and states of awareness, indicating that it is a cellular automatic process and a fundamental component of auditory processing.
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30. El biosensor HyPer 2 para la monitorización del peróxido de hidrógeno intracelular en fibras de músculo esquelético expuestas a insulina e interleukina 1 beta

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El peróxido de hidrógeno (H_2O_2) desempeña un papel esencial en numerosos procesos fisiopatológicos. Así, el H_2O_2 puede actuar como molécula de señalización y regular diferentes vías de señalización celular. Las vías de señalización de la insulina y la interleukina 1 beta (IL-1β) en el músculo esquelético podrían estar moduladas por el H_2O_2.
En este estudio, la secuencia codificante de HyPer2, un biosensor de H_2O_2 codificado genéticamente, fue microinyectada y electroporada en el músculo *flexor digitorum brevis* (FDB) de ratón. Posteriormente se procedió a aislar fibras musculares del FDB mediante una digestión enzimática y se mantuvieron en cultivo. Las fibras aisladas en cultivo se sometieron a condiciones experimentales en las que las fibras se exponían a insulina o IL-1β. Las fibras que expresaban el biosensor HyPer2 fueron monitorizadas en tiempo real mediante microscopía de fluorescencia para registrar los cambios en la concentración intracelular de H_2O_2 durante 60 minutos. Los resultados indicaron que el biosensor HyPer2 detectaba un aumento ligero pero continuo en la concentración intracelular de H_2O_2 15 minutos después de la adición de insulina o IL-1β.
Los resultados sugieren que tanto la insulina como la IL-1β provocan un leve aumento en la producción de H_2O_2 intracelular en la fibra de músculo esquelético.

31. EPIDURAL AUDITORY CORTEX ANODAL DIRECT CURRENT STIMULATION AFTER SOUND OVERSTIMULATION. A NEW APPROACH FOR OTOPROTECTION.

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In our previously work , we show an increases in auditory thresholds (measured by auditory brainstem evoqued potentials recordings) after 0.1 mA anodal epidural multisession stimulation (EMS) of the auditory cortex. Such decrease in auditory

sensitivity (up to 20 dBls) is exploited here to analyze potential otoprotective effect to minimize hearing loss induced by acoustic overstimulation.
A silver squares (1x3mm) electrode was stereotaxical implanted along the rostro-caudal axis of the auditory cortex. A week after surgery, 0.1 mA direct current was delivered in awake male Wistar rats of 250 gr in 4 daily sessions. A day after the end of stimulation protocol rats were exposed to a 2h single session of white noise at 115dB. A sham control group were stimulated by a similar EES protocol but without acoustic overstimulation. To evaluate changes in auditory thresholds ABR were recorded after surgery in parallel in sham control and sound overstimulated animal groups at 1, 7 and 14 days after the electric current stimulation protocol. Brains and cochleae of all animals was fixed for immunocytochemical analysis at the end of experiments. To assess the effects of epidural electric stimulation (EES) on the brain cortex, well characterizedfunctional anatomical markers of glial cells (GFAP/Astrocytes and Iba1/microglial cells) and neurons (c-Fos) were analyzed in alternate serial sections by quantitative immunocytochemistry. Immunostaining for c- Fos, Iba 1 and GFAP of serial sections allows to demonstrate precise localization of the electrode and also a local and a global electrolytic overactivation withoutlesional effects on the brain.
Increases of 20 to 30 dB ABR auditory thresholds was observed 4 days after the EES multisession protocol. Time course of tresholdship, wave amplitudes and latencies of the ABRS in control and sound overstimulated animal groups will discussed by the authors.

32. EPIDURAL DIRECT CURRENT STIMULATION OF THE AUDITORY CORTEX: c-FOS, GFAP AND IBA1 IMMUNOCYTOCHEMICAL QUANTITATIVE ANALYISIS.

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Epidural electrical stimulation (EES) is an invasive technique used to treat brain diseases. Applying low-intensity electrical current in the brain has been demonstrated its effectiveness in neuro-otological diseases such (e.g. tinnitus, acoustic hallucinations) andother central auditory disorders. Glial cells are known to react electricalfield. Furthermore, axons are more sensitive to the electrical stimulation than cell bodies. Here we consider multi-session EPS elicits structural anatomical changes in the cortex and global electrolytic effects in the brain. We applied multiple sessions of 1mA epidural anodal direct current stimulation directly on the auditory cortex (AC) in rat for 10 minutes and analyzed glial activation and cytoarchitectural cortical reorganization by immunocytochemistry using glial cell markers such as GFAP and Iba1, for astrocytes and microgliocytes respectively, and

the immediate early gene c-Fos. Our results show a localized glial activation (AZ) within the cytoarchitectural limits of the AC. Thus, GFAP is overexpressed locally in the electrical field. However, global glial activation also occurs surrounding the blood vessels and ventricles. Analysis of c-Fos immunoreactive neurons shows a statistically significant increase in the number of immunoreactive neurons in supragranular layers and a decrease in layer 6, as well as a loss of the canonical laminar organization of the cortex. We conclude multi-session EPS on the AC induces local and global glial activation due to an electrolytic effect and structural reorganization of cortical layers.

33. Octopus cells of the cochlear nucleus: An uncommon neural pathway for extraordinary temporal precision in auditory processing

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All natural sounds are complex because they consist of a mixture of frequencies. In the cochlea, high frequency tones are detected slightly earlier than low frequency tones. This “cochlear delay” is subsequently transmitted to the brain, which means that sounds of differing frequencies do not reach the central nervous system (CNS) simultaneously. Yet, the CNS has to interpret as a unique acoustic object the complex sounds coming from a given source. This amazing temporal integration is performed by the “octopus cells” of the posteroventral cochlear nucleus (PVCN).
To better understand how the brain integrates complex sounds, we studied the connections of octopus neurons in the rat. We labeled their axons selectively by injecting the bidirectional neuroanatomical tracer biotinylated dextran (BDA) in the region located between the motor nucleus of the facial nerve and the lateral superior olive, which is traversed by the axons of the octopus cells. The tracer taken up by the axons was transported retrogradely all the way to the cell bodies of octopus cells in the ipsilateral PVCN, thus proving the success of our experimental design. The tracer was transported also anterogradely in the axons, filling with unprecedented detail their terminal ramifications and synaptic specializations.
Our results show that octopus cell axons travel in the intermediate acoustic stria, which crosses the inferior cerebellar peduncle and then surrounds medially and laterally the spinal root of the trigeminal nerve. Moreover, octopus cells innervate the dorsal periolivary region and the superior paraolivary nucleus of both sides, as well as the contralateral ventral nucleus of the lateral lemniscus. In the latter, the axons end as large calyx-like synaptic specializations that surround the cell body of the postsynaptic neuron.

All these data could be used to improve the way next-generation hearing aids and implants perform the temporal integration of complex acoustic information.

34. CARACTERIZACIÓN DE LAS EPILEPSIAS PRESENTES EN LOS MIEMBROS DE UNA ASOCIACIÓN DE PERSONAS CON DISCAPACIDAD INTELECTUAL

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Las personas con discapacidad intelectual (DI) han sido y son un grupo de población especialmente vulnerable. En el ámbito de la salud se describen peores niveles de salud y peores resultados sanitarios que en la población general. Presentan una mayor prevalencia de entre otras, enfermedades del SNC, sobre todo epilepsia, en este trabajo pretendemos identificar la incidencia, situación clínica, necesidades asistenciales y sociales, y calidad de vida de personas con DI y síndromes epilépticos en amplio grupo de personas con DI.
Seleccionamos una muestra de pacientes de la asociación ASPRODES Ciudad de Salamanca con diagnósticos de epilepsia y discapacidad intelectual, de los que obtenemos datos generales y patológicos, patrón clínico de presentación y evolución de la epilepsia en esta población, así como el perfil psicopatológico y conductual asociado al proceso epiléptico en estos pacientes. Pretendemos determinar las necesidades asistenciales en esta población y el grado de consecución de las mismas.
Encontramos una población heterogénea en cuanto a la edad, con mucha variabilidad en las constantes vitales, y en las manifestaciones clínicas de la enfermedad, con un altísimo grado de medicación poco supervisada y con problemas de seguimiento por parte de los especialistas. También podemos observar la presencia de síntomas de comportamiento y psicopatológicos que aumenta esta poli medicación. Planteamos establecer un protocolo de atención sanitaria para población con DI y epilepsia, incluyendo medidas de mejora asistencial.

35. EFFECTS OF CONNEXIN43 REGION 266-283 IN NEURAL STEM CELLS FROM THE SUBVENTRICULAR ZONE IN AN IN VIVO GLIOMA MODEL

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Glioblastoma multiforme is one of the most aggressive brain tumours worldwide. Part of its malignancy lies in a subpopulation of tumour cells known as Glioma Stem Cells (GSCs). Aiming to eliminate these cells, the group designed the cell-

penetrating peptide TAT-Cx43$_{266-283}$, based on the interaction between connexin43 and the oncogenic protein c-Src, observing significant effects of this peptide on the reduction of tumour cell proliferation and migration. Several studies have shown a relationship between GSCs and neural stem cells (NSCs) from the subventricular zone (SVZ), one of the remaining neurogenic niches in adulthood. Therefore, in this work we aimed to investigate whether TAT-Cx43$_{266-283}$ affects SVZ NSCs in a glioma model in vivo. In order to do that we studied the number of SVZ NSCs by immunohistochemistry, using the marker nestin, as well as their differentiation to glial (astrocytes) and neural lineage using GFAP and doublecortin, respectively. Our preliminary results suggest that the implantation of tumour cells slightly increased nestin and GFAP levels within the SVZ, and that the treatment with TAT-Cx43$_{266-283}$ reverted this effect. These results suggest a relationship between the tumour and the number of astrocytes generated in the SVZ. In absence of tumour cells, TAT-Cx43$_{266-283}$ did not modify the levels of GFAP and doublecortin in the SVZ, suggesting that TAT-Cx43$_{266-283}$ did not affect differentiation of SVZ NSCs under resting conditions.

36. Alteraciones moleculares y morfológicas en la cóclea del modelo de epilepsia audiógena GASH/Sal

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Introducción: El hámster GASH/Sal (*Genetic Audiogenic Seizure Hamster from Salamanca*) es un modelo genético de epilepsia audiogéna que sufre convulsiones tónico-clónicas generalizadas en respuesta a un estímulo acústico de alta intensidad. **Objetivos**: Investigar las alteraciones moleculares y morfológicas en la estructura coclear que puedan estar relacionadas con la susceptibilidad del GASH/Sal a las convulsiones. **Métodos**: Se utilizaron 9 hámsters sirios controles y 9 GASH/Sal machos de 8 semanas de edad. Para el estudio molecular se analizaron la expresión de los genes *Gpr98*, *Cdh23* y *Pcdh15* mediante PCR cuantitativa en animales con y sin estimulación acústica. Para el análisis histológico, se tomaron microfotografías del órgano de Corti (OC) y de las células ganglionares que se analizaron con el software Image J. Todos los experimentos fueron aprobados por el Comité de Bioética de la Universidad de Salamanca. **Resultados**: En animales sin estimulación, la expresión relativa de los genes *Gpr98*, *Cdh23* y *Pcdh15* fue menor en la cóclea del hámster GASH/Sal respecto a los controles (p <0,05). Sin embargo, en los GASH/Sal estimulados, se observa un aumento en la expresión de todos los genes en relación al GASH/Sal sin estimular y al grupo control estimulado (p <0,01). Estos resultados se correlacionan con alteraciones morfológicas en las células ciliadas del OC y una reducción del número de neuronas del ganglio espiral (p <0,05) en el GASH/Sal. **Conclusiones**: En estado basal, el GASH/Sal presenta

alteraciones en la expresión de los genes implicados en la funcionalidad y estructura de las células ciliadas del OC, así como defectos morfológicos en el receptor auditivo. Estas alteraciones moleculares e histopatológicas pueden contribuir a la susceptibilidad a las convulsiones audiógenas del modelo GASH/Sal.
Agradecimientos: Universidad de Salamanca, programa de apoyo a los GIR 2019 (IP-López DE); Conserjería de Educación JCyL #SA070P17 (IP-Lopez DE); Conserjería de Sanidad #GRS 1600/A/17 (IP-Gonçalves JM).

37. Análisis y validación del transcriptoma del foco epileptogénico en el modelo de epilepsia GASH/Sal

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Introducción. El hámster GASH/Sal es un modelo de epilepsia audiógena de origen genético cuyo foco epileptogénico es el colículo inferior (CI). **Objetivos**. Analizar el transcriptoma del CI para obtener un perfil de los genes relevantes en el GASH/Sal que podrían usarse para buscar un vínculo común con la epilepsia humana. **Metodología**. Empleando la técnica RNA-Seq realizamos un estudio comparativo del transcriptoma del CI de hámsteres controles y GASH/Sal sometidos a estimulación auditiva, mediante la plataforma Illumina 75bp Single-End. Se seleccionaron estadísticamente los genes diferencialmente expresados entre ambos grupos experimentales y se clasificaron ontológicamente (GO) según su función molecular, procesos biológicos y celulares. Finalmente, un grupo representativo de estos genes fueron validados por RT-qPCR. **Resultados.** El análisis comparativo de los dos transcriptomas dio como resultado la detección de 36 genes diferencialmente expresados entre ambos grupos de hámsteres, siendo un grupo de ellos factores de transcripción y, otros, implicados en procesos de transporte y unión entre moléculas, entre otras categorías. 25 de los 36 genes diferencialmente expresados tras el análisis de los transcriptomas fueron validados mediante la técnica RT-qPCR. **Conclusiones.** Los resultados muestran algunos relevantes cambios moleculares, a nivel de variaciones de expresión génica, en el núcleo epileptógeno (el CI) del GASH/Sal tras las crisis convulsivas. El conocimiento de este conjunto de genes bien pudiera ser la génesis de una mayor comprensión de los mecanismos ictales en este modelo animal y posibles correlaciones con la epilepsia humana.
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38. Estudio sobre el mecanismo anti-inflamatorio de la VNS en el modelo de epilepsia hámster GASH/Sal

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Introducción: Aunque el efecto antiepileptógeno de la estimulación del nervio vago (VNS) está demostrado en la clínica, el mecanismo de acción es desconocido. Una de las hipótesis sugiere un efecto antinflamatorio. **Objetivos**: Determinar, en el modelo de epilepsia audiosensible GASH/Sal, si la VNS afecta a la expresión de la Interleukina 1 beta (IL-1B) en tejido nervioso, así como valorar si regula la activación de la ruta inflamatoria de las proteínas quinasas activadas por mitógenos (MAPK) y el grado de apoptosis en tejido cerebral. **Materiales y método**: en 10 animales, se implantó un sistema de VNS compuesto por un generador programable y un electrodo bipolar. Los parámetros fueron: 30 Hz de frecuencia, 1,5 mA de intensidad, 250 us de ancho de pulso, 30s ON y 5 minutos OFF. A los 14 días de estimulación, se extrajeron muestras en fresco de tejido nervioso. Se evaluó la expresión de IL-1B mediante ELISA, así como la fosforilación de p38 MAPK y Bad mediante Western Blot. **Resultados**: Además de presentar un efecto anticonvulsivo, la VNS reduce de forma significativa la expresión de IL-1B en cerebelo y corteza. En cerebelo, se correlaciona con una reducción en la fosforilación de p38 MAPK y un aumento de la fosforilación de Bad. **Conclusiones**: la VNS muestra efecto antiinflamatorio en el modelo, disminuyendo la activación de la ruta de las MAPK y la apoptosis. Estos resultados contribuyen al conocimiento de los mecanismos de acción de la VNS y nos muestran la importancia de continuar con investigaciones en la misma línea.

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39. Defects in the expression of genes associated with refractory epilepsy in the GASH/Sal model

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Abstract. The ATP-binding cassettes (ABC) are a family of membrane proteins that mediate ATP-driven transports of different substrates across the membrane. In spite of these beneficial effect, they have also been associated to drug resistance by cell rejection. The ABCB1 or P-glycoprotein (P-gp) is the most expressed drug efflux transporter at the blood-brain barrier. High P-gp levels have been detected at the epileptogenic nucleus in epilepsy-refractory patients and different experimental models of epilepsy. This correlates with the drug transporter

hypothesis, which suggests that the inefficiency of the antiepileptic drugs might be explained by the overexpression of ABC transporters at the epileptogenic tissue. Here, we used the genetic audiogenic seizure hamster from Salamanca (GASH/Sal) to assess changes in the mRNA expression levels of genes associated with resistance to antiepileptic drugs. Thus, gene expression analysis (RT-qPCR) of the epileptogenic nucleus, so-called inferior colliculus, was carried out in naïve and kindled GASH/Sal (with repetitive seizure), and compared with age-matched controls. Among the common genes differentially expressed in basal state, we found overexpression of the *abcc4*, *Hif-1alpha* and *Epo* genes in the epileptogenic nucleus of GASH/Sal in relation to controls. Conversely, repetitive convulsive seizures produced in the audiological kindling cause underexpression of the *abcc4*, *abcb1a* and *Hif-1alpha* genes, being in relation to controls in the epileptogenic nucleus. It seems that the repeated crises produce a kind of "accommodation" where, in general, the tendency is to diminish the hypoxic effects of seizures manifested by a significant decrease in hif-1α with its correlate in the decrease of the gen encoded the constitutive isoform of P-gp (*abcb1a*), and of the multiple drug resistance protein ABCC4.
New experiments will be necessary to delve into the relationship of these genes with the evolution of seizures in the GASH/Sal model of epilepsy.
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40. Behavioral effects of vagus nerve stimulation in the GASH/Sal epilepsy model

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Abstract. Vagus nerve stimulation (VNS) for the treatment of refractory epilepsy has been widely used with beneficial results. However, the morphofunctional mechanisms responsible for positive effects of this procedure are still not fully understood. We have a model of experimental epilepsy from genetic origin that is widely characterized: the Genetic Audiogenic Seizure Hamster from Salamanca (GASH/Sal). This fact considerably facilitates the realization of numerous studies. One of the most relevant ones focuses on evaluating the effects of VNS on behavioral indicators, allowing to obtain information about the occurrence of depression and anxiety. These alterations are known to be suffered by a high number of epileptic patients. In animals GASH/Sal with VNS for 15 days and without it, as well as in control animals (golden hamster) (n = 8), a behavioral study was performed by using the Open Field Test. The parameters evaluated include distance traveled, time of permanence in the central area of the arena, latency of the beginning of the exploration, number of rearing and of grooming among others. The comparison from the parameters analyzed between the study groups show VNS, in addition to the almost total elimination of seizures in GASH/Sal animals, also

causes the normalization of various behavioral indicators. This evidence allows us to conclude that the procedure of VNS could also induce beneficial effects on alterations related to depression and anxiety disorders associated with epilepsy.

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