INSTITUTO DE NEUROCIENCIAS CASTILLA Y LEÓN

VNiVERSiDAD D SALAMANCA

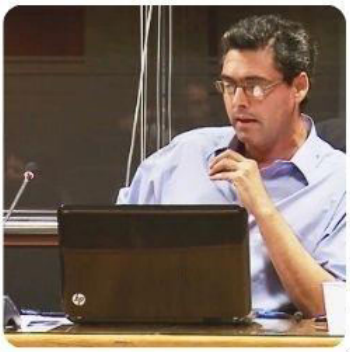
JORNADA CIENTIFÍCA DEL INCYL

Homenaje a nuestro compañero Javier Yajeya

Aula 'Pío del Río-Hortega´, INCyL

10 y 11 de octubre de 2024

PROGRAMA



Jueves, 12:00 horas

Ponencia Invitada

"Banda gamma del EEG durante la vigilia, el sueño y en un modelo farmacológico de psicosis"
Pablo Torterolo

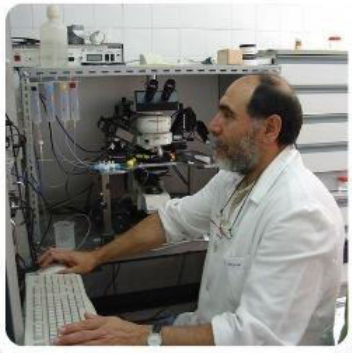
Oct 10 2024

Jueves, 16:00 horas

Sesión de Pósteres

Hall del Instituto de Neurociencias de Castilla y León a cargo de los investigadores del INCyL

Oct 10 2024



Viernes, 11:00 horas

Homenaje a Javier Yajeya

Jornada en homenaje a nuestro compañero el Prof. Yajeya

Oct 11 2024

PONENCIA INVITADA: DR. PABLO TORTEROLO

JUEVES, 10 DE OCTUBRE DEL 2024 A LAS 12:00 HORAS EN EL AULA PÍO DEL RÍO HORTEGA DEL INSTITUTO DE NEUROCIENCIAS DE CASTILLA Y LEÓN

Resumen:

Se presentarán resultados en ratones, ratas y gatos, sobre cómo las oscilaciones gamma del EEG (un ritmo clave para la función cogniKva), son moduladas por la respiración durante los estados de sueño-vigilia, y cómo la ketamina a dosis sub-anestésicas (considerado un modelo farmacológico de psicosis) afecta esta modulación.

Referencias:

Castro-Zaballa S, González J, Cavelli M, Mateos D, Pascovich C, Tort A, Hunt MJ, Torterolo P. (2024). Cortical high-frequency oscillations (» 110 Hz) in cats are statedependent and enhanced by a subanesthetic dose of ketamine. Behavioural Brain Research 476, 115231. https://doi.org/10.1016/j.bbr.2024.115231

González J, Torterolo P, Tort A.B.L (2023). Mechanisms and functions of respirationdriven gamma oscillations in the piriform cortex. eLife 2023;12:e83044. DOI: https://doi.org/10.7554/eLife.83044

González J, Cavelli M, Mondino A, Castro-Zaballa S, Brankačk J, Draguhn A, Torterolo P, Tort A.B.L. (2023). Breathing modulates gamma synchronization across species. Pflugers Arch (European Journal of Physiology). 475(1):49-63. doi: 10.1007/s00424-022-02753-0.

Cavelli M, Castro-Zaballa S, Gonzalez J., Rojas Líbano D, Rubido N, Velásquez N, Torterolo P. (2019). Nasal respiration entrains neocortical long-range gamma coherence during wakefulness. European Journal of Neuroscience, 51(6):1463-1477. doi: 10.1111/ejn.14560.

Castro-Zaballa S, Cavelli M, Gonzalez J, Nardi AE, Machado S, Scorza C, Torterolo P (2019). EEG 40 Hz coherence decreases in REM sleep and ketamine model of psychosis. Frontiers in Psychiatry 9:766. doi: 10.3389/fpsyt.2018.00766. Castro S, Falconi A, Chase M, Torterolo P (2013). Coherent neocortical 40-Hz oscillations are not present during REM sleep. European Journal of Neuroscience,37(8):1330-9. doi: 10.1111/ejn.12143.

SESIÓN DE PÓSTERES

JUEVES, 10 DE OCTUBRE DEL 2024 A LAS 16:00 HORAS EN EL HALL DEL INSTITUTO DE NEUROCIENCIAS DE CASTILLA Y LEÓN

(Libro de abstracts en la página 15)

Presentación de pósteres impares: de 16:00 a 17:00
Prestación de pósteres pares de 17:00 a 18:00

Póster N.	Título	Laboratorio	Presentador
1	The Effect Of Signal Level And Signal-To-Noise Ratio On Adaptation To Noise In Spectral And Temporal Modulation Detection	Audición Computacional y Psicoacústica	David López-Ramos
2	Design And Testing Of A Front-End Implementation Of A Binaural Audio Processing Strategy Inspired By The Medial Olivocochlear Reflex	Audición Computacional y Psicoacústica	María Milagros Jerónimo Fumero
3	Effect Of Precursor Level Statistics On Adaptation To Noise Measured With Electroencephalography	Audición Computacional y Psicoacústica	Miriam I Marrufo Pérez
4	Comparison Of Performance For Cochlear-Implant Users With Audio Processing Strategies Based On Short-Time Fft Or Spectral Feature Extraction	Audición Computacional y Psicoacústica	Peter T. Johannesen

5	The Effect Of Cochlear Synaptopathy On Adaptation To Noise	Audición Computacional y Psicoacústica	Sónia L. Coelho-de-Sousa
6	Msk1 Absence Hinders Bdnf-Dependent Striatal Neurodevelopment And Leads To Schizophrenia Symptoms	Formación de Circuitos Neuronales y enferemades cerebrales	Natalia Varela, Carlos Hernández, Alejandro Cebrián
7	Analysis Of Activity Markers As A Function Of Pi3ki + Mekico-Treatments Revealed Differences In Network Circuitry Across Glioblastoma Cells	Laboratorio de Señalización Celular y Proteómica	Elisa Arias, Alejandra Macías
8	Divergence Of Pi3k And Mapk Signaling Pathway Activities Between An Adherent Cellular Model Of Glioblastoma And Their Derived Neurospheres	Laboratorio de Señalización Celular y Proteómica	Lucía García, Elisa Arias, Alejandra Macías
9	Swimming Towards Vertebrate Central Nervous System Regeneration	Neurobiología Comparada	Almudena Velasco
10	Proteínas Adaptadoras De Ubiquitina Ligasas Como Nuevos Actores En El Mecanismo Del Dolor	Neurobiología de las neurotrofinas	Daniel Cañada-García

11	Novel Trka Mutants In The Ngf/Trka Nociceptive Pathway	Neurobiología de las neurotrofinas	Francisco José González Calvo
12	Tat-Cx43266-283 As A Potential Therapy For Glioblastoma: Treatment Response Prediction And Positive Results In Combination With Tumor Resection In Preclinical Models.	Neurobioquímica	Andrea Álvarez-Vázquez
13	Generation Of Tat-Cx43266-283- And Temozolomide-Resistant Glioma Stem Cells	Neurobioquímica	Enrique Jiménez Madrona
14	Exploring The Antitumor Effect Of The Cell-Penetrating Peptide Tat-Cx43266-283-Mod In Glioblastoma Preclinical Models	Neurobioquímica	María Martínez Fernández
15	Exploring The Effect Of Tat-Cx43266-283 In Lung Cancer Brain Metastasis Models	Neurobioquímica	Pilar Cerveró-García
16	Pharmacokinetic Properties Of The Anti-Tumor Peptide Tat-Cx43266-283 In Different Murine Brain Tumor Models	Neurobioquímica	Raquel Flores Hernández

17	Neuronal Responses To Omitted Tones In The Auditory Brain: A Neuronal Correlate For Predictive Coding	Neurociencia Auditiva y Cognitiva	Ana B. Lao-Rodríguez
18	Stimulus-Specific Adaptation In The Hippocampus Of The Anesthetized Rat	Neurociencia Auditiva y Cognitiva	Jazmín S. Sánchez
19	Auditory Stimulus-Specific Adaptation In The Primary Visual Cortex Of The Anesthetized Rat	Neurociencia Auditiva y Cognitiva	Jazmín S. Sánchez
20	Mismatch Negativity Changes Throughout Urethane Anesthesia In The Rat	Neurociencia Auditiva y Cognitiva	Laura H. Bohórquez
21	Novelty Detection And Predictive Procesing Impairments In An Animal Model Of Alzheimer Disease	Neurociencia Auditiva y Cognitiva	Laura Quintela Vega
22	Sex- And Age-Specific Effects On Subcortical Predictive Processing Of Typically Developing And Valproic Acid-Induced Rats	Neurociencia Auditiva y Cognitiva	Sara Cacciato Salcedo

23	Rons Facilitate Ampk And Akt Phosphorylation Which Drive Glut4 Translocation To Plasma Membrane In Skeletal Muscle Cells (C2c12 Myotubes)	Fisiopatología redox del músculo esquelético y envejecimiento	Martín-Prieto, Eva
24	The Inferior Colliculus Is Not Innervated By Non-Auditory Cortical Areas	Neurohistología	Mario Gómez Martínez
25	Multisession Direct Current Stimulation Targeting The Auditory Cortex Prevents Cortical Aging In A Wistar Rat Model Of Arhl	Neuroplasticidad auditiva	Inés Santos Fernández del Campo
26	Assessing The Impact Of Caffeine And Taurine From Energy Drinks On Zebrafish Embryos	Neuroquímica y Neurociencia Molecular	Verónica González Núñez
27	Neuroprotective Strategies With Neurotrophic Factors Against Selective Neuronal Loss	Plasticidad Neuronal y Neurorreparación	David Díaz López
28	Inhibition Of Microglia In An Animal Model Of Selective Neurodegeneration, The Purkinje Cells Degeneration Mutant Mouse	Plasticidad Neuronal y Neurorreparación	Eduardo Weruaga

29	Inhibition Of Microglia In An Animal Model Of Selective Neurodegeneration, The Purkinje Cell Degeneration Mutant Mouse	Plasticidad Neuronal y Neurorreparación	Eduardo Weruaga Prieto
30	Unveiling Fusion Between Bone Marrow-Derived Cells And Purkinje Cells: Patch-Seq Analysis In A Mouse Model Of Multiple Sclerosis	Plasticidad Neuronal y Neurorreparación	Pablo G. Téllez de Meneses
31	Effects Of Environmental Enrichment On Mossy Fibers And Their Relationship With Microglia	Plasticidad Neuronal y Neurorreparación	Sara Sánchez Monreal
32	Environmental Enrichment Effects On Hippocampal Microglia And Adult Neurogenesis	Plasticidad Neuronal y Neurorreparación	Teresa Cocho Curto
33	Delving Into Synaptic Activity In Autism: Nitric Oxide Pathway And Glutamate/Gaba Ratio	Plasticidad Neuronal y Neurorreparación	Valeria Lorena Cabedo Navarro

34	Modelling Fully Developed Human Retinal Pigment Epithelium Sheets Obtained From Induced Pluripotent Stem Cells (Ipsc)	Trastornos Degenerativos del Sistema Visual	Lucía San José Lassalle
35	Proteomic Profile Modifications In The Epileptogenic Nucleus Of The Genetically Audiogenic Seizure-Prone Hamster Gash/Sal After Repeated Seizures	Trastornos audiomotores y epilepsias reflejas	Carlos García Peral
36	Epilepsy Related To Ingestion In A Syngap1 Mutation Pediatric Patient	Trastornos audiomotores y epilepsias reflejas	Hilario Gómez Martín
37	Contenido Proteómico En Vesículas Extracelulares De Pacientes Epilépticos. Estudio Piloto	Trastornos audiomotores y epilepsias reflejas	Jaime Gonçalves
38	Fundamento Neurobiológico De Las Funciones Auditivas De La Musculatura Endotimpánica	Trastornos audiomotores y epilepsias reflejas	Ricardo Gómez Nieto

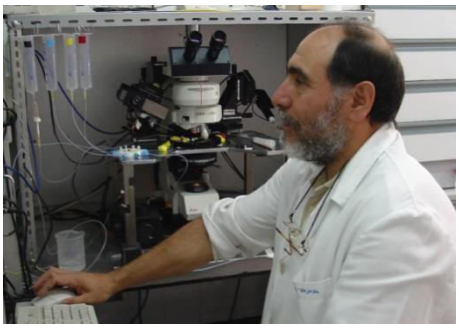
39	Hippocampal prolactin system. I. Synthesis and localization of the hormone in different age stages	Neuroendocrinology	José Carretero
40	Hippocampal prolactin system.II. Synthesis and localization of the two isoforms of Prolactin Receptor in different age stages	Neuroendocrinology	David Hernández-González y David Díez Castro
41	Influence of estrogens on prolactin synthesis and release in choroid plexuses of female mice	Neuronedocrinology	Leonardo Catalano-Iniesta

JORNADA HOMENAJE PROF. JAVIER YAJEYA

VIERNES, 11 DE OCTUBRE DEL 2024 A LAS 11:00 HORAS EN EL AULA PÍO DEL RÍO HORTEGA DEL INSTITUTO DE NEUROCIENCIAS DE CASTILLA Y LEÓN

11:00 horas	**Presentación de la sesión** Dr. Manuel Sánchez Malmierca, Director del INCYL y Dr. Miguel Merchán Cifuentes
11:10 horas	**Estudiante de Medicina en la Universidad Complutense de Madrid.** Dr. Rafael Alonso Solís. Catedrático de Fisiología, Prof. Emérito, ULL
11:20 horas	**El postdoctorado en los Estados Unidos.** Carta del Prof. Joaquín M. Fuster, UCLA Semel Institute for Neuroscience & Human Behavior. School of Medicine - University of California at Los Angeles. USA
11:25 horas	**Trayectoria científica y humana en la Universidad de Salamanca.** Dr. José María Criado. Profesor Titular de Fisiología. USAL
12:00 horas	**Hablan sus colaboradores y discípulos:** Dr. Juan Carlos Alvarado Profesor Titular de Histología, UCLM Dr. Juan de Dios Navarro Profesor Titular de Fisiología, UCLM Dra. Asunción Colino Matilla. Catedrática de Fisiología de la UCM Dr. Alfredo Sanabria Castro, Profesor. UCR Dr. José María Delgado, Catedrático de Fisiología, Prof. Emérito, UPO Dr. Rafael Coveñas, Profesor Titular, Departamento de Biología Celular, USAL Dr. Miguel Merchán. Catedrático de Biología Celular, Prof. Emérito, USAL

12:50 horas	**Habla su grupo de investigación de la Universidad de Salamanca** Dra. Adelaida Sánchez Riolobos y Dra. Margarita Heredia Chons,
13:00 horas	**Cierre del acto y entrega de fotografía del Prof. Yajeya para el INCYL.** Vicerrector de Investigación - Dr. José Miguel Mateos Roco. Director del INCyL - Dr. Manuel Sánchez Malmierca, Investigador Principal del grupo de Neurofisiología -Dr. José María Criado Miembro fundador del INCYL - Dr. Miguel Merchán Cifuentes



Obituario:

Javier Yajeya Pérez

1947-2024

Javier Yajeya nació en San Xoán de Río, un pueblecito de la Provincia de Ourense en Galicia, situado en la zona montañosa de la Comarca de Tierra de Trives.

Realizó sus estudios de bachillerato en Cuenca, donde estaba afincada su familia. En 1969 inició sus estudios de Medicina en la Universidad Complutense de Madrid. Pronto, en 1972, conoció a su compañera y esposa de toda la vida, Amelia con quien tuvo a su hija Elena.

Fue un académico carismático y muy vocacional tanto desde la perspectiva docente como investigadora. Su talento académico fue reconocido pronto, y ya desde los primeros años, como estudiante de Medicina, obtuvo una plaza de alumno interno en el Departamento de Fisiología de la Universidad Complutense, dirigido en aquel momento por el ilustre profesor D. Antonio Gallego. Enseguida descubrió que sus verdaderos intereses se hallaban en la comprensión de los mecanismos fisiológicos fundamentales del sistema nervioso. En 1975, obtuvo una plaza de profesor ayudante en el mismo Departamento, donde bajo la tutela del Prof. Gallego se impregnó del espíritu docente e investigador de su maestro, alcanzando en 1978 el grado de Doctor por la Universidad Complutense de Madrid con la Tesis: *Control talámico de la actividad eléctrica cortical*, dirigida por el Dr. D. José Andrés Sobrino Montalbán.

En 1978, se incorporó como profesor adjunto interino al Departamento de Fisiología y Bioquímica de la Universidad de Salamanca bajo la dirección, en aquel momento, del Profesor D. Antonio Fernández de Molina, obteniendo por oposición, en el año 1981, la plaza de Profesor Adjunto, posteriormente Titular, en ese mismo Departamento.

A partir de esta fecha, se desplazó a USA donde realizó una estancia de investigación de año y medio en la Universidad de Pensilvania, trasladándose posteriormente desde allí a los Ángeles, en concreto a la Universidad de California, investigando sobre el *papel de la corteza prefrontal y parietal en los procesos de integración sensorial y sensorio- motora asociados a procesos de memoria y organización temporal de la conducta*. Estudios que realizó bajo la dirección del eminente neurocientífico y humanista, Profesor Joaquín Fuster, con el que estableció fuertes lazos científicos y personales.

En 1984, se incorporó definitivamente al Departamento de Fisiología y Bioquímica de la Universidad de Salamanca, colaborando en la línea de investigación del Prof. Fernández de Molina, interesado en *el papel modulador de la amígdala sobre mecanismos hipotalámicos y mesencefálicos*.

Tras la jubilación del Prof. Fernández de Molina, asumió el papel de encargado de la cátedra de Fisiología, formando a partir de entonces un grupo de trabajo, en el que

Javier Yajeya fue un ejemplo de responsabilidad, esfuerzo, asertividad, y estableciendo confianza y cooperación entre sus miembros, consiguiendo un equipo con estrechos lazos de unión tanto en lo laboral como en lo personal, que se han mantenido a lo largo del tiempo creando escuela con numerosos investigadores actualmente distribuidos por el mundo. En este periodo realizó estudios sobre el *papel de la corteza prefrontal y motora en relación con la ejecución de conductas motoras de precisión.*

En 1989, fue elegido director del Departamento de Fisiología y Farmacología, y en 2010 obtuvo la catedra de Fisiología en la Universidad de Salamanca. Aunque nunca se mostró como un jefe, era un líder indiscutible, sobresaliente, muy reconocido, respetado y querido por todos, que logró el equilibrio adecuado entre apoyar, pero no interferir. Siempre fue afable y generoso con las ideas y los consejos, tanto en lo personal como en lo profesional. Entendió la importancia de dar a las personas la libertad y el espacio para encontrar su propio camino, a su propio ritmo. Además, tenía el raro don de reconocer lo que era importante y lo que no lo era tanto. Su interés por la investigación del sistema nervioso, en todos sus aspectos académicos, le llevó a ser protagonista principal, en estrecha colaboración con su compañero y buen amigo Miguel Merchán, en la promoción del Instituto de Neurociencias de Castilla y León.

Era un científico con absoluto dominio de las técnicas de electrofisiología aplicadas al estudio del sistema nervioso tanto en animales agudos como crónicos, así como en experimentos *in vitro*. Durante su carrera, Javier publicó 65 artículos y muchas otras comunicaciones y capítulos de libro, orientadas hacia el estudio del complejo amigdalino y de las cortezas motoras y prefrontal. En los últimos años, su interés se dirigió a estudiar los *mecanismos que subyacen a la enfermedad de Alzheimer y el papel que en ella juega el péptido beta-amiloide*.

Más allá de la investigación, Javier fue además un excelente docente y un profesor muy popular entre sus alumnos, dada su gran capacidad para comprender y empatizar con los jóvenes. En sus clases siempre predominaba el interés, no solo en contar, explicar y demostrar, sino especialmente inspirar y motivar a sus alumnos. Era un excelente comunicador.

Las buenas personas son valiosas, y Javier era una buena persona. Le echaremos siempre de menos.

José María Criado

"Cuando la muerte se precipita sobre el hombre, la parte mortal se extingue; pero el principio inmortal se retira y se aleja sano y salvo"

Platón

LIBRO DE ABSTRACTS

The effect of signal level and signal-to-noise ratio on adaptation to noise in spectral and temporal modulation detection

***Laboratorio**: Audición Computacional y Psicoacústica*

Presenter: David López-Ramos

Authors: David López-Ramos, Luis E. López-Bascuas, Almudena Eustaquio-Martín, Miriam I. Marrufo-Pérez, Enrique A. Lopez-Poveda

Keywords: adaptation to noise, spectrotemporal modulation perception

Abstract:

Background. The term 'noise adaptation' refers to an improvement in auditory function as the signal of interest is delayed in the noise. We have previously shown that when the signal is a modulated ripple noise, adaptation occurs for spectral (SM) and temporal modulation (TM) detection, but not for the detection of spectro-temporal modulations combined. Here, we investigate the effect of stimulus level and signal-to-noise ratio (SNR) on noise adaptation for temporal and spectral modulation detection.

Methods. The signal was a 200-ms spectrally (2 cycles/oct) or temporally (10 Hz) modulated ripple noise embedded in white noise. The noise-signal onset delay was 50 ms (early condition) and 800 ms (late condition). The modulation depth (dB) was varied adaptively to measure a modulation detection threshold. Adaptation was calculated as the threshold improvement in the late relative to the early condition. In Experiment 1, adaptation for SM (N=16) and TM (N=11) detection was measured for signal levels of 45, 60 and 75 dB SPL while the SNR was fixed at 0 dB. In Experiment 2, adaptation was measured for SM (N=10) and TM (N=10) detection for SNRs of -10, -5, 0, 5 and 10 dB while the background noise level was fixed at 60 dB SPL.

Results. SM detection thresholds improved significantly in the late condition at the three levels (1.8 dB at 45 dB SPL; 2.2 dB at 60 dB SPL, and 3.0 dB at 75 dB SPL). For TM detection, significant adaptation was observed at 60 and 75 dB SPL but not at 45 dB SPL (2.9 dB, 2.0 dB, and 1 dB, respectively). The effect of signal level on adaptation was not significant for SM or TM detection. Adaptation for SM detection was significant at all SNRs except 5 dB (1.2 dB at 10 dB SNR; 1.7 dB at -5 dB SNR; 2.2 dB at 0 dB SPL and 2.9 dB at +10 dB SNR). For TM detection, adaptation was significant at - 10 dB SNR (2.4 dB) and at 0 dB SNR (3.0 dB), but not at the other SNRs.

Conclusions. Noise adaptation for SM detection tended to increase with level. For TM detection, adaptation tended to be greater at mid-levels than at lower or higher levels. However, the stimulus level did not affect adaptation significantly for SM or TM detection. Although noise adaptation in SM and TM detection seems to be greater at 0 dB SNR, the effect of SNR on adaptation was not significant. We discuss the implications of the results for elucidating the mechanisms involved in noise adaptation.

[Work supported by the University of Salamanca, Banco Santander, and the Spanish Ministry of Science and Innovation (grant PID2019-108985GB-I00)].

Nº 2

Design and testing of a front-end implementation of a binaural audio processing strategy inspired by the medial olivocochlear reflex

***Laboratorio**: Audición Computacional y Psicoacústica*

Presenter: María Milagros Jerónimo Fumero

Authors: Lopez-Poveda EA, Eustaquio-Martín A, Fumero MJ, Gorospe JM, Wirtz C, Schatzer R, Stohl J, Nopp P

Keywords: Binaural sound processing, Cochlear implant, Medial Olivocochlear (MOC) Reflex, Speech-in-noise recognition

Abstract:

Objectives. Users of cochlear implants (CIs) still find it challenging to understand masked speech or to localize sound sources, even with two devices. We have previously argued that this could be partly because CI users lack the effects and benefits of the medial olivocochlear (MOC) reflex, and shown that, compared to using two functionally independent devices, binaural CI audio processing inspired by the MOC reflex can improve hearing in noise (Lopez-Poveda et al., 2016, Ear Hear 37:e138-e148) and sound localization for CI users (Lopez-Poveda et al., 2019, Hear Res 379:103-116). Though promising for improving CI outcomes, however, the MOC strategy may be hard to implement in clinical devices because it involves time-varying, contralateral control of the compressive electric-to-acoustic maps. Here, we present a version of the MOC strategy designed to operate in the frequency domain and at the front-end of processing, referred to as the MOCFE.

Design. The MOCFE strategy was implemented and tested in combination with a pair of functionally independent MED-EL FS4 audio processors, one per ear. CI user performance was compared for MOCFE as well as for two reference strategies: the standard FS4 (STD) strategy and a back-end MOC strategy. The MOCFE and MOC strategies were implemented with fast contralateral inhibition (time constants = 2 ms). Tests included (1) speech reception thresholds for sentences in fluctuating and steady-state noise, in unilateral and bilateral listening modes, and for three different speech levels; and (2) sound source localization in quiet and in noise. Five users of bilateral CI participated in the tests.

Results. (1) For speech intelligibility in a fluctuating masker, the MOCFE was as beneficial (re STD) as the MOC strategy, except for one specific spatial configuration (S-60N-60), where the MOCFE produced no benefits while the MOC strategy did. (2) Neither the MOCFE or the MOC strategies improved intelligibility in a steady-state noise, possibly because they involved fast rather than slow contralateral inhibition. (3) The binaural MOCFE and MOC strategies tended to improve sound source localization slightly relative to the STD strategy.

Conclusion. The MOCFE can become a successful alternative to the MOC strategy, as it can be more easily implemented in clinical devices.

Work supported by MED-EL GmbH.

Nº 3

Effect of precursor level statistics on adaptation to noise measured with electroencephalography

***Laboratorio**: Audición Computacional y Psicoacústica*

Presenter: Miriam I Marrufo Pérez

Authors: Miriam I. Marrufo-Pérez, Deborah A. Vickers, Enrique A. Lopez-Poveda

Keywords: amplitude modulation detection, electroencephalography

Abstract:

Background. In noisy backgrounds, the recognition of isolated words, the detection of pure tones, and the detection of amplitude modulation (AM) all improve as the target sounds are delayed in the noise. It has been hypothesized that this 'adaptation to noise' occurs because auditory neurons shift (adapt) their dynamic range towards the most common level in the noise preceding the target sound. The hypothesis is supported by behavioural experiments that show that adaptation occurs when the level of the noise preceding the target is steady but not when it is very fluctuating. However, physiological evidence is still lacking in support of this mechanism. Here, we investigate whether adaptation as measured with electroencephalography depends on the precursor level statistics.

Methods. Envelope- (EFR) and frequency-following responses (FFR) were obtained for normal-hearing listeners using a 64-channel Biosemi system (16 kHz sampling). Participants were presented monaurally with AM tones (70 dB SPL, 250 ms, 576 Hz carrier, 84 Hz modulation frequency, 100% modulation depth) in steady noise (75 dB SPL; 0.1-10 kHz) that started and finished 50 ms before and after the tone. The stimulus could be preceded by a noise precursor (350 ms in duration) with the same spectrum and average level as the simultaneous masking noise. The precursor was steady or fluctuating in level (from 30 to 83 dB SPL every 50 ms). Adaptation was estimated as the difference in the EFR and FFR with and without precursor. Participants were asked to remain awake and with their eyes closed while recording. 2400 tones in alternating polarity (1200 for each polarity) were presented in each measure. Recordings were added to obtain the response to the modulating frequency (EFR), or subtracted to obtain the response to the carrier frequency (FFR). The inter-stimulus interval was 500 ms.

Results. Preliminary results for four participants suggest that the EFR and FFR are larger with than without the steady precursor (~2 dB on average). This provides physiological evidence for adaptation to noise, i.e., for an objectively improved neural representation of the AM tones in continuous versus gated noise. More data are necessary to shed light on the effect of precursor level statistics on noise adaptation.

[Supported by the Spanish Ministry of Universities, Unión Europea NextGenerationEU/PRTR, and University of Salamanca to MIMP, Spanish Ministry of Science and Innovation (grant PID2019-108985GB-I00) and the European Regional Development Fund to EALP, and the MRC Senior Fellowship in Hearing Research (MR/S002537/1) to DAV].

Nº 4

Comparison of performance for cochlear-implant users with audio processing strategies based on short-time FFT or spectral feature extraction

***Laboratorio**: Audición Computacional y Psicoacústica*

Presenter: Peter T. Johannesen

Authors: Peter T. Johannesen; Yue Zhang; Behnam Molaee-Ardekani; Aswin Wijetilake; Alejandro Soler Valcarcel; Manuel Segovia-Martinez; Enrique Lopez-Poveda

Keywords: Cochlear implant, Deafness, Hearing, Phonetic feature, Sound coding strategy, Spectral processing.

Abstract:

Background. We investigated if and to what extent a spectral feature extraction (SFE) audio processing strategy for cochlear implants (CIs) improves hearing performance and comfort compared to Crystalis, a short-time FFT based strategy currently implemented in Oticon Medical CIs. In the SFE strategy, acoustic events (or spectral peaks) were extracted using a synthetic feature extractor and mapped into the 20 CI stimulation channels. SFE was hypothesized to reduce frequency smearing and improve frequency resolution because spectral peaks are detected and narrower filter spacing can be achieved without the constrains of the FFT bin width.

Methods. For six users of Oticon Medical Digisonic CIs, we compared performance with the SFE and Crystalis strategies on various aspects: word recognition in quiet, sentence reception threshold in noise (SRT), consonant discrimination in quiet, listening effort, melody contour identification (MCI), and subjective sound quality. Word recognition and SRTs tests were conducted on the first and last day of testing to assess potential learning and/or accustomization effects. Listening effort was assessed in quiet and in a low-noise condition (individual SRT+15 dB) by measuring pupil dilation. MCI involved identifying a pattern of five tones among five possible patterns that formed either a constant, rising, falling, rising-falling, or falling-rising melody. MCI was measured for tone distances of two and four semitones and for fundamental frequencies of 131 and 262 Hz. Subjective sound quality was assessed using the multiple stimulus with hidden reference and anchor (MUSHRA) paradigm for three groups of sounds: sentences, music, and ambient sounds.

Results. Word recognition was similar for the two strategies on the first day testing, while it became (unexpectedly) better for Crystalis than the SFE strategy on the last day of testing. SRTs were worse with the SFE strategy than Crystalis on the first day of testing but became comparable for the two strategies on the last day of testing. Consonant discrimination scores were higher for Crystalis than for the SFE strategy. MCI scores were similar for the two strategies in all test conditions. Subjective sound quality scores tended to be lower for the SFE strategy than for Crystalis. Listening effort was not substantially different across strategies.

Conclusions. We conclude that CI-user performance is similar with SFE as with a more standard short-time FFT based approach. However, longer accustomization times may be required to reveal the full potential of feature extraction.

[Work supported by Oticon Medical].

Nº 5

The effect of cochlear synaptopathy on adaptation to noise

***Laboratorio**: Audición Computacional y Psicoacústica*

Presenter: Sónia L. Coelho-de-Sousa

Authors: Sónia L. Coelho-de-Sousa, Miriam I. Marrufo-Pérez, Marcelo Gómez-Álvarez, Enrique A. Lopez-Poveda

Keywords: synaptopathy; adaptation to noise; ABR; ageing

Abstract:

Background. Adaptation to noise refers to the improvement in word-in-noise recognition as words are delayed a few hundred milliseconds from the noise onset. This adaptation is thought to reflect one or more physiological mechanisms that can adjust the dynamic range of auditory nerve fibers, such as statistical adaptation to the most frequent noise level preceding the words and/or noise activation of olivocochlear efferent reflexes. The loss of cochlear synapses (or synaptopathy) could impair these mechanisms, hence adaptation to noise. The aim of the present study was to investigate the impact of synaptopathy on adaptation to noise. Because synaptopathy predominantly reduces the number of cochlear synapses for auditory nerve fibers with high thresholds, we expected a larger effect of synaptopathy on adaptation to high level noise.

Methods. For 48 participants with normal-hearing (pure-tone average thresholds at 500-2000 Hz <25 dB HL), we measured (1) speech reception thresholds (SRTs; signal-to-noise ratios at 50% recognition) for disyllabic words delayed 50 or 800 ms in stationary, speech-shaped noise; (2) high-frequency thresholds (HFTs) at 12 kHz; and (3) auditory brainstem responses (ABRs) for clicks presented at 95 and 110 dB ppeSPL. SRTs were measured for fixed noise levels of 55 and 78 dB SPL by adaptively varying the speech level. Adaptation to noise was calculated as the SRT improvement in the 800-ms versus the 50-ms delay condition. Because adaptation is known to be greater for vocoded than for natural words, words were processed through a tone vocoder. The amplitudes of ABR wave I for the two click levels and its rate of growth with increasing level (slope) were used as proxies for cochlear synaptopathy.

Results. Adaptation occurred at the two noise levels (55 dB SPL, mean=0.88 dB, p=0.001; and 78 dB SPL, mean = 1.89 dB, p<0.001). At 78 dB SPL, adaptation was correlated with wave I slope [r(46)=0.089, p=0.039] but not with wave I amplitude [at 95 dB ppeSPL: r(46)=0.024, p=0.30; at 110 dB ppSPL: r(46)=0.013; p=0.43]. At 55 dB SPL, adaptation was not significantly correlated with any ABR measure. Results were similar when the potential confounding effects of HFTs were partialled out.

Conclusions. Cochlear synaptopathy (as assessed by wave I slope) could reduce adaptation to high-level noise. More data are necessary to corroborate these findings.

[Work supported by the Spanish Ministry of Science and Innovation (grant PID2019-108985GB-I00), and the European Regional Development Fund.]

Nº 6

MSK1 absence hinders BDNF-dependent striatal neurodevelopment and leads to schizophrenia symptoms

***Laboratorio**: Formación de Circuitos Neuronales y enferemades cerebrales*

Presenter: Natalia Varela, Carlos Hernández, Alejandro Cebrián

Authors: Natalia Varela, Carlos Hernández, Alejandro Cebrián, Inés S. Fernández del Campo, Sandra García Losada, Noelia Martín Ávila, Juan Carlos Arévalo, Miguel A. Merchán, Manuel Sánchez Martín, Rubén Deogracias

Keywords: BDNF, MSK1, GABAergic development, schizophrenia

Abstract:

Brain-derived neurotrophic factor (BDNF) plays a critical role in postnatal development by modulating the architecture of specific neuronal populations and brain areas. However, the precise molecular program controlling this differential responsiveness to BDNF is still unclear. In the present study, we describe that this program is governed by the restricted expression of the mitogen- and stress-activated protein kinase-1 (MSK1) in GABAergic neurons. Also, we show that while Msk1 expression declines in cortical interneurons along early postnatal development, its expression in striatal neurons increases until adulthood. Utilizing a novel MSK1 loss-of-function mouse model, we reveal its essential role in postnatal growth of the striatum, as it interacts with and modulates the BDNF-dependent phosphorylation of the methyl-CpG binding protein-2 (MeCP2). Furthermore, these mutant mice exhibit an altered transcription pattern of genes involved in the control of the dopamine and GABAergic signalling pathways. Consequently, MSK1 knockout mice behaviour is markedly altered, showing social dysfunction, altered anxiety- and depressive-like responses unequally manifested in males and females. These results elucidate how disruptions in the BDNF/MSK1 pathway impact GABAergic neurite outgrowth and contribute to behaviours reminiscent of schizophrenia in humans.

Nº 7

Analysis of activity markers as a function of PI3Ki + MEKico-treatments revealed differences in network circuitry across glioblastoma cells

***Laboratorio**: Laboratorio de Señalización Celular y Proteómica*

Presenter: Elisa Arias, Alejandra Macías

Authors: Maruan Hijazi, Elisa Arias, Alejandra Macías

Keywords: Glioblastoma, kinase inhibitors, co-treatments, glioblastoma stem cells

Abstract:

PI3K and MAPK are frequently dysregulated pathways in glioblastoma. Consequently, inhibitors against members of this network are actively pursued by pharmaceutical companies to treat this malignancy and other types of cancer. However, these pathways converge to regulate downstream functions and often compensate each other, leading to drug resistance. Here by treating cellular models of glioblastoma with PI3K and MEK inhibitors we show different patterns of PI3K-MEK signaling network circuitry across the profiled cell lines. For example, we found an increase of activity markers for PI3K pathway after MEK inhibitor treatment, indicating that the PI3K pathway is inhibited by the MAPK in some models. These effects demonstrate that therapies based on cotreatments with PI3K and MEK/MAPK inhibitors are necessary to combat compensation mechanisms and transient responses to therapy. Infact, clinical trials are evaluating combination therapies with inhibitors against PI3K and MAPK signaling members, which are approved to treat different cancers in combination with other agents. Our results will advance in the knowledge of this aggressive brain cancer that remains extremely difficult to treat.

Nº 8

Divergence of PI3K and MAPK signaling pathway activities between an adherent cellular model of glioblastoma and their derived neurospheres

***Laboratorio**: Laboratorio de Señalización Celular y Proteómica*

Presenter: Lucía García, Elisa Arias, Alejandra Macías

Authors: Maruan Hijazi, Lucía García, Elisa Arias, Alejandra Macías

Keywords: glioblastoma multiform, glioma-initiating cells, neurospheres, kinase inhibitors, co-treatments

Abstract:

Survival of patients with multiform glioblastoma (GBM) goes from 12 to 18 months after diagnosis, as they have a high rate of relapse and resistance to treatment. Furthermore, we know that GBM is characterized by a heterogeneous set of cancer cells, including glioma-initiating cells (GICs), so therapeutic strategies specifically targeting them could be critical to improve clinical outcomes in GBM patients. After the evaluation of the expression of certain stem cell marker proteins in two cell models, adherent GL261 and neurospheres, we concluded that neurospheres acquired stem cell characteristics. We also treated these two models with kinase inhibitors of PI3K (Alpelisib and Copanlisib) and MAPK (Trametinib) signaling pathways, which both are deregulated in GBM, and we observed significant differences in their activity: the adherent cells showed higher PI3K pathway activity, while neurospheres had higher MAPK pathway activity. Moreover, we found that co-treatments visibly decreased cell viability, being lower in neurospheres. In conclusion, this study suggests that neurospheres and adherent cells differ in potential therapeutic strategies for tumor treatment and that co-treatments of both pathways are necessary to combat compensatory mechanisms.

Nº 9

Swimming towards vertebrate central nervous system regeneration

***Laboratorio**: Neurobiología Comparada*

Presenter: Almudena Velasco

Authors: Cristina Pérez-Montes, Laura DeOliveira-Mello, Almudena Velasco, Adrián Santos-Ledo* and, Rosario Arévalo*

Keywords: visual system, oligodendrocyte, Sox10, Sox2, optic nerve, zebrafish

Abstract:

The visual system of teleost fish shows continuous growth and regenerative capacities throughout their whole life. Thus, it can be very useful as a model to study the vertebrate central nervous system (CNS) neurogenesis. Several studies have demonstrated the participation of astrocytes and oligodendrocytes to support, guide, nourish, and myelinate the constantly added axons. Therefore, our study focused on how glial cells de-differentiate and differentiate during the regeneration process.

We have tracked the oligodendrocytes in the visual system of adult zebrafish before and after the optic nerve (ON) crush (6, 24 and 72 hours post-lesion, and 7 days post-lesion) using the sox10:tagRFPt transgenic line. We have also analyzed the expression pattern of Sox2 using immunohistochemistry in these fully differentiated oligodendrocytes. 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. Before ON crush, sox10:tagRFPt cells are found in the mature retina, the optic nerve head (ONH) and through all the ON. While Sox2 cells are localized in the peripheral germinal zone (PGZ), the mature retina and the ON. After ON crush, sox10:tagRFPt cells appears in the same areas mentioned before except in the ON crush zone, suggesting that they die or de-differentiate. Concomitantly, Sox2 cells extend towards the ONH, invading the ON crush zone. Interestingly, we have also found sox10:tagRFPt cells positive for Sox2 in the regenerative ON, which is characteristic of immature oligodendrocytes. Also, some but not all sox2 cells localized in the ON, the ONH and the retina were positive for the proliferation marker PCNA.

Our results suggest that the oligodendrocytes present in the crushed area respond to damage, proliferating and contributing to the structural and functional restoration of the injured visual system.

Nº 10

Proteínas adaptadoras de ubiquitina ligasas como nuevos actores en el mecanismo del dolor

***Laboratorio**: Neurobiología de las neurotrofinas*

Presenter: Daniel Cañada-García

Authors: Daniel Cañada-García, Cristina Vicente-García, Ana Hernández-García, Jorge Valero, Sharad Kumar, Juan Carlos Arévalo

Keywords: Osteoatrosis, Nocicepción, NGF/TrkA, Ubiquitinación, Ndfip2

Abstract:

La osteoartritis (OA) es una enfermedad degenerativa que causa dolor crónico en las articulaciones. El factor de crecimiento nervioso (NGF) y su receptor tirosina quinasa (TrkA) juegan un papel crucial en la patogénesis del dolor. La vía de señalización NGF/TrkA está regulada por la ubiquitinación, mediada por enzimas como Nedd4-2, que controla el tráfico intracelular y la degradación lisosomal de TrkA. Estudios previos han demostrado que una mutación en el sitio de unión de Nedd4-2 a TrkA (TrkAP782S) reduce la ubiquitinación de TrkA, aumentando su señalización, lo que provoca hipersensibilidad al dolor osteoartrítico inducido químicamente en ratones mediante inyección de monoiodoacetato (MIA). Hemos analizado los cambios en el patrón de expresión génica durante el desarrollo de la OA en ratones silvestres y mutantes de TrkA, identificando proteínas como Ndfip2, un adaptador que regula la función de Nedd4-2 junto con Ndfip1. Para estudiar su función, generamos un ratón knockout condicional para Ndfip2 en neuronas que expresan TrkA, observando una reducción dependiente del sexo en los niveles del receptor y en el número de neuronas que lo expresan. Tanto en ratones machos como hembras, se observó un descenso en la nocicepción mecánica y en la sensibilidad al frío, junto con un incremento moderado en la respuesta nocifensiva durante la primera fase del test de formalina. En cultivos de neuronas sensoriales, Ndfip1 y Ndfip2 interactúan con Nedd4-2 y TrkA respectivamente, regulando el sistema de manera diferente: Ndfip1 participa en la degradación de TrkA, mientras que Ndfip2 afecta a su biosíntesis y activación. Con estos hallazgos, buscamos profundizar en el mecanismo de ubiquitinación de TrkA que regula las funciones de NGF, y explorar el potencial de Ndfip2 como una nueva diana para el tratamiento del dolor crónico.

Nº 11

Novel TrkA mutants in the NGF/TrkA nociceptive pathway

***Laboratorio**: Neurobiología de las neurotrofinas*

Presenter: Francisco José González Calvo

Authors: González-Calvo, Francisco J., Cano-Arguelles, A. L, Martín-Zanca, D., Arévalo, J. C.

Keywords: TrkA, SNP, Pain, Nociception, NGF.

Abstract:

Neurotrophins are a family of growth factors that participate in the development, maintenance, survival and differentiation of neurons in the central and peripheral nervous system. The prototype of neurotrophins is nerve growth factor or NGF, which exerts its functions through of two types of transmembrane tyrosine kinase receptor: the tyrosine kinase receptor TrkA and the TNFR family receptor, p75. The activation of TrkA receptor by NGF binding leads to a signaling cascade mediated by 3 main proteins: PI3K, PLC-ɣ and MAPK, carrying out functions as neuronal survival and differentiation during development. Besides, it has been documented a straight relation between the NGF-TrkA signaling and pain processing. This way, genetic modifications in the TrkA and NGF coding sequences have been directly related to alterations in pain sensitivity, as in Congenital Insensitivity to Pain with Anhidrosis (CIPA) and in Hereditary Sensory and Autonomic Neuropathy type V (HSANV). In collaboration with the Bioinformatics service of the USAL, we have identified Single Nucleotide Polymorphisms (SNPs) in the NTRK1/TRKA gene which are present in volunteers of the UK Biobank who report different types of pain. We have observed that there are pain-related SNPs which appears to elicit a higher activation of TrkA, survival and differentiation in PC12-TrkA-KO cell cultures. We also made a SNP2 KI mice in collaboration with Lino Tessarollo's lab, in which we are performing different behaviour assays to study the pain sensibility of those animals.

Nº 12

TAT-Cx43266-283 as a potential therapy for glioblastoma: treatment response prediction and positive results in combination with tumor resection in preclinical models.

***Laboratorio**: Neurobioquímica*

Presenter: Andrea Álvarez-Vázquez

Authors: Andrea Álvarez-Vázquez, Josephine Volovetz, Laura San-Segundo, Pilar Cerveró-García, Raquel Flores-Hernández, Claudia Ollauri-Ibáñez, Berta Segura-Collar, Christopher G. Hubert, Pilar Sánchez-Gómez, Steven M. Pollard, Justin D. Lathia, Arantxa Tabernero.

Keywords: Glioblastoma, therapy, antitumor peptide, translational research

Abstract:

Glioblastomas (GBM) are the most malignant primary brain tumors, and they remain incurable. Despite the current standard of care (surgical resection of the tumor and administration of chemotherapy and radiotherapy) some GBM stem cells (GSCs) remain in the brain parenchyma causing tumor recurrence. The Src-inhibitory peptide TAT-Cx43266-283 has shown promising antitumor results in GBM preclinical models, reducing GSC viability and increasing survival in GBM-bearing mice. Here we have investigated some of the main gaps to overcome when translating preclinical results to the clinic, to promote the progress of TAT-Cx43266-283 as a clinical therapy to fight GBM.

First, we investigated biomarkers of treatment response to design a more targeted therapy. We assessed cell viability after treatment with TAT-Cx43266-283 in 13 patient-derived GSC lines and we found a stronger treatment response in those GSCs that had alterations in the Epidermal Growth Factor Receptor (EGFR). This was further confirmed in several murine GBM lines. Importantly, we found that treatment with TAT-Cx43266-283 was more effective in vitro than temozolomide or the classical EGFR inhibitor, erlotinib. Moreover, we showed that EGFR participates in TAT-Cx43266-283 response together with Src, by decreasing EGFR and EGFRvIII activity in some GSCs and increasing survival in mice bearing tumors with EGFR alterations.

Next, we studied the effect of TAT-Cx43266-283 in a clinical context by administering it in a murine model of tumor resection. We analyzed the histopathology of this GBM model, uncovering histological features typical of human GBM. We found that resection alone improved, although not significantly, GBM-bearing mice survival. However, tumors that regrew after resection exhibited more aggressive features. Importantly, the combination of tumor resection and TAT-Cx43266-283 achieved better survival outcomes and showed reduced invasive features.

Altogether, these results serve as a base for future clinical investigations and support the therapeutic potential of TAT-Cx43266-283 as a treatment for GBM.

Nº 13

Generation of TAT-Cx43266-283- and temozolomide-resistant glioma stem cells

***Laboratorio**: Neurobioquímica*

Presenter: Enrique Jiménez Madrona

Authors: Enrique Jiménez-Madrona, Laura García-Vicente, Andrea Álvarez-Vázquez, Arantxa Tabernero

Keywords: Glioma stem cells, drug resistance

Abstract:

Introduction:

Glioblastoma is the most common malignant primary brain tumor. Despite maximum safe tumor resection, radiotherapy and adjuvant chemotherapy with temozolomide (TMZ), patients diagnosed with glioblastoma have a median survival of 10 to 15 months. Glioblastoma stem cells (GSCs) have the ability to withstand current treatment standards and self-renew, causing fatal relapses. Our lab designed the cell penetrating peptide TAT-Cx43266-283 (TAT-Cx43), which has shown promising results in preclinical GBM models, targeting GSCs and increasing glioblastoma-bearing mouse survival. Because tumor cells inevitably acquire resistance to most treatments, in this study we aim to develop and study TAT-Cx43- and TMZ-resistant GSCs.

Material and method:

For this purpose, we used mouse GL261- and SB28-GSC subpopulations and treated them with growing concentrations of TAT-Cx43 (6.25 – 200 µM) and TMZ (25 – 1600 µM) for 6 to 8 passages (4-5 months). Dose-response was assessed in basal, TAT-Cx43- and TMZ-resistant GSCs. EC50 values were calculated at 72 and 144 h of treatment in at least 3 independent experiments.

Results and discussion:

Our results show that both SB28- and GL261-GSCs acquired resistance to TAT-Cx43 and TMZ after prolonged treatment with increasing concentrations. We are currently studying TMZ-resistant GSCs. Regarding TAT-Cx43-resistant cells, we found that EC50 values increased 5.36 (27.19 – 145.8 µM) and 1.96 times (62.19 – 121.8 µM) in TAT-Cx43-resistant compared to basal SB28-GSCs, at 72 and 144 h of treatment, respectively. The same trend was observed in GL261-GSCs. Thus, EC50 values for TAT-Cx43 increased 5.11 (34.36 – 175.5 µM) and 4.23 times (21.58 – 91.30 µM) in TAT-Cx43-resistant compared to basal GL261-GSCs, at 72 and 144 h of treatment, respectively. Interestingly, TAT-Cx43-resistant SB28-GSCs seemed to be more sensitive to TMZ. Thus, EC50 values for TMZ decreased 1.80 (2032 – 1126 µM) and 1.29 times (891.9 – 690.3 µM) in TAT-Cx43-resistant compared to basal SB28-GSCs at 72 and 144 h, respectively. Surprisingly, EC50 values for TMZ decreased 7.37 (6606 – 896.5 µM) but increased 6.54 times (47.89 – 313.6 µM) in TAT-Cx43-resistant compared to basal GL261-GSCs at 72 and 144 h, respectively. Although these results are intriguing, they should be interpreted cautiously, since non-lineal adjustment was not optimal for TMZ-EC50 in GL261-GSCs.

Conclusion:

We generated TAT-Cx43- and TMZ-resistant SB28- and GL261-GSCs by exposing them to increasing concentrations of treatment for extended periods of time. Our results show that TAT-Cx43 might sensitize SB28-GSCs to TMZ. However, this trend of TMZ sensitization was not reliably shown in TAT-Cx43-resistant GL261-GSCs. Furter studies are required to confirm these results and test the potential benefits of TMZ and TAT-Cx43 combination.

Conflict of Interest:

TAT-Cx43266–283 is a patent (ID: ES2526109B1) from the University of Salamanca.

Nº 14

Exploring the antitumor effect of the cell-penetrating peptide TAT-Cx43266-283-mod in glioblastoma preclinical models

***Laboratorio**: Neurobioquímica*

Presenter: María Martínez Fernández

Authors: M. Martínez-Fernández, A. Álvarez-Vázquez, E. Jiménez-Madrona, R. Flores-Hernández, A. Tabernero

Keywords: Glioblastoma, glioma stem cells, connexin-43, growth-associated protein 43

Abstract:

Introduction:

Glioblastoma (GBM) is the most common and aggressive primary brain tumor. Part of its malignancy lies in a subpopulation of cells within the tumor that have stem-cell properties (glioma stem cells, GSCs), which are resistant to standard therapies and responsible for tumor recurrence. Since GSCs constitute an interesting therapeutic target, our laboratory has designed a cell-penetrating peptide, TAT-Cx43266-283, that has shown promising antitumor effects in preclinical models of GBM, reducing GSCs proliferation, migration and invasion and increasing the survival of GBM-bearing mice by inhibiting c-Src oncoprotein activity in these cells. Recently, several modifications have been made in this peptide in order to improve its antitumor efficacy. Therefore, we aimed to compare the effect of this modified peptide (TAT-Cx43266-283-mod) with that of TAT-Cx43266-283 and the chemotherapeutic temozolomide (TMZ) in different GBM preclinical models. Furthermore, it has been shown that infiltrative GBM cells that evade tumor surgical resection are highly tumorigenic, so that recurrent tumors turn out to be more invasive than non-resected ones. Growth-associated protein 43 (GAP43) is a major structural protein of tumor microtubes, which have been identified as relevant cellular structures involved in intercellular communication, invasion and therapy resistance in GBM. In this sense, we also wanted to study the effect of TAT-Cx43266-283-mod treatment in GAP43 expression.

Materials and methods:

For these purposes, we first assessed cell viability of two murine GBM stem-cell lines (GL261 and SB28) after treatment with TAT-Cx43266-283-mod. Next, as resection is the first-line treatment against GBM, we studied the effect of administering TAT-Cx43266-283-mod in a murine model of tumor resection. To this aim, the murine GBM stem-cell line GL261 was intracranially injected into the brains of C57BL/6 mice, and once the tumors had already developed, animals were subjected to GBM resection or sham operation and treated either with TAT-Cx43266-283-mod or saline until the end of the experiment. Finally, immunofluorescence analysis for GAP43 staining were performed in brain sections of these mice.

Results and discussion:

On one hand, we observed that TAT-Cx43266-283-mod had a higher antitumor effect than TMZ and its counterpart TAT-Cx43266-283 in GL261 and SB28 stem-cell lines. On the other hand, although the results are preliminary and we are exploring them further, the effect of TAT-Cx43266-283-mod in combination with tumor resection on mice survival is promising, and GAP43 expression seems to be lower in the recurrent tumors of mice treated with TAT-Cx43266-283-mod compared to those treated with saline, which would explain the longer survival of these animals.

Conclusion:

Altogether, these results bring to light the therapeutic potential of TAT-Cx43266-283-mod as a possible treatment for GBM.

Conflict of Interest:

TAT-Cx43266–283mod is a patent (ID: PCT1367.115) from the University of Salamanca.

Nº 15

Exploring the effect of TAT-Cx43266-283 in lung cancer brain metastasis models

***Laboratorio**: Neurobioquímica*

Presenter: Pilar Cerveró-García

Authors: Pilar Cerveró-García, Laura García-Vicente, Andrea Álvarez-Vázquez, Raquel Flores-

Hernández, Claudia Ollauri-Ibáñez, María Paniagua-Sancho, Rocío Talaverón, Sandra M. Martín-Guerrero, Pedro Casado, Vinothini Rajeeve, Tommy Shields, Maruan Hijazi, Pedro R

Keywords: Brain metastasis, lung cancer, Src, ERK, mass spectrometry

Abstract:

Brain metastasis is the most common type of brain cancer and metastasis in general accounts for 90% of the deaths caused by cancer. Although considerable efforts have been made in recent years to develop effective treatments to improve the prognosis of patients with brain metastasis, the current treatments implemented in the clinic are still insufficient, and life expectancy is still poor. In the case of brain metastasis, lung cancer is the most common primary tumor to generate metastasis in this tissue. Cancer stem cells have been characterized as a key target for metastasis therapy, as they are able to acquire invading properties that allow them to escape the primary cancer site, migrate to other tissues through the bloodstream, and proliferate in a new tissue. In previous studies, our laboratory developed an antitumoral peptide based on Cx43 sequence, the TAT-Cx43266-283 peptide, which inhibits the activity of the oncoprotein Src, reducing proliferation, migration and invasion properties of glioblastoma stem cells, improving survival in glioblastoma models. Given the relevance of Src for lung cancer brain metastasis, in this work we analyzed the potential of TAT-Cx43266-283 for lung cancer brain metastasis therapy.

First, in vitro models showed how TAT-Cx43266-283 was able to either impair migration and invasion capacities or to reduce cell viability of human and mouse lung cancer cell lines. Using an in vivo model of lung cancer brain metastasis based on the implantation of mouse lung cancer cells in the brain parenchyma of immunocompetent mice, we showed that TAT-Cx43266-283 improved their survival. However, the molecular mechanism by which TAT-Cx43266-283 exerted these effects appeared to be different from previous findings made in glioblastoma models.

To identify the molecular mechanism of TAT-Cx43266-283 in lung cancer brain metastasis, we relied on a phosphoproteomic analysis, a high-throughput technique to determine the kinases and pathways altered in lung cancer brain metastasis models after TAT-Cx43266-283 treatment. With this approach, we unveiled ERK as a key mediator of TAT-Cx43266-283 effect in both types of models. ERK might participate in the changes observed in pathways related to cytoskeleton and vascularization functions in the treated 3LL-derived brain tumors. In addition, we studied the effect of TAT-Cx43266-283 in combination with inhibitors of several kinases unveiled by the phosphoproteomic study, and we found that TAT-Cx43266-283 together with inhibitors of MEK1/2, PKC, GSK-3ß and CaMKII showed promising results that support their combined use.

Overall, the results presented in this work set the basis for future studies to advance in the proposal of TATCx43266- 283 as a candidate for brain metastasis treatment, alone or in combination with other therapies.

Nº 16

Pharmacokinetic properties of the anti-tumor peptide TAT-Cx43266-283 in different murine brain tumor models

***Laboratorio**: Neurobioquímica*

Presenter: Raquel Flores Hernández

Authors: Raquel Flores Hernández, Andrea Álvarez-Vázquez, Rocío Talaverón, Pilar Cerveró-García, María Paniagua-Sancho, Carmen García-Macías, Arantxa Tabernero

Keywords: Glioblastoma, peptide-based drug, penetrance, stability, toxicity

Abstract:

Malignant brain tumors remain very difficult to treat due, among other causes, to the blood brain barrier (BBB). Our group designed a cell-penetrating peptide (TAT-Cx43266-283) based on connexin43 that inhibits the oncogenic activity of Src. TAT-Cx43266-283 enhances the survival of glioma-bearing mice and exerts important anti-tumor effects in preclinical models of glioblastoma in vitro and in vivo.

One of the main advantages of peptide-based drugs is their low toxicity and high specificity. However, poor plasma stability may result in suboptimal pharmacokinetic profile. In the present study we investigated the toxicity, plasma stability and BBB penetrance of TAT-Cx43266-283 peptide.

Glioma-bearing mice were intraperitoneally administered 5 days/week with 8 nmol/g/day of TAT-Cx43266-283. After 3 weeks, the histology of kidney, liver, spleen, heart, lung, and thymus was analyzed, showing no signs of toxicity, except for a small alteration in the renal medulla.

For stability and BBB penetrance studies, we used biotinylated-TAT-Cx43266-283. Glioma-bearing mice were intraperitoneally administered with biotinylated-TAT-Cx43266-283 and blood samples were taken at different time points. Western blots showed the stability of the peptide for at least 3h. We used a glioblastoma model and a lung brain cancer metastasis model (a common brain metastasis type) to assess the BBB penetrance. Histochemistry analyses showed that biotinylated-TAT-Cx43266-283 was present in these tumors at least 1,5 h after its administration.

So far, our preliminary results show that TAT-Cx43266-283 is not toxic in mice organs, remains stable in plasma for at least 3h, which suggests a good pharmacokinetic profile, and crosses the BBB, which supports its clinical application.

Nº 17

Neuronal responses to omitted tones in the auditory brain:

A neuronal correlate for predictive coding

***Laboratorio**: Neurociencia Auditiva y Cognitiva*

Presenter: Ana B. Lao-Rodríguez

Authors: Ana B. Lao-Rodríguez, David Pérez-González, Manuel S. Malmierca

Keywords: Electrofisiología auditiva

Abstract:

The human brain detects perceptual mismatches between expected and actual sensory inputs. These responses have been widely recorded in all sensory systems and have been interpreted in terms of predictive processing. Predictive processing is a leading and unifying theory of how the brain performs probabilistic inference. According to this framework, the brain extracts the regularities from the environment and uses them to actively predict what should happen next. When the prediction and input do not match, a prediction error signal is generated. It has been argued that the omission response provides conclusive, empirical evidence of the predictive process, as it occurs in the absence of sensory input (Wacongne et al., 2011, 2012). Nevertheless, to date, empirical evidence of omission responses at the neuronal level remains elusive. We investigated whether auditory neurons were signaling the omission deviant in an oddball paradigm context by measuring the evoked neuronal activity. The recordings were conducted in the inferior colliculus and the auditory cortex of

anesthetized and awake rodents. Our results reveal a subset of neurons that robustly increases their activity during the omission of an expected tone. These responses are evident, although weak, at anesthetized preparations and become stronger and distinct at the cortical level. Omission responses also show a higher probability of occurrence with shorter SOAs which aligns with the highest probability of omission responses at short latencies in humans (Raij et al. 1997; Hughes et al. 2001; SanMiguel et al., 2013a, 2013b). A deeper analysis based on individual stimulation sequences unveiled the so-called omission-selective responses (Fiser et al., 2016) and its distribution across the auditory cortex fields. Our findings suggest that neurons in the auditory system detect a deviation from expectations without the need for an external stimulus (Bendixen, et al 2012) and gives a decisive empirical support to the theory of predictive processing.

Nº 18

Stimulus-Specific Adaptation in the Hippocampus of the Anesthetized Rat

***Laboratorio**: Neurociencia Auditiva y Cognitiva*

Presenter: Jazmín S. Sánchez

Authors: Jazmín S. Sánchez* 1,2,3, David Pérez-González1,2,4 and Manuel S. Malmierca1,2,3

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Keywords: Hippocampus, Auditory, Stimulus-Specific Adaptation, multichannel recordings

Abstract:

The hippocampus is well known for its role in spatial and episodic memory. A broader function has been proposed including aspects of perception and relational processing. Recently, the hippocampus has also been associated with the detection of auditory novelty. Neural bases of sound analysis have been thoroughly described along the auditory pathway up to the auditory cortex (AC), and beyond in the prefrontal cortex (PFC). However, wider networks supporting auditory cognition are still elusive.

The human brain can automatically detect auditory changes, as indexed by the mismatch negativity (MMN) event related potential. MMN is a key biomarker of automatic deviance detection, thought to emerge from the AC and PFC. At the neuronal level, stimulus-specific adaptation (SSA) is considered the cellular counterpart of MMN. It is well established that the hippocampus has pathways that interconnect with the AC and PFC. The existence of these pathways further emphasizes the potential role of the hippocampus in the cortical manifestation of MMN. Recent studies showing similarities between neuronal SSA and behavioral habituation hint at the possibility that SSA interacts with, and may be part of, the brain's memory systems.

In the current study, we used the classical oddball paradigm, as well as two control sequences (many-standards and cascade) in anesthetized rats. Stimuli were pure tones 75 ms long, presented at a rate of 1 Hz. We recorded neuronal spiking activity related to auditory mismatch responses, in the hippocampal regions CA1 and Dentate Gyrus (DG), with 32-channel probes. We found that a subset of neurons in the hippocampus show long-lasting auditory responses with latencies ranging from 125-325 ms after stimulus onset. Approximately 35% of the neurons registered show an auditory response, less than 5% of said neurons present significant SSA.

These results demonstrate that a small subset of neurons in the hippocampus plays a significant role in SSA modulation and is part of a distributed network for deviance detection.

Financial support was provided by the Spanish Ministry of Science and Innovation (MICIN; grant # PID2019-104570RB-I00); and Consejería de Educación, Junta de Castilla y León (grant # SA218P23); JSS held a fellowship PRE2020-095617 funded by MCIN/AEI/ 10.13039/501100011033 and by "European Union NextGenerationEU/PRTR".

Nº 19

Auditory Stimulus-Specific Adaptation in the primary visual cortex of the Anesthetized Rat

***Laboratorio**: Neurociencia Auditiva y Cognitiva*

Presenter: Jazmín S. Sánchez

Authors: Jazmín S. Sánchez* 1,2,3, David Pérez-González 1,2,4 and Manuel S. Malmierca 1,2,3

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Keywords: Stimulus specific Adaptation, multichannel neurophysiology, Visual Cortex

Abstract:

As humans and other living organisms interact with their environment, the brain tries to generate an elaborate model of the world in the most efficient manner, continuously integrating information from different sensory inputs and adapting to change in their surroundings. It is known that primary sensory cortices respond to cross-modal stimuli, such as auditory responses in the primary visual cortex (V1), which may be relevant for multisensory representation.

The human brain can automatically detect auditory changes, as indexed by the mismatch negativity (MMN) event related potential. At the neuronal level, stimulus-specific adaptation (SSA) is considered the cellular counterpart of MMN. Recent studies of cross-modal MMN in animal models suggests that cross-modal information processing influences MMN without significant involvement of strong top-down effects, such as those stemming from prior knowledge and attention.

In an effort to understand the role of V1 neurons in auditory deviance detection, first, we recorded frequency response areas to auditory stimuli in V1 neurons to check if they were sensitive to auditory stimuli and then, we used the classical auditory oddball paradigm, and two control sequences (many-standards and cascade) to test deviance detection. Stimuli were pure tones 75 ms long, presented at a rate of 1 Hz. We recorded neuronal spiking activity related to auditory mismatch responses in the lower layers of V1 using 32-channel probes. We found distinct and significant SSA responses to pure tones, as well as long-lasting auditory responses with latencies around 175 ms after stimulus onset, with a maximum peak latency at around 400 ms. Approximately 60% of the recorded V1 neurons exhibited this type of auditory response, with a fifth of these neurons displaying a significant SSA index. When comparing the random and periodic oddball responses, there is significant difference between the responses, showing that neurons in V1 are able to distinguish between simple auditory patterns.

The existence of significant auditory SSA in V1 demonstrate that V1 neurons are part of a distribute network for deviance detection across sensory modalities and evidence the intricate nature of the circuits engaged in sensory integration and MMN.

Financial support was provided by the Spanish Ministry of Science and Innovation (MICIN; grant # PID2019-104570RB-I00); and Consejería de Educación, Junta de Castilla y León (grant # SA218P23); JSS held a fellowship PRE2020-095617 funded by MCIN/AEI/ 10.13039/501100011033 and by "European Union NextGenerationEU/PRTR".

Nº 20

Mismatch negativity changes throughout urethane anesthesia in the rat

***Laboratorio**: Neurociencia Auditiva y Cognitiva*

Presenter: Laura H. Bohórquez

Authors: Laura H. Bohórquez, Adam Hockley and Manuel S. Malmierca

Keywords: MMN, ECoG, Predictive coding, Urethane, Local Field Potentials, Prefrontal Cortex, Auditory Cortex

Abstract:

The mismatch negativity (MMN) is an auditory response elicited when a regular pattern in stimuli is disrupted by an event that breaks the regularity of that pattern. Is thought to emerge from two cortical sources: the auditory cortex (AC) and the prefrontal cortex (PFC). The generation of the MMN is consistent with the predictive coding hypothesis in which the AC sends afferent signals to the PFC, which generates a predictive model that it sends by efferent projections to the AC. This causes anticipated stimuli to be inhibited, and unpredictable stimuli create a prediction error to be transmitted bottom-up to update the model in the PFC. MMN has become a useful biomarker for several diseases because it has the advantages of being a reliable neurological signal that does not require attention or consciousness, it can be recorded by electroencephalography, electrocorticography (ECoG) or by invasive recording of individual neurons (neuronal mismatch). We studied how MMN changes under anesthesia and under different stimuli paradigms. Long Evans female rats were implanted with ECoG arrays consisting of silver-ball electrodes placed over left and right rostral and caudal AC, and two more electrodes over right and left PFC. We recorded local field potentials (LFPs) from anesthetized animals at different times points after induction of anesthesia with urethane (4, 6, 8, 10, 12 & 16 hours). Stimuli consisted of oddball, with a frequency pair of 10 kHz and 10 kHz + ½ octave, and cascade control paradigms, using different stimulus onset asynchrony (SOA; 125, 250, 500 & 1000 ms). We observe that longer SOAs (500 & 1000 ms) produce greater amplitude deviant responses, and over 16 hours there is a progressive decrease in the amplitude of the deviant response, producing a decreased prediction error and consequently in the MMN. In contrast, at shorter SOAs (125 & 250 ms), showed a smaller amplitude of the deviant response without changes observed over the 16 hours. These results show that, larger SOAs (500 & 1000 ms) are preferable for observing the MMN by ECoG in anesthetized rat. This method of MMN recording offers a global study of the different areas of the cerebral cortex and can be more transferable to human studies.

Nº 21

Novelty detection and predictive procesing impairments in an animal model of alzheimer disease

***Laboratorio**: Neurociencia Auditiva y Cognitiva*

Presenter: Laura Quintela Vega

Authors: L. Quintela Vega, D. Pérez-González, M. S. Malmierca

Keywords: Alzheimer disease, behaviour, novelty detection

Abstract:

Novelty detection and predictive procesing impairments in an animal model of alzheimer disease

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Age-related hearing loss is a widespread disability, affecting communication and social participation in the older population. Recent research highlights hearing loss as a significant risk factor for dementia, such as Alzheimer's disease (AD).

As individuals age, peripheral processing accuracy diminishes, leading to a greater dependence on predictions over sensory input. The reliability of auditory predictions may be significantly impaired in dementia. AD patients show affected temporo-parietal areas, which may lead to a worse adaptation to auditory stimuli. Consequently, aging alters the predictive process both cognitively and behaviorally.

We trained 20 freely moving rats (16-months-old, TgF344-AD and control) from both sexes, to discriminate deviant tones in an oddball paradigm presented in an operant conditioning chamber. TgF344-AD rats manifest age-dependent AD related lesions including amyloid plaques, neurofibrillary tangles with hyperphosphorylated tau, and neuronal loss. We divided the animals into two groups, based on the pair of frequencies presented as standard and oddball in the different tasks and neuronal recordings (4.8-6.7 and 8.0-11.3 kHz).

Various iterations of the oddball paradigm were presented to evaluate the animals' discrimination ability. We varied the interstimulus interval (1.5, 2 and 4 seconds) and tested the influence of frequency contrast relative to the standard tone (0.5, 1.0 and 1.25 octaves between tones). We also presented a modified oddball paradigm where the frequency contrast between standard and deviant tone randomly varied between 9 possibilities and different standard/deviant probability ratios (90/10 and 70/30 %) were tested. Animals' responses were evaluated using the d’ index.

During training, we observed differences between the young healthy animals (results from previous studies in our laboratory), the aged and the AD ones. These disparities were mainly influenced by Alzheimer's disease-related lesions development and distribution, age of the subjects and the sex, leading to considerable individual variability. In conclusión, age and dementia affect the detection of deviant stimuli differently, depending on the disease progression and sex.

Work supported by project PID2019-104570RB-I00 funded by MCIN/AEI/10.13039/501100011033/ to MSM and The Foundation Ramón Areces grant CIVP20A6616 to MSM/LQ/DPG.

Nº 22

Sex- and age-specific effects on subcortical predictive processing of typically developing and valproic acid-induced rats

***Laboratorio**: Neurociencia Auditiva y Cognitiva*

Presenter: Sara Cacciato Salcedo

Authors: Cacciato Salcedo S, Lao-Rodríguez AB, Malmierca MS

Keywords: Autism, Valproic acid, Sex, Age, Neurodevelopment

Abstract:

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterized by restricted, repetitive interests, and atypical sensory processing. Altered sensory processing would compromise environmental experience, hindering the acquisition of progressively more elaborate cognitive abilities. Particularly, when stimuli (e.g. sounds) and contexts (e.g. social) become dynamic and unpredictable. The predictive coding theory frames these symptoms. It postulates that the brain constantly compares prior predictions with upcoming sensory information. In case of mismatch, a prediction error arises, updating the predictions to the new environmental experience, facilitating adaptive behaviour. The predictive coding theory of ASD suggests an atypical predictiveb processing of sensory stimuli, compromising adaptability to non-routine events. To study unusual predictability in ASD, autistic traits were induced to pups born of pregnant rats that received an i.p injection of valproic acid solution (400 mg/kg) at the gestational day 12. Female and male prepubertal (PD30-45) and adult (PD65-120) animals were included, addressing developmental and sex biases.

We studied the predictive processing of auditory stimuli at the subcortical level, recording single neurons in the lemniscal and nonlemniscal inferior colliculus (IC). Five auditory conditions were presented: A classical oddball paradigm was used to study mismatch negativity; the brain response to the disruption of the regularity, and control sequences were used to further analyse predictive indexes.

Results support the notion of atypical predictive processing in ASD, which explain limited adaptive behaviour in unexpected situations.

Nº 23

RONS facilitate AMPK and AKT phosphorylation which drive GLUT4 translocation to plasma membrane in skeletal muscle cells (C2C12 myotubes)

***Laboratorio**: Fisiopatología redox del músculo esquelético y envejecimiento*

Presenter: Martín-Prieto, Eva

Authors: Martín-Prieto, Eva; Fernández-Puente, Escarlata; Palomero, Jesús

Keywords: Reactive Oxygen and Nitrogen Species (RONS) ; Redox signaling ; Oxidative Stress ; GLUT4; Glucose Uptake;Insulin Resistance; C2C12 myotubes; Skeletal Muscle

Abstract:

Reactive Oxygen and Nitrogen Species (RONS) at low or moderate levels -oxidative eustress- may be essential for redox signaling processes that regulate cellular homeostasis. Glucose uptake in skeletal muscle may be affected by RONS homeostasis. The phosphorylation of AMPK (P-AMPK) and/or AKT (P-AKT) is the key in signaling pathways that lead glucose uptake. Thus, this phosphorylation prompts the translocation of GLUT4 from the cytosolic vesicles to the plasma membrane, which facilitate glucose internalization into the cell. We have explored the influence of RONS homeostasis on glucose uptake in skeletal muscle and how these RONS promote AMPK and AKT phosphorylation, GLUT4 translocation, and glucose internalization. Our experimental model was C2C12 myotubes that were exposed to different pro-oxidant reagents. Immunodetection (western-blotting) methodology was performed to detect P-AMPK and P-AKT. In addition, immunocytochemistry combined with confocal fluorescence microscopy techniques were undertaken to detect GLUT4 translocation to the plasma membrane. The results showed that insulin does not affect P-AMPK but induce P-AKT. H_2O_2 (1 and 100 µM) might facilitate P-AMPK and produce more P-AKT than insulin. Nitric oxide donors, SNAP and SNP, might not affect P- AMPK however, they enhance P-AKT. Angiotensin II would evoke P-AKT, and only 2,5 µM Angiotensin II appeared to facilitate P-AMPK. Furthermore, the immunodetection for co- localization of GLUT4 in the plasma membrane showed a clear translocation of GLUT4 under a pro-oxidative environment, which might be mediated upstream by P-AMPK or P-AKT signalling pathways. In conclusion, the RONS-induced phosphorylation of AMPK and AKT may be an alternative, different to the insulin-dependent pathway, which may facilitate the glucose uptake in C2C12 myotubes.

Nº 24

The inferior colliculus is not innervated by non-auditory cortical areas

***Laboratorio**: Neurohistología*

Presenter: Mario Gómez Martínez

Authors: H. Rincón, M. Gómez-Martínez, J. Martín, and E. Saldaña

Keywords: Inferior colliculus, Visual cortex, Auditory cortex, Somatosensory cortex, Motor cortex

Abstract:

The inferior colliculus (IC), a major center for the integration of auditory information, is the lowest level in the auditory pathway where prediction error occurs. It is known that the IC receives this predictive information through descending projections from the auditory cortex. Recently, two works have described that the IC also receives descending projections from several non-auditory cortical areas (Olthof et al. 2019 and Gartside et al. 2024). This observation has strong functional implications, e.g., it could suggest that the IC receives predictive information from other sensory modalities.

In the work by Olthof et al. (2019), numerous retrogradely labeled neurons were found in the visual, somatosensory, motor, and prefrontal cortical areas on both sides after injecting retrograde tracers into the IC. Anterograde tracer injections were also performed in these same cortical areas, which resulted in densely labeled puncta in all regions of the IC on both sides. However, given that there are evident contradictions between these results and those described in the extensive literature on cortico-collicular projections, and the strong functional implications derived from these findings, we decided to replicate this work.

To this end, we injected a well-known tracer that is transported in an anterograde direction, the biotinylated dextran (BDA), in the auditory, visual, somatosensory and motor cortical areas of adult rats. In our experiments, only the cases with injections of BDA in the auditory cortex revealed labeled fibers in the IC. Therefore, we conclude that the IC is not innervated by non-auditory cortical areas. We do not know the origin of the results shown by Olthof et al. (2019) and Gartside et al. (2024), but it is possible that they are due, at least in part, to technical artifacts. Our study reinforces the importance of reproducibility of research, which is essential for the advancement of the discipline.

Nº 25

Multisession direct current stimulation targeting the auditory cortex prevents cortical aging in a Wistar rat model of ARHL

***Laboratorio**: Neuroplasticidad auditiva*

Presenter: Inés Santos Fernández del Campo

Authors: Inés S. Fernández del Campo, A. Fuente, Iván Díaz, I. Plaza, Miguel A. Merchán

Keywords: Aging, c-fos, Inhibition, age-related hearing loss

Abstract:

We have previously communicated that multisession epidural direct current anodic stimulation (ES) of the auditory cortex (AC) preserves hearing thresholds (Fernández del Campo, 2024) and the loss of neurons in the olivary brainstem efferent system (Alvarado, 2024) after aging. Although it is known that the descending pathway is the main effector for hearing preservation in our model, intrinsic mechanisms through which ES preserves cortical regulation must be analyzed. Our hypothesis is that our ES protocol regulates AC activity and prevents alterations in inhibitory circuitry, thereby stabilizing hearing thresholds during aging.

To test this hypothesis, after applying our previously tested multisession anodal epidural direct current stimulation protocol in a Wistar rat model, we analyzed auditory cortical evoked potentials and c-fos quantitative immunocytochemistry. Also, effects on the loss of inhibitory interneurons were explored through GAD-67 and Parvalbumin quantitative immunocytochemistry.

Compared to young rats, animals with ARHL, exhibit changes in wave amplitude, latency, and morphology of auditory evoked potentials, along with a significant decrease in c-fos, GAD-67, and Parvalbumin immunoreactive neurons in the AC. However, after ES we observed better preserved cortical evoked responses and no substantial changes in c-Fos and inhibitory immunocytochemical markers.

These findings suggest that multisession ES induces the stabilization of cochlear responses by preserving AC function and the survival of inhibitory micro-circuitry. Present results can help to design future potential therapeutic strategies for brain ES that may benefit the prevention of presbycusis, one of the most prevalent age-related pathologies.

Nº 26

Assessing the Impact of Caffeine and Taurine from Energy Drinks on Zebrafish Embryos

***Laboratorio**: Neuroquímica y Neurociencia Molecular*

Presenter: Verónica González Núñez

Authors: Elena González Moraga and Verónica Gonzalez Nuñez

Keywords: cafferine, taurine, energy drinks, zebrafish

Abstract:

Energy drinks contain large amounts of stimulants, such as caffeine, and other additives as taurine, two biologically active compounds. At present, the consumption of energy drinks has significantly increased, especially among teenagers and other people aged under 30. The OEDA has issued an alert on the consumption of these drinks, as it may become a Public Health problem in a near future. While Caffeine Use Disorder is listed as one of the ten classes of Drug and Substance Use Disorders in the DSM-V, there are hardly any studies on the long-term effects of taurine consumption, especially at high doses.

Zebrafish embryos provide a unique model to in vivo analyse the neurophysiological effects of certain drugs of abuse, as they are small and transparent, allowing real-time observations of the effects of these substances. Previous work carried out by our research group has shown that several 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 group is studying the potential effects of caffeine and taurine on zebrafish embryos. To achieve this, we have carried out several in vivo tests to analyse the toxicity of these compounds, as well as to study the biochemical mechanisms responsible for their biological actions. These analyses comprise: the Zebrafish Embryo Toxicity Test (ZFET); the EROD assay to determine the induction of CYP1a liver detoxification pathway; the Neuromast Assay to evaluate their potential neurotoxicity; Acridine Orange staining to determine cell viability; Measurement of ROS levels using DCFH-DA; Immunohistochemical staining to evaluate the effect of these compounds on cell proliferation during the embryonic development and in the activation of glial cells. Our results indicate that we have validated an experimental model to in vivo test the biological effects of components found in energy drinks, and that they may exhibit a certain degree of toxicity and deleterious effects during the embryonic development.

Nº 27

Neuroprotective strategies with neurotrophic factors against selective neuronal loss

***Laboratorio**: Plasticidad Neuronal y Neurorreparación*

Presenter: David Díaz López

Authors: David Díaz López, Laura Pérez-Revuelta, David Pérez-Boyero, Carmelo A. Ávila-Zarza, Jesús García Briñón, Eduardo Weruaga Prieto

Keywords: Cell therapy, cerebellum, IGF1, Neurotrophic factors, olfactory bulb, PCD mouse, VEGF-B

Abstract:

PCD mice suffer the postnatal death of cerebellar Purkinje neurons and mitral cells of the olfactory bulb (OB). They are also defective in IGF1, but little is known about other neurotrophic factors, which may act as neuroprotective molecules.

The expression of different neurotrophic factors (IGF1, BDNF, VEGF-A and VEGF-B) was analyzed by qPCR and ELISA around cerebellar and bulbar degenerations of PCD mice. Then, we applied two different therapies depending on the affected region.

1. We refined healthy bone marrow transplants to reduce the degeneration of mitral cells. PCD mice were transplanted with 7.5 million bone marrow stem cells, supplemented with genetically modified hematopoietic cells for overexpressing the Igf1 gene. At P150, animals were sacrificed and their olfactory bulbs were analyzed by immunofluorescence and quantitative PCR.

2. Recombinant human IGF-1 or VEGF-B were administered in PCD mice depending on their expression around Purkinje cell loss. After motor tests, cerebella were analyzed at P25 or P30 by immunofluorescence.

Only IGF1 and VEGF-B presented variations around the neurodegenerative processes of PCD mice. Concerning OB, the transplantation of a genetically modified healthy bone marrow stopped the mitral cell loss of PCD mice. IGF1-enriched transplants changed the inflammatory pattern and prevented DNA damage. Regarding cerebellar degeneration, we observed an improvement in both motor coordination and Purkinje cell survival in PCD mice administered with VEGF-B. qPCR analyses revealed an inhibition of the intrinsic pathway of the apoptotic process.

In conclusion, neurotrophic factors are very suitable candidates to develop different neuroprotective therapies.

Nº 28

Inhibition of microglia in an animal model of selective neurodegeneration, the Purkinje Cells Degeneration mutant mouse

***Laboratorio**: Plasticidad Neuronal y Neurorreparación*

Presenter: Eduardo Weruaga

Authors: Pérez-Boyero D, Téllez de Meneses PG, Reverte I, Ragozzino D, Weruaga E*, Díaz D*

Keywords: cerebellum, microglia, neuroinflammation

Abstract:

The Purkinje Cell Degeneration (PCD) mutant mouse presents a mutation in the Ccp1 gene that produces the selective post-natal death of Purkinje cells. Concomitantly with this neuronal loss, a strong microgliosis takes place in the cerebellum, but it is not fully understood whether it presents a beneficial or a harmful role in the development of the pathology. Moreover, it is also unknown if this gliosis is a direct consequence of the neurodegeneration or if the pcd mutation itself causes an aberrant microglial behavior. Therefore, our study focused on the influence of the aberrant microglial behavior in the cerebellar neurodegeneration of the PCD mouse.

For this purpose, we used wild-type (WT) animals, and both untreated PCD and PCD mice administered with the microglial inhibitor PLX5622 to remove cerebellar microglia. Behavioral analyses were performed along with a series of immunohistochemically techniques.

Our results indicate that PCD mice with removed microglia exhibit an improved motor behavior compared to untreated PCD mice. We also observed that treated animals have a higher number of Purkinje cells compared to untreated PCD mice.

Therefore, we could confirm that the exacerbated microgliosis occurring in the PCD mouse fosters the loss of Purkinje cells, which triggers the loss of motor skills.

Nº 29

INHIBITION OF MICROGLIA IN AN ANIMAL MODEL OF SELECTIVE NEURODEGENERATION, THE PURKINJE CELL DEGENERATION MUTANT MOUSE

***Laboratorio**: Plasticidad Neuronal y Neurorreparación*

Presenter: Eduardo Weruaga Prieto

Authors: D. Pérez-Boyero, P. González Téllez de Meneses, I. Reverte Soler, D.

Ragozzino, E. Weruaga Prieto, D. Díaz López

Keywords: Inflammation, Microglia, Neurodegeneration

Abstract:

The Purkinje Cell Degeneration (PCD) mutant mouse presents a mutation in the

Ccp1 gene that produces the selective post-natal death of Purkinje cells. Concomitantly with this neuronal loss, a strong microgliosis takes place in the cerebellum, but it is not fully understood whether it presents a beneficial or a harmful role in the development of the pathology. Moreover, it is also unknown if this gliosis is a direct consequence of the neurodegeneration or if the pcd mutation itself causes an aberrant microglial behavior. Therefore, our study focused on the influence of the aberrant microglial behavior in the cerebellar neurodegeneration of the PCD mouse.

For this purpose, we used wild-type (WT) animals, and both untreated PCD and PCD mice administered with the microglial inhibitor PLX5622 to remove cerebellar microglia. Behavioral analyses were performed along with a series of immunohistochemically techniques.

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Therefore, we could confirm that the exacerbated microgliosis occurring in the PCD mouse fosters the loss of Purkinje cells, which triggers the loss of motor skills.

Nº 30

Unveiling fusion between bone marrow-derived cells and Purkinje cells: Patch-Seq analysis in a mouse model of multiple sclerosis

***Laboratorio**: Plasticidad Neuronal y Neurorreparación*

Presenter: Pablo G. Téllez de Meneses

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Keywords: Bone marrow-derived cells, Multiple sclerosis, Purkinje cell, Cell fusion, Patch-Seq

Abstract:

Neurodegenerative diseases pose significant challenges for society due to limited treatments. Among the potential therapies, cell therapy stands out as a particularly promising one. We know that bone marrow-derived cells (BMDCs) can infiltrate the brain and transdifferentiate to different cell types, or fuse, for example, with Purkinje cells. These fusion events are unusual, but they increase in some pathologies, such as multiple sclerosis. In the Experimental Autoimmune Encephalomyelitis (EAE), a mouse model of multiple sclerosis, fused cells are functional and maintain two transcriptionally independent nuclei. This suggests that BMDCs might have a therapeutic potential, e.g. as vehicles for the expression of therapeutic agents. Nevertheless, fusion mechanisms and the characteristics of fused cells are still unclear.

We developed a protocol to study cell fusion events in EAE mice. We first transplanted mice with GFP-positive BMDCs to identify fused cells. Then, we

optimized the time window between the transplant, EAE induction, and sacrifice. Once optimized, we used Patch-Seq technology to compare individual fused Purkinje cells and non-fused neighbours. Cells were recorded, filled with biocytin for morphological studies, and their cytoplasm was collected for single-cell RNASeq analysis.

Using this multimodal approach, we could assess functional, morphological, and gene expression differences in fused and non-fused cells to unravel the cell type responsible for these events and possible fusion mechanisms.

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Nº 31

Effects of environmental enrichment on mossy fibers and their relationship with microglia

***Laboratorio**: Plasticidad Neuronal y Neurorreparación*

Presenter: Sara Sánchez Monreal

Authors: Sara Sánchez-Monreal, Teresa Cocho, Noelia Rodríguez-Iglesias, Luis A. de Castro, Carmelo Ávila-Zarza, Eduardo Weruaga, José Ramón Alonso, Jorge Valero

Keywords: Environmental enrichment, mossy fibers, hippocampus, microglia, adult hippocampal neurogenesis

Abstract:

The hippocampus is one of the most plastic regions of the central nervous system due to the constant remodeling of synaptic contacts and because neurogenesis continues throughout adult life. Newly generated granular cells incorporate into the hippocampal circuitry and extend their axons, known as mossy fibers, establishing new contacts with CA3 pyramidal cells. Lifestyle, and in particular environmental enrichment, modulates brain function by promoting neuro- and synaptogenesis in the hippocampus. Furthermore, microglia have been proposed as a modulator of the environmental effects on adult neurogenesis. In this project, we optimized a protocol for the analysis of new mossy fibers and their glutamatergic terminals in CA3 by using the specific markers PSA-NCAM and vGlut1, respectively. We also analyzed the relationship between the axonal fibers and terminals with microglia. The application of this protocol allowed us to detect differences between CA3 regions and the increase of excitatory terminals of the new mossy fibers after environmental enrichment

Nº 32

ENVIRONMENTAL ENRICHMENT EFFECTS ON HIPPOCAMPAL MICROGLIA AND ADULT NEUROGENESIS

***Laboratorio**: Plasticidad Neuronal y Neurorreparación*

Presenter: Teresa Cocho Curto

Authors: Cocho, T., Sánchez-Monreal, S., Rodriguez-Iglesias, N., Valero, J., Sierra, A., Ávila-Zarza, C., Weruaga, E. & Alonso, J.R.

Keywords: Adult neurogenesis, environmental enrichment, hippocampus, microglia, molecular layer.

Abstract:

Physical, social and cognitive stimulation provided by an active lifestyle induces changes in the structure and function of the brain that promote healthy aging. This phenomenon has been widely studied in the hippocampus, as it is a brain structure that exhibits high plasticity, including the production of new neurons in the dentate gyrus (DG) during adulthood (adult hippocampal neurogenesis: AHN). Most previous studies have focused on the effects of environmental stimulation on the production of new neurons. However, the cellular mechanisms by which lifestyle modulates AHN are not completely understood yet. In this study, we investigated the effect of environmental stimulation on microglía within the molecular layer (ML) of the DG, as microglia have the potential to detect changes in its microenvironment and consequently modulate AHN. We housed two-month-old mice in an enriched environment (environmental enrichment: EE) for six weeks and we compared them with animals housed in standard conditions (non-enriched: NE). Our results indicate that EE increases the size of the DG and the dendritic density of immature neurons located in the ML. Additionally, EE reduces the area occupied by microglia in the ML without changing their number. Furthermore, our colocalization analyses show that EE decreases microglial surveillance of new dendrites. These findings indicate that EE affects the interaction between microglia and newly generated neurons.

Nº 33

DELVING INTO SYNAPTIC ACTIVITY IN AUTISM: NITRIC OXIDE PATHWAY AND GLUTAMATE/GABA RATIO

***Laboratorio**: Plasticidad Neuronal y Neurorreparación*

Presenter: Valeria Lorena Cabedo Navarro

Authors: Cabedo Navarro, V. L.; Pérez-Boyero, D.; Téllez de Meneses, P. G.; Weruaga Prieto, E.; Alonso Peña, J. R.; Díaz López, D.

Keywords: Autism, nitric oxide pathway, GABA, glutamate

Abstract:

Autism is a complex and heterogeneous neurodevelopmental condition. One of the major pathophysiological alterations in this disorder is an excitatory/inhibitory (E/I) neurotransmission imbalance. However, the underlying mechanisms of this imbalance remain poorly understood. The neuromodulator nitric oxide (NO) constitutively participates in E/I activity regulation by interacting with both glutamate and GABA. Little is known about how nitrergic system works in the autistic brain, although several studies suggest that dysregulations in this system may be involved in the pathogenesis of autism.

We investigated the E/I imbalance and the nitrergic system functioning in autism, using the valproic acid-induced model (VPA). Our area of interest was the olfactory bulb (OB), a fundamental region of the olfactory system, which is richly interconnected with areas involved in cognition and emotion. Previous studies demonstrated olfactory impairments and, specifically, changes in the OB in people with autism and in the VPA mouse. Another important point is the remarkable production of NO in the OB. We implemented cellular and molecular techniques to study GABAergic and glutamatergic synapses and activity/expression of NO as well as elements involved in its synthesis-degradation process.

Our analyses indicate significant alterations in the E/I ratio and aberrant levels of nitrergic pathway elements in the VPA animal. This new information supports the E/I imbalance hypothesis and emphasises the potential role of nitrergic system, as modulator of neuronal excitability, in this synaptic mismatch. As a next step, we will directly investigate the relationship between NO and glutamate/GABA, thus shedding further light on this field.

Nº 34

Modelling fully developed human retinal pigment epithelium sheets obtained from induced pluripotent stem cells (IPSC)

***Laboratorio**: Trastornos Degenerativos del Sistema Visual*

Presenter: Lucía San José Lassalle

Authors: San José-Lassalle, L., Aldavero-Muñoz, I., Segurado, A., Velasco, A., Lillo, C.

Keywords: iPSC; retinal pigment epithelium; cell culture; age-related macular degeneration

Abstract:

Age-related macular degeneration (AMD) constitutes the main cause of blindness in people over 50 years old. There are currently no efficient treatment options for this disease. Human induced pluripotent stem cells (hiPSCs) offer new opportunities for an accessible and reproductible in vitro model for retinal diseases, as they can differentiate to retinal pigment epithelium (RPE), which plays an important role in vision and retinal function maintenance. Our objective is, first, to develop a protocol for generating pure RPE sheets from hiPSCs with high efficiency and reproducibility, and secondly, to study the effects of the deletion of CRB2, a protein involved in the physiological maturation and polarization of the RPE, in the establishment of a functional RPE cell layer. The starting point was the PGP1 line, a hiPSC commercial cell line obtained from primary fibroblasts from a control donor with a well-defined genetic and clinical background. Then, control cells and a pool of hiPSCs CRISPR-edited to remove CRB2 were differentiated to RPE cells. This method is based in the manipulation of the insulin growth factors (IGF), basic fibroblast growth factor (FGF-2; FGF-basic), transforming growth factor beta (TGF-β), and WNT pathways, that leads to an increased efficiency for RPE differentiation. Thus, it enables RPE sheet generation at high purity without manual selection. This protocol produced a homogeneous, pigmented, polarized, phagocytic, and polygonal monolayer of human RPE cells. We expect that this differentiation protocol could be useful for the efficient production of RPE sheets for regenerative cell therapies, as well as serve as a model for the testing of potential treatments for the AMD and other retinal diseases in which the RPE is affected. However, more data is needed to determine how key proteins like CRB2 impact both healthy and pathological RPE.

Nº 35

PROTEOMIC PROFILE MODIFICATIONS IN THE EPILEPTOGENIC NUCLEUS OF THE GENETICALLY AUDIOGENIC SEIZURE-PRONE HAMSTER GASH/SAL AFTER REPEATED SEIZURES

***Laboratorio**: Trastornos audiomotores y epilepsias reflejas*

Presenter: Carlos García Peral

Authors: Carlos García-Peral, Laura Zeballos-Fernández, Martín Manuel Ledesma, Alberto Lazarowski, Dolores E. López García

Keywords: GASH/Sal, seizures, proteomic, bioinformatics

Abstract:

The Genetic Audiogenic Seizure Hamster, Salamanca (GASH/Sal) is a model of audiogenic seizures with the epileptogenic focus localized in the inferior colliculus. The sound-induced seizures promote transcriptomic and proteomic disturbances in the inferior colliculus. Here, we aim to compare the epileptogenic foci protein profiles of the GASH/Sal after repetitive stimulations with the naïve GASH/Sal counterpart. Protein samples from the inferior colliculus were processed for enzymatic digestion and then analyzed by mass spectrometry in Data-Independent Acquisition mode. After identifying the proteins using the UniProt database, we selected those with differential expression and performed over-representation gene ontology and pathway enrichment analyses using bioinformatics tools. We identified 5139 proteins, among them, 286 were differentially expressed proteins (DEPs), with 57 upregulated and 229 downregulated proteins, and 24 of the DEPs directly related to epilepsy. Furthermore, 9 and 14 proteins were exclusively found in naïve GASH/Sal or stimulated GASH/Sal respectively. To mention, the taperin protein, uniquely found in the GASH/Sal naïve group. Mutations in the gen enconding this protein cause progressive hearing impairment without vestibular dysfunction. In the GASH/Sal stimulated group, the protocadherin gamma-C5 isoform was detected, a potential calcium-dependent cell-adhesion protein that may be involved in the establishment and maintenance of specific neuronal connections in the brain. The results indicate a protein profile alteration in the epileptogenic nucleus of stimulated animals. Interestingly, we observed enriched biological process related to regulation of neurotransmitter levels, metabolic pathways and energy production, as well as enriched KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways associated with neurodegenerative disorders such as Parkinson and Alzheimer disease. In summary, this study reports protein alterations in the epileptogenic nucleus of a convulsive animal model submitted to repetitive epileptic crisis.

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Nº 36

EPILEPSY RELATED TO INGESTION IN A SYNGAP1 MUTATION PEDIATRIC PATIENT

***Laboratorio**: Trastornos audiomotores y epilepsias reflejas*

Presenter: Hilario Gómez Martín

Authors: Hilario Gómez, María Dolores Calabria, Aránzazu Hernández Fabián, Raúl Sastre, Gemma Vázquez, Ricardo Gómez-Nieto, María Justel

Keywords: epilepsy, EEG, eating chewing, SYNGAP1,

Abstract:

The *SYNGAP1* gene (6p21.32) encodes a synaptic protein that activates Ras GTPase and is predominantly expressed in the synapses of excitatory neurons. It plays a critical role in the NMDA receptor-activated Ras signaling cascade, which regulates postsynaptic density, as well as the formation, development, and maturation of dendritic spines. Loss-of-function mutations in SYNGAP1 have significant consequences for neuronal development, resulting in a clinical phenotype that includes global developmental delay or intellectual disability, often associated with epilepsy, autism spectrum disorder (ASD), and other behavioral disturbances. Notably, some patients experience epileptic seizures triggered by eating.

We report the case of a 13-year-old male patient with no family history of epilepsy or developmental delay. His early development was unremarkable until the age of 5-6 months when a noticeable slowing in neurodevelopment occurred. He was subsequently diagnosed with intellectual disability, ASD, and ataxia.

Comprehensive genetic testing and video electroencephalography (EEG) were conducted to establish a definitive diagnosis. A 1-hour and 24-minute awake-state EEG, incorporating activation maneuvers such as intermittent light stimulation and food intake, was performed due to suspicion of epilepsy triggered by eating. Genetic analysis revealed a *SYNGAP1* mutation. Although some EEGs appeared normal, the patient exhibited generally affable and calm behavior. In addition, he was diagnosed with celiac disease. More recently, his mood began to deteriorate, particularly during meals and in the afternoons. Initially, these mood changes were attributed to gastrointestinal issues or sensory responses related to ASD.

Given the potential link between seizures and eating, another EEG was performed during food consumption. This EEG revealed sporadic polyspike-wave activity, accompanied by a minor clinical event of disconnection, leading to a diagnosis of generalized epilepsy with absences, likely of reflex origin triggered by eating. Treatment with valproic acid was initiated, resulting in a marked improvement in mood and the cessation of clinical epileptic events.

However, epileptic activity persisted, occurring more frequently during non-REM sleep and occasionally during meals, although without overt clinical manifestations.

In conclusion, it is crucial to actively investigate epileptic activity in patients with developmental encephalopathy and *SYNGAP1* mutations. The relatively high incidence of reflex epilepsy associated with chewing warrants thorough diagnostic evaluation to guide appropriate treatment, thus preventing significant diagnostic delays.

Nº 37

CONTENIDO PROTEÓMICO EN VESÍCULAS EXTRACELULARES DE PACIENTES EPILÉPTICOS. ESTUDIO PILOTO

***Laboratorio**: Trastornos audiomotores y epilepsias reflejas*

Presenter: Jaime Gonçalves

Authors: Jaime Gonçalves, Manuel Martín Ledesma, David Sánchez-Benito, Laura Zeballos, Dolores E. López DE, Jesús M. Gonçalves-Estella, Calabria Gallego

Keywords: epilepsia, exosomas, proteómica, bioinformatica

Abstract:

Introducción. Los exosomas son vesículas extracelulares liberados por numerosos tipos celulares. Desempeñan un papel clave en múltiples funciones celulares, incluyendo coagulación y señalización intercelular. Recientemente, se ha incrementado el interés del estudio de los exosomas como biomarcadores no invasivos en epilepsia.

Objetivos. Estandarizar la evaluación de exosomas en pacientes epilépticos, buscando proteínas comunes que ayuden a comprender los mecanismos implicados en la susceptibilidad convulsiva, buscando nuevas dianas terapéuticas.

Metodología. Se aislaron exosomas de plasma de pacientes epilépticos mediante el kit comercial EV Pan (Miltenyi Biotec). Posteriormente, se analizó el contenido proteico mediante nano HPLC-MS/MS. Se utilizaron las bases de datos KEGG, GO, Disease Ontology y DisGeNet para hacer análisis de sobre-representación de vías y enfermedades en cada una de las muestras.

Resultados. Se identificaron 447 proteínas, incluyendo un alto número de marcadores proteicos exosomales, lo que confirma que es una muestra enriquecida en exosomas. Aproximadamente, un 35% del contenido proteico es el mismo entre los distintos pacientes analizados.

En todos los pacientes, se encuentran sobre-representadas significativamente vías relacionadas con el sistema de complemento y la cascada de coagulación, así como vías relacionadas con la respuesta inmune. El análisis de sobre-representación de enfermedades según DisGeNET indicó que las proteínas encontradas se relacionan significativamente con trombofilia, síndrome coronario agudo, desórdenes del sistema de coagulación e inflamación.

Conclusión. Aunque hay una variabilidad en el contenido de proteínas entre los pacientes, las vías KEGG y GO sobre-representadas son esencialmente las mismas y se relacionan con la sobre-representación de enfermedades. Hay que estudiar la clínica de los pacientes para evaluar si el incremento de los problemas circulatorios tiene relación con el tratamiento farmacológico.

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Nº 38

FUNDAMENTO NEUROBIOLÓGICO DE LAS FUNCIONES AUDITIVAS DE LA MUSCULATURA ENDOTIMPÁNICA

***Laboratorio**: Trastornos audiomotores y epilepsias reflejas*

Presenter: Ricardo Gómez Nieto

Authors: Ricardo Gómez-Nieto, Elisa Gil-Carcedo, Dolores E. López y Luis Ángel Vallejo Valdezate

Keywords: atención auditiva selectiva, impedancia, motoneuronas, oído medio,

trazadores neuronales.

Abstract:

Introducción. El cerebro humano posee una habilidad sorprendente y vital para nuestra interacción con el entorno: la capacidad de concentrarse selectivamente en estímulos auditivos, incluso en ambientes ruidosos. Esta facultad se sustenta en la musculatura endotimpánica, la cual modula las vibraciones de la cadena de huesecillos, permitiendo una percepción sonora precisa. Aunque el músculo tensor del tímpano se reconoce principalmente por su función protectora frente a traumas acústicos y la atenuación de sonidos autogenerados, aún persiste una pregunta intrigante: ¿juega también un rol activo en funciones auditivas complejas, como la atención auditiva selectiva?

Objetivos. Nuestro estudio se enfoca en dos objetivos clave. Primero, analizamos si el núcleo motor del tensor del tímpano presenta una organización citoarquitectónica y conexiones nerviosas compatibles con la ejecución de tareas auditivas complejas.

Metodología. Para ello, empleamos técnicas avanzadas de trazado de vías nerviosas e inmunohistoquímica en ratas Wistar. Segundo, investigamos si la contracción de los músculos endotimpánicos se ve influenciada por la presencia de conversaciones en entornos ruidosos cuando se requiere atención selectiva. Mediante la medición de la impedancia y la frecuencia de resonancia en el oído medio de individuos con audición normal, evaluamos cómo la atención afecta la respuesta auditiva.

Resultados. Nuestros resultados muestran que las motoneuronas del tensor del tímpano presentan una organización en columna, lo que sugiere una interacción funcional con otros núcleos auditivos y una capacidad de recibir modulaciones. Además, las pruebas auditivas en humanos demostraron que la impedancia del oído medio cambia en función del nivel de atención prestado a estímulos auditivos específicos.

Conclusión. La convergencia de estos hallazgos aporta una nueva visión sobre el papel de los músculos del oído medio como moduladores dinámicos de frecuencias antes del procesamiento coclear. Estos descubrimientos no solo amplían nuestra comprensión de la fisiología auditiva, sino que también abren nuevas vías para mejorar prótesis auditivas y tratar patologías del oído medio, ofreciendo un avance significativo en el campo.

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