VNiVERSiDAD D SALAMANCA

INSTITUTO DE NEUROCIENCIAS CASTILLA Y LEÓN

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THE ROOTS OF AUDITORY NEUROSCIENCE IN SPAIN: FROM PAST TO FUTURE

An International Symposium on Auditory Neuroscience.

A tribute to Prof. Miguel A. Merchán

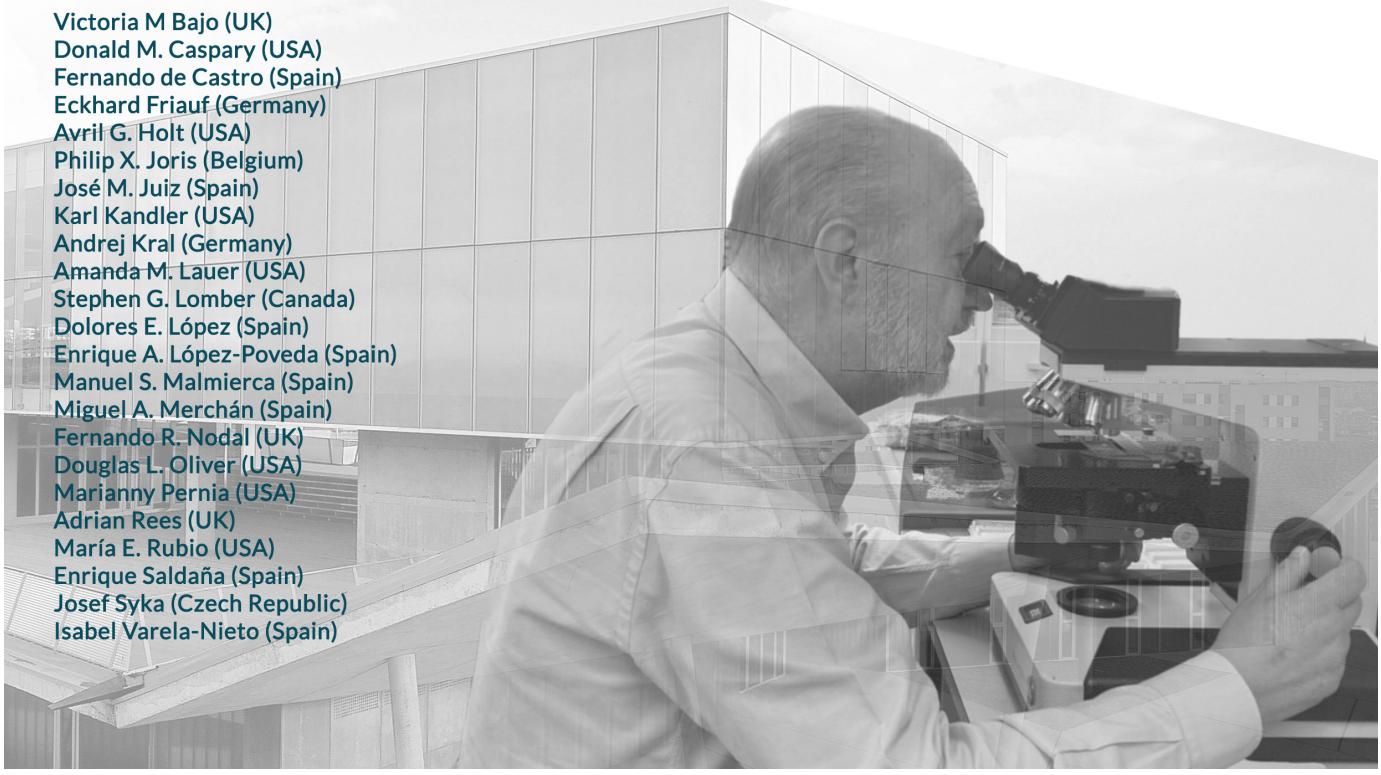
Aula 'Pío del Río-Hortega'

Institute for Neuroscience of Castilla y León

Salamanca, 8th - 9th of June 2023

Speakers:

Victoria M Bajo (UK)
Donald M. Caspary (USA)
Fernando de Castro (Spain)
Eckhard Friauf (Germany)
Avril G. Holt (USA)
Philip X. Joris (Belgium)
José M. Juiz (Spain)
Karl Kandler (USA)
Andrej Kral (Germany)
Amanda M. Lauer (USA)
Stephen G. Lomber (Canada)
Dolores E. López (Spain)
Enrique A. López-Poveda (Spain)
Manuel S. Malmierca (Spain)
Miguel A. Merchán (Spain)
Fernando R. Nodal (UK)
Douglas L. Oliver (USA)
Marianny Pernia (USA)
Adrian Rees (UK)
María E. Rubio (USA)
Enrique Saldaña (Spain)
Josef Syka (Czech Republic)
Isabel Varela-Nieto (Spain)



AN INTERNATIONAL SYMPOSIUM ON AUDITORY NEUROSCIENCE

A TRIBUTE TO PROF. MIGUEL A. MERCHÁN.

THE ROOTS OF AUDITORY NEUROSCIENCE IN SPAIN: FROM PAST TO FUTURE

SALAMANCA – SPAIN June 8th – 9th, 2023

LOCAL ORGANIZING COMMITTEE:

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I. PLAZA

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Department of Cell Biology and Pathology, School of Medicine

TIME TABLE June 8th	
09:00 - 09:30	INTRODUCTION & WELCOMING
09:30 – 10:00	M. S. Malmierca
10:00 – 10:30	E. Friauf
10:30 – 11:00	D. Caspary
11:00 – 11:30	M. Pernia
11:30 – 12:00	COFFE BREAK
12:00 – 12:30	D. L. Oliver
12:30 – 13:00	A. Kral
13:30 – 13:30	I. Varela-Nieto
13:30 – 14:00	A. G. Holt
14:00 - 15:30	LUNCH BREAK
15:30 – 16:00	E. Saldaña
16:00 – 16:30	K. Kandler
16:30 – 17:00	V.M. Bajo
17:00 – 17:30	A. Rees
17:30 – 18:00	J.M. Juiz
18:00 – 18:30	F. De Castro
21:00	DINNER AT CASINO

TIME TABLE June 9th	
09:30 - 09:50	D.E. López
09:50 – 10:15	E. López- Poveda
10:15 - 10:45	M. E. Rubio
10:45 – 11:15	J. Syka
11:15 – 11:45	S.G. Lomber
11:45 – 12:00	COFFE BREAK
12:00 – 12:30	A.M. Lauer
12:30 – 13:00	F.R. Nodal
13:30 – 13:30	P.X. Joris
13:30 – 14:00	M. Merchán
14:00 - 14:30	CONCLUDING REMARKS
14:30	LUNCH BREAK

LIFE IS FULL OF SURPRISES: THE NEURONAL BASIS DEVIANCE DETECTION AND PREDICTIVE CODING

Manuel S. Malmierca, Adam Hockley, Sara Cacciato, Laura Quintela, Jazmin Sánchez, Warren Bakay, Ana Belen Lao-Rodriguez, Guillermo V. Carbajal, Lorena Casado-Román, Camilo J. Morado-Díaz, Javier Nieto-Diego, Gloria G. Parras, Catalina Valdés-Baizabal, Yaneri A. Ayala, Daniel Duque, Xin Wang, Lucy Anderson, Flora M. Antunes, David Pérez-González‡

‡Author order is reverse chronological of lab start date, except for Manolo, who is "el jefe" and insisted he go first.

Cognitive and Auditory Neuroscience Laboratory (CANELab)
Institute of Neuroscience of Castilla y León (INCYL)
University of Salamanca, Salamanca, Spain

Mismatch negativity (MMN) is one aspect of the auditory evoked potentials that occur when expectations are violated. It is correlated with behavioral and perceptual measures of deviance detection. Furthermore, the amplitude of the MMN response represents the magnitude of the expectancy violation. The MMN response is widely considered to be a neurophysiological correlate of perceptual prediction error that results from the comparison between: the actual sensory input (bottom-up) and a memory input, so-called prediction, encoded in top-down activity. Prediction error arises when there is a difference between the internal prediction and the sensory input.

In this talk, I will summarise the work carried out in CANELAB on the neuronal MMN for the past 20 years and how this evolved from the perspective of being one of Miguel Merchán's disciples.

This work was supported by Spanish Agencia Estatal de Investigación grant (AEI), PID2019-104570RB-I00 and The Foundation Ramón Areces grant CIVP20A6616

SYNAPTOPHYSIOLOGY IN THE AUDITORY SYSTEM

Eckhard Friauf

Animal Physiology Group, Department of Biology
Technical University of Kaiserslautern, Germany

The auditory system offers several excellent (and extraordinary) opportunities to investigate information processing between neurons. In several instances, the auditory system resembles a high-tuned sports car rather than an unadventurous farm tractor. This talk will focus on three aspects from the superior olivary complex.

1) Synaptic robustness during long-lasting high-frequency activation
2) Synaptic performance upon loss of neurotransmitter re-uptake
3) Synaptic performance upon loss of synaptic vesicle filling

AGING AND DEGRADED TEMPORAL RELIABILITY ENGAGE TOP-DOWN RESOURCES ENHANCING MGB UNIT RESPONSES

Donald M. Caspary

Departments of Pharmacology and Surgery Southern Illinois University School of Medicine, Springfield, Illinois 62702, USA

The prevalence of age-related hearing loss and loss of speech understanding doubles with every decade of life, affecting at least 30% of the population aged 65 to 74 years and at least 50% of the population over 75 years of age. In public settings, seniors frequently have great difficulty understanding speech, which can lead to withdrawal from social activities, depression and cognitive decline. Adults with mild-to-moderate age-related hearing loss show substantially impaired processing of speech, likely due to a temporally degraded ascending code. All individuals can compensate for difficulty in speech understanding by using top-down, mnemonic and cognitive resources, to help disambiguate the ascending acoustic message. In a series of single-unit recording studies from auditory thalamus (MGB) in awake rats, we asked, how do top-down resources shape/alter coding of temporally degraded ascending acoustic signals?

Using predictable/repeating 450ms modulated (SAM) broadband signals, MGB unit response properties showed that aging and temporal salience can alter coding of SAM stimuli. We observed adaptive-suppression in unit responses to predictable, temporally "clear" SAM stimuli from young rat MGB. In aged rat MGB, unit responses shifted from adaptive-suppression toward repetition-enhancement when presented predictable SAM stimuli. Simulating the aged ascending code, by presenting temporally degraded, predictable SAM stimuli while recording from units in young rat MGB, resulted in a change toward repetition-enhancement. Repetition-enhancement could be reversed toward a more adaptive code by optogenetic blockade of corticothalamic terminals. We postulate that top-down/corticothalamic glutamatergic excitation reflects mnemonic/cognitive expectations/predictions onto ascending responses of MGB neurons, enhancing responses to expected stimuli.

VOCALIZATION CATEGORIZATION DEFICITS AFTER TEMPORARY THRESHOLD SHIFTS IN GUINEA PIGS

Pernia M. [1,4], Kar M. [1,4,5] , Williams K. [1], Sadagopan S. [1,2,3,4,5]

Departments of [1]Neurobiology, [2]Bioengineering and [3]Communication Science and Disorders, [4]CNUP, [5]CNBC
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Exposure to moderate-to-intense sounds can cause temporary threshold shifts (TTS) in the audiogram, and is hypothesized to contribute to lasting auditory perceptual deficits. To gain insight into possible cortical circuit pathologies underlying such deficits, we induced TTS in a guinea pig animal model to determine its effects on a complex sound recognition behavior (vocalization categorization). Since the loss of high-threshold auditory nerve fibers has been proposed as an underlying cause of these deficits, we further hypothesized that vocalization categorization deficits would be pronounced at loud sound levels. We induced TTS in guinea pigs, using 2–8 kHz noise at 106 to 109 dB SPL for 2 hours. Auditory brainstem response recordings revealed elevated thresholds that returned to within 10 dB of baseline 30 days after noise exposure, accompanied by permanently decreased wave I amplitudes in all animals. Using pupillometry as a correlate of bottom-up processing, we estimated behavioral thresholds for call categorization-in-noise and found that categorization was impaired primarily at loud sound levels. Surprisingly, animals did not show performance deficits in an operant vocalization categorization task. Deeper analyses of behavioral data suggested that guinea pigs might adapt their behavioral strategies to compensate for bottom-up deficits. Taken together, these results suggest that top-down influences can mask the effects of deficits in the ascending auditory pathway. In ongoing experiments, we are performing neural recordings in the auditory cortex to probe the effects of TTS on the neural representation of vocalizations across cortical laminae.

DEVELOPING AN OBJECTIVE ELECTROPHYSIOLOGICAL TEST FOR TINNITUS

Douglas L. Oliver, Emily Fabrizio-Stover, and Alice L. Burghard

Department of Neuroscience, University of Connecticut Health Center, Farmington, CT 06030-3401, USA.

Subjective tinnitus is the perception of a sound in the absence of an external acoustic stimulus. There is no clinical diagnostic test for tinnitus, and its diagnosis is based on the patient's report since subjective tinnitus cannot be heard by others. Research on tinnitus is hampered by the lack of an objective test. Here, we report an objective electrophysiological test for tinnitus that can be used on both animal and human subjects. The test is based on the observation of a subpopulation of neurons in the inferior colliculus (IC) that have a long-duration sound-induced afterdischarge. After the offset of a sound with a duration of more than 20 seconds, these neurons continue to fire for up to several minutes. The hypothesis is that these neurons become a signal generator in chronic noise-induced tinnitus. The basis of the electrophysiological test is to use a Novel Stimulus Paradigm (NSP, patent pending) to evoke responses from the auditory brainstem before and after a long-duration sound. We postulated that the ongoing signal generation in tinnitus will produce tinnitus-specific differences in the response to the NSP.

Mice were trained in an active avoidance test for tinnitus, then a unilateral, high frequency hearing loss was induced in awake mice by exposure to a loud bandpass noise (16 kHz center, 2 kHz-wide, 113-116 dB SPL, 1 hr). Mice were retested in active avoidance to determine whether they had tinnitus 8 weeks after noise exposure. The response to the NSP at the frequency of the behaviorally identified tinnitus and at lower frequencies was obtained in both ABR and deep brain extracellular recordings from the IC in the same mice. Comparison of tinnitus and non-tinnitus mice revealed tinnitus specific differences in both sets of recordings. In general, tinnitus subjects displayed less suppression after the long-duration sound than non-tinnitus subjects with both electrophysiological measurements. The changes were most significant in the late ABR waves (mimicking the results of the IC recordings) in response to high frequency stimuli in the range of the tinnitus pitch. These results are consistent with the notion that the IC generates a detectable, recurrent signal in tinnitus that may prove useful as a non-invasive, objective electrophysiological test for tinnitus.

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BRAIN´S CONNECTOME IS SHAPED BY DEVELOPMENTAL HEARING EXPERIENCE

Andrej Kral

Institute of AudioNeuroTechnology & Dept. of Experimental Otology, ENT Clinics, Hannover Medical School, Hannover, Germany

Australian Hearing Hub, School of Medicine and Health Sciences, Macquarie University, Sydney, Australia

A popular model of prelingual deafness are congenitally deaf cats, where plasticity with chronic electrostimulation through cochlear implants have been studied (Kral et al., 2019, Ann Rev Neurosci). Congenital deafness had extensive influence on the organization of the auditory system, with predominant consequences in the cerebral cortex (Kral et al., 2016, Lancet Neurol). In recent years, mathematical tools exploiting analysis of oscillatory cortical activity allowed to decipher specific effects of deafness on cortical processing. So-called induced responses, indicative of corticocortical interactions, were most prominently reduced in the auditory cortex of congenitally deaf cats (Yusuf et al., 2017, Brain). In adult hearing cats (HC) and congenitally deaf cats (CDCs), cortical responses to acoustic and electric stimulation (through a cochlear implant) were compared in the primary auditory field (A1) and the higher order posterior auditory field (PAF). Recordings were performed using multielectrode arrays and the penetrations were histologically reconstructed. For effective connectivity pairwise phase consistency, weighted phase-lag index and nonparametric Granger causality were used as connectivity measures. CDCs demonstrated a substantially reduced stimulus-related corticocortical coupling in the connectivity measures used. Largest deficits were observed in sensory-related top-down interactions, in the alpha and beta band. The data document that corticocortical interactions are dependent on developmental hearing experience. The result suggest that the congenitally deaf brain cannot incorporate top-down prediction information into auditory processing and thus have a deficient mechanism of predictive coding.

Supported by Deutsche Forschungsgemeinschaft (Exc 2177) and MedEl Comp., Innsbruck, Austria.

TO HEAR OR NOT TO HEAR: OXIDATIVE STRESS, NEUROINFLAMMATION AND AGEING

Isabel Varela-Nieto and Silvia Murillo-Cuesta

Neuropathology of Hearing and Myelinopathies Group. Institute for Biomedical Research "Alberto Sols" (IIBM), Spanish National Research Council-Autonomous University of Madrid (CSIC-UAM), 28029 Madrid, Spain

Rare Diseases Biomedical Research Networking Centre (CIBERER), The Institute of Health Carlos III (ISCIII), 28029 Madrid, Spain

Presbycusis or age-related hearing loss (ARHL) is a common chronic condition affecting more than 30% people over the age of 65, thus being the most prevalent sensory deficit. It precedes and accelerates cognitive frailty and, in some cases, triggers neurodegeneration. ARHL is characterized by a complex multifactorial etiology, its debut and evolution are influenced by genetic and environmental components, such as exposure to noise and ototoxics. Using animal and cellular models, we have studied the molecular keys of auditory aging to better characterize the contribution of oxidative stress to ARHL, to identify novel targets and to test anti-oxidant small molecules in preclinical models of presbycusis.

Celaya et al., IGF-1 Haploinsufficiency Causes Age-Related Chronic Cochlear Inflammation and Increases Noise-Induced Hearing Loss. Cells. 2021 Jul 3;10(7):1686.

Bermúdez-Muñoz et al., G6PD overexpression protects from oxidative stress and age-related hearing loss. Aging Cell. 2020 Dec;19(12):e13275.

Celaya et al., Deficit of mitogen-activated protein kinase phosphatase 1 (DUSP1) accelerates progressive hearing loss. Elife. 2019 Apr 2;8:e39159.

PERIPHERAL DAMAGE CHANGES THE INHIBITORY BALANCE IN THE RAT INFERIOR COLLICULUS

Avril Genene Holt, Soo D. Lee, Ronald D. Griffith, Jr., Mikiya Asako, Richard A Altschuler

Dept. Ophthalmology, visual and anatomical sciences, Wayne State Univ., Detroit, MI, USA

Kresge Hearing Research Institute, Dept. Otolaryngology, Univ. Michigan, Ann Arbor, MI, USA ,

and Dept. of Otolaryngology Kansai Medical University, Moriguchi, Osaka, Japan.

Several studies suggest that hearing loss results in changes in the balance between inhibition and excitation in the inferior colliculus (IC). The IC is an integral nucleus within the auditory brainstem. The majority of ascending pathways from the lateral lemniscus, superior olivary complex (SOC), and cochlear nucleus synapse in the IC before projecting to the thalamus and cortex. Many of these ascending projections provide inhibitory innervation to neurons within the IC. However, the nature and the distribution of this inhibitory input have only been partially elucidated in the rat. The inhibitory neurotransmitter, gamma aminobutyric acid (GABA), from the ventral nucleus of the lateral lemniscus, provides the primary inhibitory input to the IC of the rat with GABA from other lemniscal and SOC nuclei providing lesser, but prominent innervation. We have previously demonstrated changes in the expression of GABA related genes in the IC following deafness. Those results are consistent with reports of neuronal dysfunction in the IC with aging and after trauma. In the current study the number, intensity, and density of GABA positive axon terminals in the IC were compared in normal hearing and deafened rats. While the number of GABA immuno labeled puncta did not differ between groups, the intensity of labeling was significantly reduced. The localization and distribution of labeling was also examined. In deafened animals, the number of immuno gold particles was reduced by 78% in axodendritic and 77% in axosomatic GABAergic puncta. The affected puncta were primarily associated with small IC neurons. These results suggest that reduced inhibition to small IC neurons may contribute to the increased neuronal excitability observed in the IC following noise or drug induced hearing loss.

THE NUCLEI OF THE LATERAL LEMNISCUS FRON THE SPANISH PERSPECTIVE

M. Gómez-Martínez, H. Rincón, M. Gómez-Álvarez, R. Gómez-Nieto, E. Saldaña

University of Salamanca, Neuroscience Institute of Castilla y León (INCyL), Salamanca, Spain

In the 1980's, when Miguel Merchán set up his research group in Salamanca, Spain, the nuclei of the lateral lemniscus (NLL) were pretty much terra incognita. The work carried out by Dr. Merchán and his Spanish coworkers over more than 30 years has provided a major impetus in understanding the organization and the neural connections of the mammalian NLL. Highlights of this prolific harvest include the description of the projections of the rat dorsal NLL (DNLL), the characterization of the laminar, concentric organization of the rat and cat DNLL, the controversial 'spiral' organization of the rat ventral NLL (VNLL), the mosaic-like organization of the cat VNLL, the distribution of the inhibitory neurons of the rat VNLL, and the stereological estimate of the number of neurons in the rat NLL. The Spanish quest to understand the NLL continues. Although the NLL have traditionally been thought to exclusively participate in the bottom-up transmission of auditory information, data from our laboratory obtained with retrograde neuroanatomical tracers demonstrate that numerous NLL neurons innervate the superior olivary complex (SOC) of the rat.

We made large injections of FluoroGold into the SOC to determine to what extent NLL neurons contribute to descending projections, and focal injections of biotinylated dextran (BDA) to pinpoint the SOC nuclei innervated by each NLL.

The SOC is innervated by thousands of neurons located in four nuclei or regions associated with the lateral lemniscus: the ipsilateral classical ventral and intermediate nuclei of the lateral lemniscus (VNLL and INLL); the medial paralemniscal region (PL) of both sides; and the ipsilateral semilunar nucleus (SLN), a previously unrecognized nucleus that wraps around the INLL dorsally, medially, and caudally. In some experiments, at least 30% of the VNLL and INLL neurons were labeled. All SOC nuclei, except the medial and lateral superior olives, are innervated by abundant lemniscal neurons, and each SOC nucleus receives a unique combination of lemniscal inputs. The primary target of the projections from the VNLL is the ventral nucleus of the trapezoid body (VNTB), followed by the superior paraolivary nucleus, and the medial nucleus of the trapezoid body (MNTB). The INLL selectively innervates the VNTB. The PL innervates dorsal periolivary regions bilaterally. The SLN preferentially innervates the MNTB and may provide the first identified non-calyceal excitatory input to MNTB neurons.

These findings may shape our understanding of acoustic information processing in the lower brainstem. Given the proportion of lemniscal neurons involved in all these projections, the NLL should be considered major players in the descending auditory pathway.

ROLES OF NEUROTRANSMITTER CO-RELEASE IN THE DEVELOPMENT OF INHIBITION IN THE LATERAL SUPERIOR OLIVE

Karl Kandler

Department of Neurobiology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Encoding interaural sound level differences in the lateral superior olive (LSO) relies on a strong glycinergic inhibition from the medial nucleus of the trapezoid body (MNTB). In mature animals, individual LSO neurons are innervated in a precise tonotopic manner by very few MNTB neurons. This organization arises gradually during development via activity-dependent synaptic silencing and strengthening, followed by structural pruning. During this refinement period, MNTB neurons not only release glycine but also co-release GABA and glutamate. I will present results from my laboratory that show that the co-release of these additional neurotransmitters plays important and specific roles in the refinement and physiological fine-tuning of the developing MNTB-LSO pathway.

MODULATION OF CORTICAL NEURAL EVOKED ACTIVITY BY SLEEP STATUS IN A FERRET MODEL OF TINNITUS

Victoria M Bajo Lorenzana

Department of Physiology, Anatomy and Genetics, University of Oxford, UK

Subjective tinnitus is a phantom auditory perception not linked to a sound stimulus. It affects 14% of the global population and is associated with mental ill health and poor quality of life. To date, there is no effective treatment for tinnitus. We used adult ferrets exposed to mild noise trauma as an animal model of induced tinnitus. We assessed their phantom percept using two operant paradigms sensitive to tinnitus before and up to 6 months after the mild noise trauma. The integrity of the auditory brainstem was assessed over the same time using auditory brainstem recordings. Following noise overexposure, ferrets developed lasting, frequency specific impairments in their operant behaviour paradigms and evoked brainstem activity. To explore the interaction of sleep and tinnitus, in addition to tracking the behavioural markers of noise-induced tinnitus and hearing impairment after noise overexposure, we evaluated their sleep-wake pattern and spontaneous and auditory evoked EEG activity across vigilance states. Behavioural performance and auditory evoked activity after noise overexposure suggested distinct degrees of tinnitus and hearing impairment between individuals, with some exhibiting an early and others a late onset. Animals that developed signs of tinnitus also developed sleep impairments, suggesting a link between the emergence of noise induced tinnitus and sleep disturbance. Moreover, neural markers of tinnitus were reduced during sleep, suggesting that sleep may transiently mitigate tinnitus. Overall, these results highlight the potential of natural brain states dynamics to investigate tinnitus and uncover new avenues for future treatments.

DESCENDING CONNECTIONS FROM NON-AUDITORY CORTICAL AREAS AND THE HIPPOCAMPUS TO THE IC

Adrian Rees, Bas Olthof, Fiona LeBeau, Gavin Clowry and Sasha Gartside

Biosciences Institute, Faculty of Medical Sciences, Newcastle University, UK

Mounting evidence suggests that that descending information from the forebrain is important in the function of the inferior colliculus. Anatomical projections from the auditory cortex to the inferior colliculus are well described but given that sound processing is influenced by information derived from other senses, motor activity, and cognitive processes, the origin of inputs to the IC may be more diverse. Here we discuss evidence from anatomical and physiological experiments that the inferior colliculus is also the recipient of projections from non-auditory cortical areas and the hippocampus.

Retrograde tracing using Retrobeads, or a viral vector encoding green fluorescent protein (GFP), injected into the IC in rat, labelled pyramidal cells bilaterally in the visual, auditory, somatosensory, motor and prefrontal cortices. Retrograde labelling was also seen in hippocampal neurons which immunolabelled for GAD 67, demonstrating that they are GABAergic inhibitory neurones. Cortical injection of an anterograde tracer (dextran) labelled terminals in all three subdivisions of the IC with terminals associated with both GABA positive and negative neurons. Physiological studies in anaesthetised animals using optogenetic or electrical stimulation of somatosensory, motor, and prefrontal cortices combined with multielectrode recording in the IC, revealed activation of IC neurons. The response to electrical stimulation frequently consisted of fixed latency event at 4-5 ms following stimulus onset followed by more variable, longer latency activity. Such a pattern is consistent with a monosynaptic followed by a polysynaptic response within the IC.

Our findings suggest that multisensory and cognitive influences on sound processing are not limited to intra-cortical interactions but occur at an earlier stage in the auditory pathway.

…AND NOW ON THE ROAD OF AGING IN THE AUDITORY SYSTEM: A HOMAGE TO PROFESSOR MIGUEL MERCHÁN

José M. Juiz

Auditory Neuroscience Laboratory, Medical School in Albacete, University of Castilla-La Mancha, 02008 Albacete Spain

This symposium offers us a special opportunity to celebrate over four decades of Prof. Miguel Merchan´s uninterrupted dedication to advancing knowledge of the auditory system. Prof. Merchán's career is an inspiring reflection of the progression of our captivating, dynamic, characteristically border-free field of research since the late seventies of the past century.

Initial work of Prof. Merchán at the Universidad Complutense of Madrid under the direction of his mentor and brother, the much-remembered Prof. Jaime Merchán, tackled the ultrastructure of the auditory receptor with meticulous descriptive detail and observational insight, landmarks of the purest neurohistological style rooted in the School of Santiago Ramón y Cajal.

Further, he and his students at the University of Salamanca, nowadays acknowledged auditory scientists, investigated in depth nuclei and connections of the central auditory pathway, putting forth coherent and testable functional predictions, once again characteristics of well-designed and executed neurohistology.

More recently, the solid body of knowledge after decades of observations on the structure and functional organization of the auditory system stimulated Prof. Merchán's proverbial restlessness to guide his lab efforts towards the experimental study of plastic reorganization after injury to the auditory pathway to understand mechanisms and cellular adaptations to deafness. Prof. Merchán´s evolution towards experimental interrogation of the role of neural auditory structures on hearing mechanisms, based on a coherent and interrelated plethora of previous observational data on the auditory system, reflects one central hallmark of current auditory research.

For almost two decades, my lab has had the privilege of consolidating long-time links with Prof. Merchán, through a successful flow of shared competitive funding. Currently, our focus is on auditory aging. I will share recent, on-going work showing histological changes related to aging processes in the cochlea, highlighting oxidative stress, inflammation, and the role of the stria vascularis. Interconnections between peripheral and central aging will be stressed, along with evidence, based on novel animal models, on how Alzheimer disease pathologies and age-related hearing loss may biologically potentiate each other. Finally, possible therapeutic implications emanating from this work will be discussed.

THE CAJALIAN ROUTE TO MIGUEL MERCHÁN

Fernando de Castro

Instituto Cajal-CSIC, Madrid, Spain

Not so long ago and from a geography very close to we are now meeting to honor Prof. Miguel Merchán, Santiago Ramón y Cajal (1852-1934) landed in the hostile and dark Reticular World to illuminate it and lay the foundations of modern Neuroscience. The best way to leave the XIXth century behind was to cross the Cajalian and, therefore, more than "sublime door", and begin to understand how our brain is organized. Since then, it has become a kind of mantra and it is that all neuro-sailings do not lead to, but rather start from Cajal. In the case of Miguel Merchán, this is doubly true: with regard to his research work in Neuroaudiology, Miguel´s path starts from Cajal but, in addition, his own formative pilgrimage was a totally Cajalian path, following the steps of the last direct disciple of Cajal himself, Fernando de Castro (1896-1967) and his last student in the Histology chair, the ever-remembered Jaime Merchán Cifuentes (1948-2011), Miguel's only brother. It is now time to remember all these and some other anecdotes here, in Salamanca, and toast all for Miguel Merchán.

UNRAVELING THE BEGINNING OF THE SEIZURE-PRONE NEURONAL NETWORK: INSIGHTS INTO THE COCHLEAR ALTERATIONS OF AN AUDIOGENIC SEIZURE MODEL

López DE [1,2,3], Damasceno S[4], Sánchez-Benito D[1,2,3], Zeballos L[1,2,3] and Gómez-Nieto R[1,2,3]

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Recent evidence suggests that defects in the primary acoustic pathway, from the inner ear to the inferior colliculus, may be common among audiogenic seizure models. The genetic audiogenic seizure hamster from Salamanca (GASH/Sal) exhibits a complex hearing impairment with severe hearing loss and cochlear neuropathy, which makes this strain susceptible to sound-induced convulsions due to alterations in bottom-up auditory inputs to the inferior colliculus (the so-called epileptogenic nucleus). Here, we further investigated the molecular and morphological alterations of the GASH/Sal cochlea using auditory brainstem responses (ABR) testing, immunohistochemistry, confocal microscopy, and gene expression analysis. Compared to wild-type animals, the GASH/Sal exhibits high auditory thresholds and a drastic decrease in the amplitude of ABR wave I, which may be associated with auditory nerve fiber reductions. Confocal microscopy analysis reveals marked stereocilia distortion in inner and outer hair cells as well as defects in supporting cochlear cells. At the molecular level, our gene expression analysis shows upregulation of Gipc3 and Ptpqr genes, while Cdh23, Gjb2, Kcnq1, Kcnq4, Otoa, Pcdh15, Pou4f3, Prestin, Slitrk6, Tecta, and Tmc2 are down-regulated. The gene expression of Gpr98 did not show any differences compared to wild-type animals. Furthermore, we found that the GASH/Sal model exhibits one missense mutation in the Otoa gene. Our work sheds light on how acoustic cues are not faithfully transmitted from the inner ear to the brainstem auditory pathway, ultimately leading to susceptibility to audiogenic seizures in the GASH/Sal model. In sum, our findings support the idea that the cochlear receptor of the GASH/Sal may be considered the cradle of the complex seizure-prone neural network, which results in impaired auditory perception and susceptibility to audiogenic seizures.

COCHLEAR SYNAPTOPATHY IS UNLIKELY TO CONTRIBUTE TO THE HEARING DEFICITS REPORTED BY OLDER PEOPLE WITH NORMAL AUDIOGRAMS

Enrique A. Lopez-Poveda[1,*], Peter T. Johannesen[1], Byanka C. Buzo[1], Marcelo Gómez-Álvarez[1], Sonia L. Coelho-de-Sousa[1], Georg M. Klump[2]

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Many older people with clinically normal hearing report difficulties understanding speech in noise and show a poorer-than-normal ability to process auditory temporal cues. Because cochlear synaptopathy comes with aging and does not cause hearing losses, and because synaptopathy can degrade the encoding of sound in the auditory nerve, it has been hypothesized that the hearing deficits of older people with normal audiograms could be due to cochlear synaptopathy. Here, I will jointly present the results from two separate studies conducted in our laboratory aimed at testing this hypothesis. The two studies involved reasonably large cohorts of participants (N=66 and N=30) with normal hearing and covering a wide age range. The two data sets showed that (1) the thresholds for detecting silent gaps, frequency modulation, or speech in noise worsened with increasing age, as expected; (2) the slope of wave I of the auditory brainstem response (ARB) decreased with increasing age, consistent with the notion that the older listeners in our cohorts were more likely to suffer from synaptopathy. However, we found no correlation between the performance in the hearing tasks and the slope of ABR wave I. Altogether, the two studies showed that even though synaptopathy comes with aging, it is unlikely to contribute to the hearing deficits of older people with normal audiograms. The second study provided evidence that for these people, their hearing deficits are more likely due to a reduction of inhibition in their brain. [Work supported by the Oticon Foundation, the MINECO (grants BFU2015-65376-P and PID2019-108985GB-I00), Junta de Castilla y León (grants SA023P17 and SA252P20), and the European Regional Development Fund].

THE YIN AND YANG OF AMPA GLUTAMATE RECEPTORS AT AUDITORY NERVE SYNAPSES

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All information about the acoustic environment is carried from the inner ear to the CNS by the afferent fibers of the cochlear nerve, most of which (95%) receive synaptic input only from inner hair cells (IHC). Rapidly gating AMPA-type glutamate receptors (AMPAR; GluA2, GluA3 and GluA4 subunits) mediate synaptic transmission at the mature synapse between the IHC and the afferent fibers of the cochlear nerve (IHC-ribbon synapse). The influence of AMPAR subunit composition to afferent excitability in the cochlea is poorly understood. Understanding this process is important because glutamate excitotoxicity through AMPAR has been implicated in the pathogenesis of hearing loss caused by noise, ischemia, and aging. Sex differences in the vulnerability to hearing loss occur in humans and in mice, but the underlying molecular mechanisms are unknown. Our published studies led us to begin investigating the contribution of AMPAR subunits to transmission at the IHC-ribbon synapse. In addition, work in progress show that sex-specific differences in AMPAR subunit composition contribute to sound-induced cochlear damage and hearing loss. The overall important question we are addressing is what are the differences in AMPAR subunit composition that underlie sex differences in hearing loss.

AGE-RELATED HEARING LOSS

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The typical signs of the age-related hearing loss – presbycusis - comprise increasing hearing thresholds at high frequencies and the deterioration of hearing in a noisy environment, which in humans hinders speech comprehension. Besides the deterioration of the function of inner and outer hair cells in the inner ear that culminates in their loss due to apoptosis, significant age-related changes occur in the central auditory system, too. The decrease of function particularly concerns the inhibitory transmission, as demonstrated by means of immunocytochemical detection of decreased levels of glutamate decarboxylase. Age-related decreases appear in the central auditory system as well in the levels of calcium-binding proteins, such as calbindin, calretinin and parvalbumin. In addition, an age-related loss of the temporal function of neurons has been demonstrated in experimental animals with a decline in the precision and reliability of both the rate code and the temporal code. Audiological examinations in humans show that age-related hearing loss starts with increasing thresholds in high frequencies of sound and slowly spreads to low frequencies. Presbycusis is accompanied by decreases of the amplitudes of oto-acoustic emissions, decreases in the speech comprehension especially in the presence of background noise and decreases of the temporal processing of sound, expressed as increases of the gap detection thresholds. In addition, deteriorates space hearing based on interaural time delay. The results of more detailed audiological tests signalize that presbycusis must not be considered as a single pathological unit but that it may have several forms, depending on what part of the auditory system does not function normally. New insights into the mechanisms of presbycusis offers magnetic resonance imaging. MR spectroscopy showed that aging is accompanied with lower concentrations of glutamate, GABA and N-acetylaspartate in the auditory cortices of elderly subjects. MRI morphometry studies demonstrated that the gray matter thickness in the Heschl's gyrus, planum temporale and gyrus frontalis superior, decreases with aging. However, aging did not influence the laterality of these structures, i.e. dominance of the left side. Concerning the white matter, recent study with fixel-based morphometry showed that aging is accompanied with a reduction of fibers in the pathways connecting the structures of the central auditory system, but the connections of these structures with limbic structures, such as amygdala or hippocampus, are even more reduced. Acoustically-evoked activity recorded by BOLD fMRI from an area centered on Heschl's gyrus, was found to be more pronounced in the elderly subjects than in young subjects. In addition, the activation by acoustical stimuli was more expressed in the right temporal lobe than in the left temporal lobe in the elderly. These results suggest that aging not only has negative effects on the structure and function of the inner ear, but also on the central auditory system.

AUDITORY CORTEX PLASTICITY FOLLOWING HEARING LOSS

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Cortical plasticity is the neural mechanism by which the cerebrum adapts itself to its environment, while at the same time making it vulnerable to impoverished sensory or developmental experiences. Like the visual system, auditory development passes through a series of sensitive periods in which circuits and connections are established and then refined by experience. Current research is expanding our understanding of cerebral processing and organization in the deaf. In the congenitally or perinatally deaf, areas across "deaf" auditory cortex demonstrate significant crossmodal plasticity with neurons responding to visual and/or somatosensory stimuli. This crucial cerebral function results in compensatory plasticity. Not only can the remaining inputs reorganize to substitute for those lost, but this additional circuitry also confers enhanced abilities to the remaining sensory systems. In this presentation we will review our present understanding of the structure and function of “deaf” auditory cortex using psychophysical, electrophysiological, and connectional anatomy approaches and consider how this knowledge informs our expectations of the capabilities of cochlear implants in the developing brain.

ROLE OF THE OLIVOCOCHLEAR SYSTEM IN HEARING ACROSS THE LIFESPAN

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Contributions of the ascending peripheral and central auditory pathways to age-related and noise-induced hearing deficits have been well-characterized, but we know comparatively little about how the descending projections from the brain to the cochlea contribute to hearing deficits. Emerging studies have shown dynamic structural changes in the olivocochlear system associated with acoustic experience and age. Here I will summarize our efforts to understand how these pathways may be involved in hearing dysfunction measured using physiological and behavioral assays and highlight areas for future research.

THE ROLE OF AUDITORY CORTEX IN MEDIATING NEURAL PLASTICITY

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The ability to modify the neural activity evoked by sensory experience is the basis of learning. Descending projections from cortical areas are among the most prominent pathways in any sensory system, suggesting their role in modulating subcortical processing. In the auditory system, one of the main descending pathways connects the auditory cortex with the inferior colliculus (IC) in the midbrain. Silencing optogenetically ArchT-expressing neurons in adult ferrets, we show that within-trial activity in the auditory cortex is required for training-dependent recovery in sound-localization accuracy following monaural occlusion. Furthermore, this learning-induced auditory plasticity requires the functional integrity of the corticocollicular pathway. The selective elimination of the large coticocollicular pyramidal cells in layer V impairs the ability of animals to adapt to changes produced by unilateral ear occlusion. We use neuropixel probes for high-density recordings in the inferior colliculus of ferrets in two experimental paradigms. In anaesthetized animals, we assess the modulation of cortical input in shaping spatial and spectral properties of collicular neurons. Optogenetic activation of corticocollicular neurons broadens the IC neurons' spatial tuning, sharpens their frequency tuning curves, and shifts their best frequency. In awake ferrets trained in sound localization, we evaluate the temporal changes of the spatial tuning of IC neurons when perceptual learning occurs. Our results show the essential role of the auditory cortex and the corticocollicular pathways in auditory plasticity.

COCHLEAR NUCLEUS CEPHALOPOD PROJECTION NEURONS

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Octopus cells are one of the most striking cell types in the mammalian cochlear nucleus, both anatomically and physiologically, but they have been little studied in vivo. We recorded from octopus cells in the anesthetized gerbil using different approaches: axonal recordings with sharp electrodes, intracellular recordings with sharp electrodes, and whole-cell recordings using the patch clamp method. In all cases, the electrodes contained a tracer to attempt labeling of the cell and its projections. We discovered that “octopus cells” in the posterior aspect of the posteroventral cochlear nucleus in fact consist of two subpopulations, which we refer to as octopus and squid. This finding explains several morphological and physiological observations in the literature. Both cell types show onset responses to short pure tones, but whereas octopus cells usually have pure onset responses (Oi), squid cells tend to have a low rate of sustained firing (OL). Octopus cells have wider tuning and higher thresholds than squid cells. In response to stimuli containing fast frequency sweeps, octopus cells show selectivity to direction and speed of the sweeps, while squids cells do not. The two cell types also differ in their intrinsic physiological properties, with octopus cells showing smaller spikes and lower membrane resistance than squid cells. Most strikingly, both cell types are found in the OCA but whereas octopus project via the intermediate acoustic stria (IAS), squid cells project via the trapezoid body (TB). Although the projection target of squid cells remains unknown, the list of physiological and anatomical differences strongly suggests different functional roles for the two populations.

MULTISESSION ELECTRICAL STIMULATION IN THE AUDITORY CORTEX STABILIZES THRESHOLD-SHIFTS IN A WISTAR RAT MODEL OF SPONTANEOUS AGE-RELATED HEARING LOSS

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Presbycusis (age-related hearing loss - ARHL) is one of the most prevalent diseases in developed countries, characterized by increases in auditory thresholds and a loss of frequency discrimination which cause disabling hearing. It can be accepted that the loss of OHC and its effects on cochlear amplification is one of the first and most prominent alteration in hearing and frequency discrimination in ARHL. Here, we hypothesize that cortico- fugal regulation of the MOC efferent system by AC electric activation allows to stabilize thresholds along time in a model of spontaneous ARHL in Wistar rat. We have used multisession anodal stimulation of the auditory cortex (AC) to prevent auditory threshold increases in spontaneously aged Wistar rats.

Click and pure tone (4, 8, 16 and 30 kHz) auditory brainstem responses (ABRs) of Wistar rats were recorded monthly from 6 months of age. A spontaneous significant increase of thresholds was first found at 16 months (64 weeks). At that moment, a silver ball electrode was epidurally implanted on the auditory cortex, and after two weeks of recovery (68 weeks – 17 months) rats received seven 10-minute sessions of 0.1 mA anodal electric stimulation on alternating days. Five days after the last stimulation session (72 weeks -18 months), click and pure tone ABRs were recorded, and rats were sacrificed.

Click and pure tone ABRs analysis show that at 18 months, sham controls rats (implanted and non-stimulated) had significantly higher auditory thresholds than electrically stimulated ones. Pure tone ABRs show a preserved frequency discrimination between 17 (post-surgery recording) and 18 months (sacrifice) in the EES group. Therefore, epidural electric stimulation minimizes age related threshold shifts and contributes to preserve tone discrimination.

Some preliminary results from analysis in course in our lab (cortical auditory and visual evoked potential recordings and acetylcholine esterase immunocytochemistry of VNTB – MOC neurons) will be discussed to set light into the central effects of AC activation in an ARHL rat model.

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