ASSOCIATION FOR RESEARCH IN OTOLARYNGOLOGY

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2023 ARO MIDWINTER MEETING

Abstract Book

Conclusions: Our study illustrates how neuronal diversity in the CNC is generated by origin in different rhombomeres and different microdomains. Our comparative analysis strongly suggests that the DGP for the cerebellum was both used and modified by that for the CNC.
Take-Home Message:
The evolutionary novel auditory nucleus CNC was most likely generated by the use and modification of the pre-existing DGPs to generate different neurons in the developing brainstem and the cerebellar cortex.

SA73. Co-Release of GABA and ACh from Medial Olivocochlear Efferent Fibers During Development
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Category: Development: Cellular/Systems
Background: During development, inner hair cells (IHCs) in the mammalian cochlea are unresponsive to acoustic stimuli but instead present intrinsic electrical activity, crucial for the normal development of the auditory pathway. During this same period, neurons originating from the medial olivocochlear complex (MOC) transiently innervate the soma of IHCs. This synapse is mediated by acetylcholine (ACh), activating nicotinic receptors assembled by alpha9 and alpha10 subunits and ultimately controlling IHC excitability. Although it is a cholinergic synapse, previous evidence indicated the presence of abundant GABA and presynaptic GABAB receptors (GABAB-R). Moreover, application of GABAB-R agonists generated a reduction in ACh release.
Methods: Transgenic Chat tm2(crc)Lowl/J-Cre (ChAT-cre) and Gad2 tm1(cre/ERT2)Zjh/J-Cre (GAD2-cre) mice of either sex were mated with a floxed channel rhodopsin 2 (ChR2) line, Ai32, to drive specific ChR2 expression. Whole cell patch clamp was performed in P9-11 IHCs while either cholinergic (ChAT-Cre/ChR2) or GABAergic (GAD2-Cre/ChR2) fibers were optogenetically stimulated. Additionally, immunohistochemistry techniques were used to characterize cholinergic and GABAergic expression in B6.Cg-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J (tdTomato) crossed with GAD2-Cre. Furthermore, we performed calcium imaging experiments at the same age range on Balb-C mice. For this, IHCs were dialyzed with a fluorescent calcium indicator (Fluo 4) and MOC electrically stimulated during the application of a GABAB-R antagonist (CGP 36216, 200 μM).
Results: Firstly, eIPSCs were optogenetically triggered in ChAT-cre/ChR2 mice, and these responses could be partially blocked with alpha-bungarotoxin (60.8 ± 4.4%, N=5). Optogenetic experiments were also performed in GAD2-cre/ChR2 mice producing eIPSC that could also be blocked by alpha-bungarotoxin (82.3 ± 7.2%, N=4). This result strongly suggests that a cholinergic response could be elicited in IHC in GAD-expressing neurons. Also, immunohistochemistry experiments done at the base of IHCs showed approximately 30% co-localization of GABAergic and cholinergic labelling.
Finally, calcium imaging experiments were performed with stimulation of MOC fibers, allowing us to resolve the activation of single synaptic sites. The application of the GABAB-R antagonist, CGP 36216, produced an increase in the activation probability of individual calcium hotspots in the context of heterogeneous responses in different sites of a single IHC.
Conclusions: In conclusion, here we provide evidence suggesting that GABA and ACh could be co-released from MOC terminals. Whereas ACh acts postsynaptically activating α9α10 receptors, the role of GABA is presynaptic, as a negative feedback signal to locally regulate cholinergic inhibition of IHCs. Calcium imaging experiments suggest that GABA modulation operates differently at each synaptic site.

SA74. The Role of Nfe2 in Development and Oxidative Stress Response in Zebrafish Inner Ear Function
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Category: Development: Cellular/Systems
Background: Noise exposure is one of the most common reasons for auditory dysfunction. Noise mechanically destructs sensitive structures of the inner ear including hair cells, and it can intensify their metabolic activity, which leads to the excess production of reactive oxygen species (ROS). ROS production then leads to cell death pathways in mammalian cochlear and vestibular hair cells. Since the inner ear depends on hair cells to properly function, the death of these sensory receptors leads to hearing loss. Thus, understanding cellular pathways involved in combating oxidative stress is important for enhancing our