Avian Cochlear Nucleus Neurons Exhibit Tonotopic Specializations Across Development

Kristine McLellan[1,2] & Jason Tait Sanchez[1,2,3]

[1]Roxelyn and Richard Department of Communication Sciences and Disorders, Northwestern University [2]Department of Neurobiology, Northwestern University, [3]Knowles Hearing Research Center

Background

- From auditory periphery to cortex, tonotopy is a hallmark organizational pattern of auditory structures.

-In nucleus magnocellularis (NM), the avian analog to the anteroventral cochlear nucleus (AVCN), high frequency neurons develop synaptic connections earlier than low frequency neurons[1].

- NM neurons express some intrinsic differences across the tonotopic axis after embryonic development[2].

- Still, it is unclear when these differences arise and whether they are caused by a developmental delay or are conserved over time.

- Here, we used whole-cell patch clamp electrophysiology to record intrinsic differences between high (HF) and low frequency (LF) neurons at various stages across development.

Methods

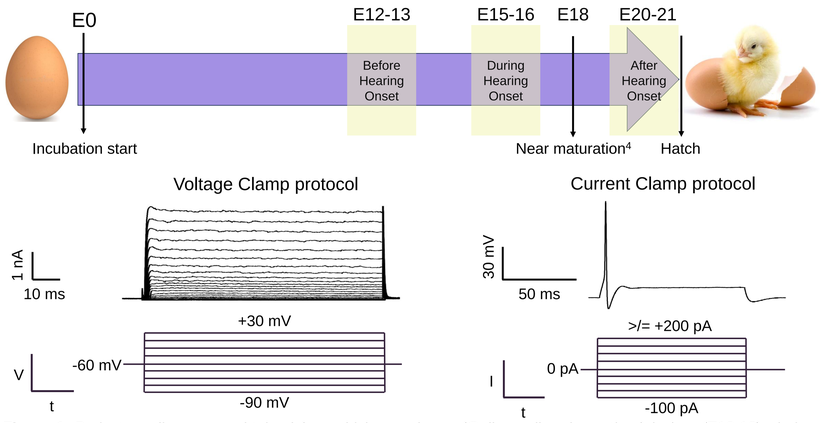

Figure 1: Brainstem slices were obtained from chicken embryos (*Gallus gallus domesticus*) before (E12-13), during (E15-16), and after hearing onset (E20-21). Whole-cell patch clamp electrophysiology experiments were conducted on neurons in rostral (i.e., high frequency) or caudal (i.e., low frequency) NM slices. Current Clamp and Voltage Clamp tests (above) recorded intrinsic neuronal properties. Synaptic activity was pharmacologically blocked. Parametric t-tests determined significance. ns = not significant, >0.05, * = <0.05, ** = <0.01, *** = < 0.001, **** = < 0.0001.

Before Hearing Onset (E12-13)

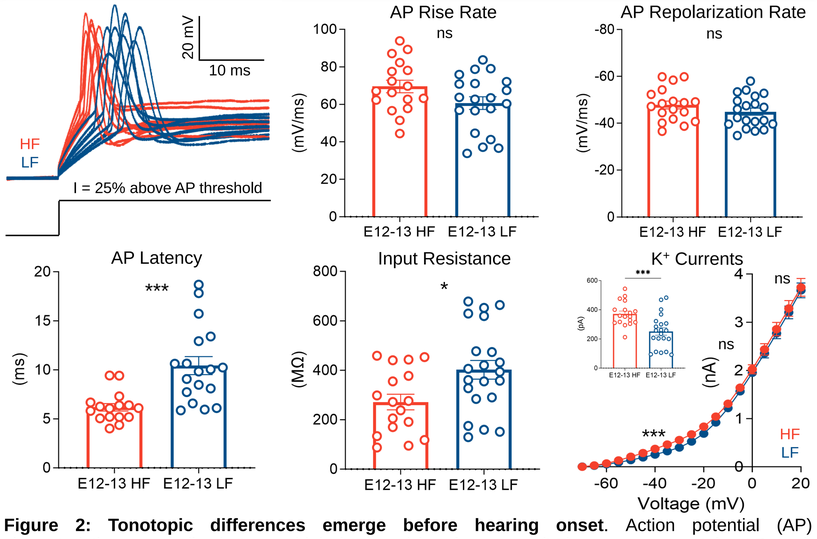

Figure 2: Tonotopic differences emerge before hearing onset. Action potential (AP) characteristics are similar between high (HF) and low frequency (LF) neurons except for AP peak latency. HF neurons have a lower input resistance and larger low-voltage activated potassium (K^+) current. No difference in total K^+ current between HF and LF >-10 mV. Inset is K^+ current at -40 mV.

During Hearing Onset (E15-16)

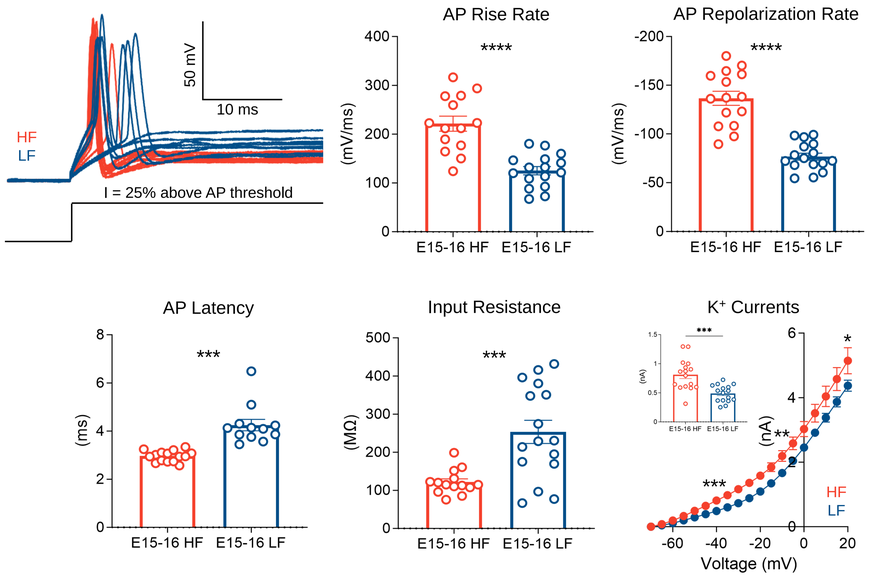

Figure 3: Tonotopic differences are highly dynamic during hearing onset. High frequency (HF) neurons continue to have earlier AP peak latencies but also have faster AP rise and repolarization rates compared to low frequency (LF) neurons. HF neurons have larger potassium (K^+) currents at low and high holding voltages (*e.g.*, -40, -10, & 20 mV), highlighting a crucial period of intrinsic development, where tonotopic properties are robustly significant across high and low frequency regions. Inset is K^+ current at -40 mV.

After Hearing Onset (E20-21)

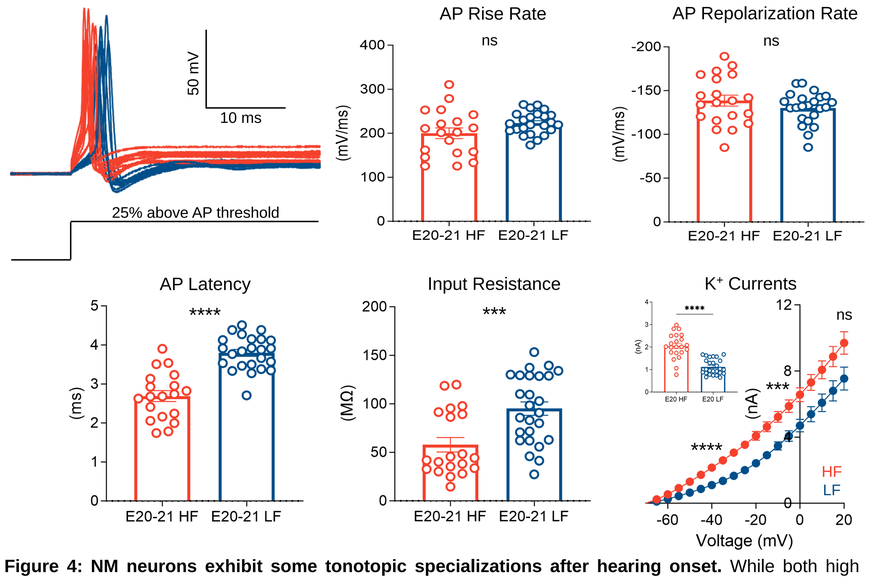

Figure 4: NM neurons exhibit some tonotopic specializations after hearing onset. While both high frequency (HF) and low frequency (LF) neurons fire precise APs after hearing onset, HF neurons still fire APs with earlier peak latencies than LF neurons. Unlike during hearing onset, the rise and repolarization rates of the APs are similar across frequencies. HF neurons have a significantly lower input resistance and larger low-voltage activated potassium (K^+) current magnitude than LF neurons, suggesting that HF neurons have more Kv1 channels than LF neurons. Inset is K^+ current at – 40 mV.

References: . [1]Jackson H, Hackett JT, & Rubel EW (1982). Organization and Development of Brain Stem Auditory Nuclei in the Chick: Ontogeny of Postsynaptic Responses. *J Comp Neurol* 210(1):80-86. [2]Fukui I & Ohmori H (2004). Tonotopic Gradients of Membrane and Synaptic Properties for Neurons of the Chicken Nucleus Magnocellularis. *J Neurosci* 24(34): 7514-23. [3]Jones TA, Jones SM, & Paggett KC (2006). Emergence of Hearing in the Chicken Embryo. *J Neurophys* 96(1):128-41. [4]Rubel EW, Smith DJ, & Miller LC (1976). Organization and Development of Brain Stem Auditory Nuclei of the Chicken: Ontogeny of N. Magnocellularis and N. Laminaris. *J Comp Neurol* 166(4):469-89.

Funding: NIDCD R01 DC017167 (JTS), NIDCD 1F31DC02136601A1 (KM)

Tonotopy Across Development

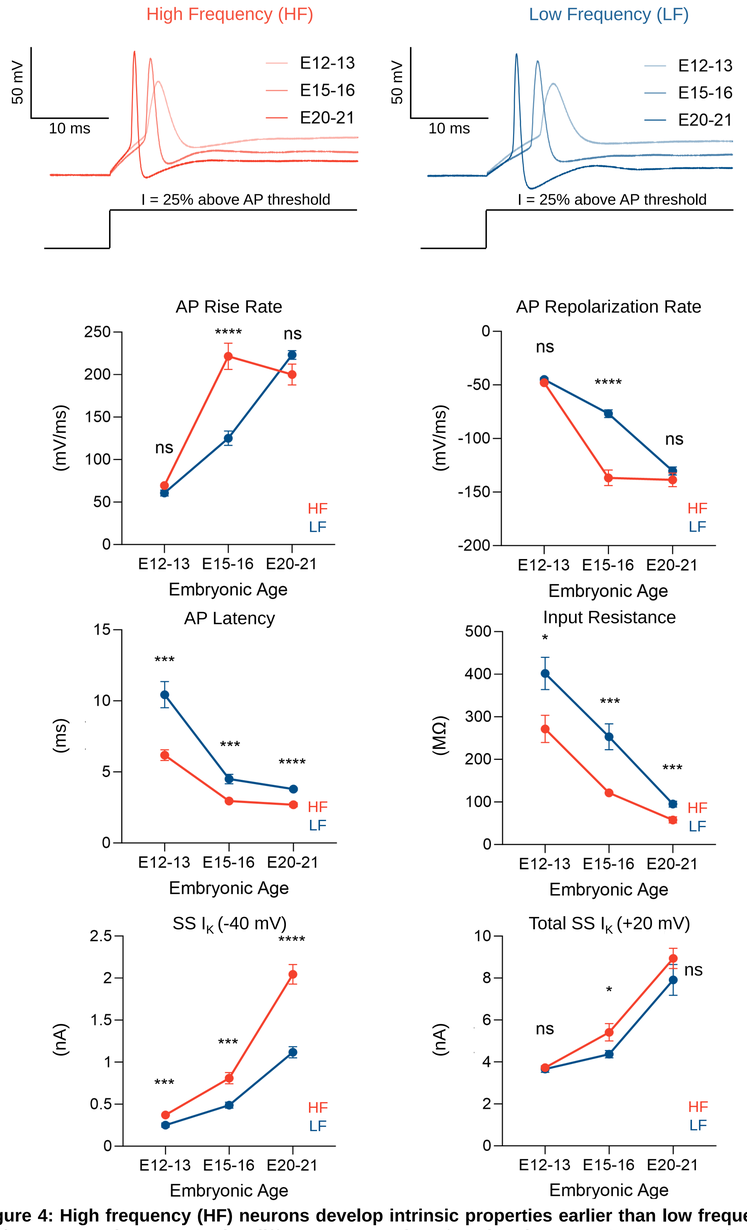

Figure 4: High frequency (HF) neurons develop intrinsic properties earlier than low frequency (LF) neurons, but tonotopic differences remain after hearing onset. Across embryonic development, both HF and LF neurons exhibit sharpened AP properties. Some tonotopic differences are minimized at embryonic maturity, but others remain. I_K = potassium (K^+) current.

Conclusions

1. Low frequency neurons demonstrate delayed development compared to high frequency neurons for action potential (AP) properties such as AP rise rate (caused by Na^+ channel contributions) and AP repolarization rate (caused by high-voltage activated K^+ channel contributions, *e.g.*, Kv3).
2. However, some functional characteristics, like AP peak latency, input resistance, and some K^+ channel currents, show distinct tonotopic differences across embryonic development.
3. While low frequency neurons do develop slower than high frequency neurons, they still retain unique tonotopic differences at all embryonic stages that cannot solely be explained by delayed development.