Neurotrophin Signaling Supports the Development of Neurons in the Avian Cochlear Nucleus

Kristine McLellan[1,2], Ann Lamptey[2], Sandesh Mohan[1], Hui Hong[3], & Jason Tait Sanchez[1,2,4]

[1]Roxelyn and Richard Department of Communication Sciences and Disorders, Northwestern University [2]Department of Neurobiology, Northwestern University, [3]Oregon Health and Science University, [4]Knowles Hearing Research Center

Northwestern University Interdepartmental Neuroscience

Background

-Brain-derived neurotrophic factor (BDNF) is a neurotrophin that mediates normal neuronal development using spatiotemporal signaling gradients.

-Neurotrophin signaling between BDNF and its high-affinity receptor, TrkB, is spatiotemporally regulated in the chicken nucleus magnocellularis (NM), a region analogous to the mammalian anteroventral cochlear nucleus.

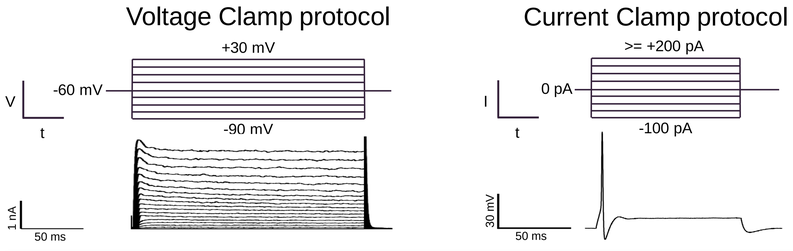
-TrkB is highly expressed in NM early in embryonic development (E9) and decreases significantly by neuronal maturation (~E18) (Cochran et al., 1999)

-It is unknown how auditory brainstem neurons within NM respond to BDNF-TrkB signaling. It is also unclear if BDNF-TrkB signaling differs across development or across the tonotopic gradient.

-Here, we exogenously applied BDNF on NM neurons *ex vivo* and studied the neurons' excitability properties with whole-cell patch clamp electrophysiology.

Methods

Brainstem slices were obtained from chicken embryos (*Gallus gallus domesticus*) at early (embryonic day 13) and late (embryonic days 19-21) developmental stages. In experimental conditions, coronal slices were bathed with BDNF and/or ANA-12 (TrkB antagonist) for at least two hours before recording. Whole-cell patch clamp electrophysiology experiments were conducted on neurons found in caudal (i.e., low frequency) or rostral (i.e., high frequency) NM slices. Neurons were bathed in pharmacological blockers during all recordings to inhibit synaptic activity. Current was injected into the soma of NM neurons using an Axon Multiclamp 700B amplifier. Neurons with a series resistance greater than 10 MΩ were excluded. Results were analyzed using Clampfit 11.0 analysis software. Significance was determined either using parametric/non-parametric t-tests or one-way ANOVA with multiple comparisons. * = <0.05, ** = <0.01, *** = < 0.001, **** = < 0.0001. Below are example electrophysiology protocols used.



Early embryonic effects of BDNF

E13 Low Frequency NM

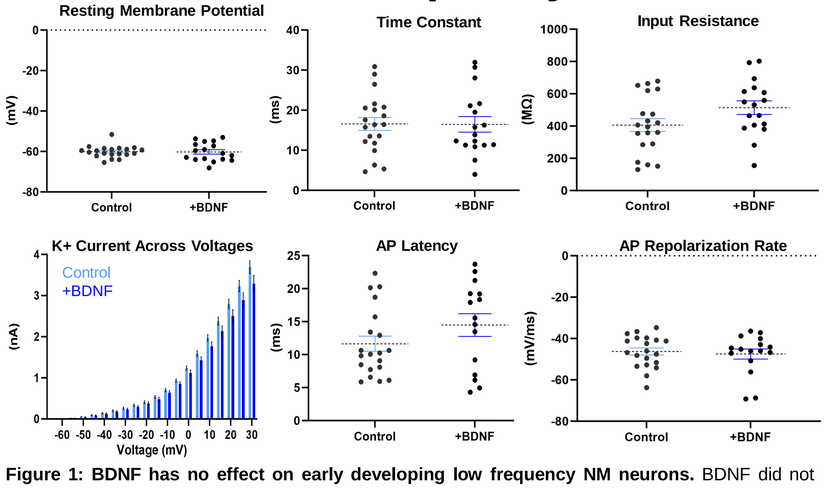


Figure 1: BDNF has no effect on early developing low frequency NM neurons. BDNF did not significantly affect passive or active properties for low frequency early developing NM neurons.

E13 High Frequency NM

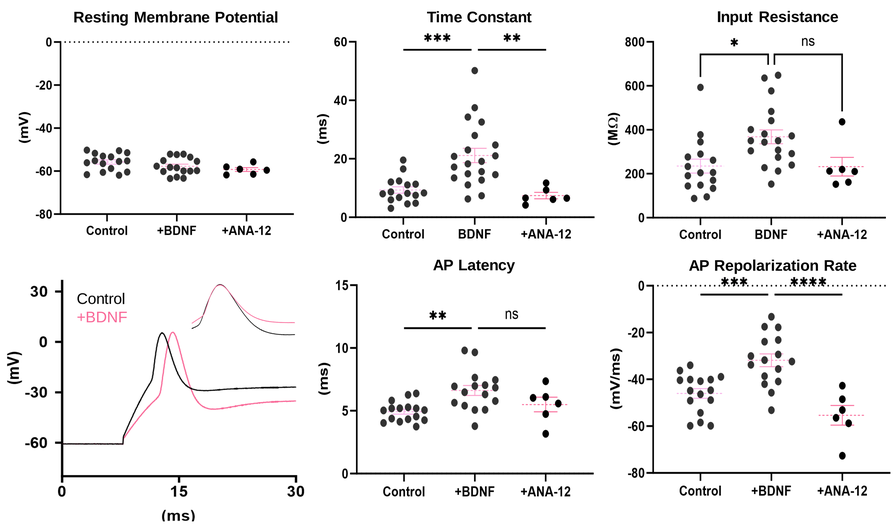


Figure 2: BDNF-TrkB activation alters the intrinsic properties of high frequency NM neurons earlier in development. Population data demonstrating active and passive neuronal properties for all conditions. Representative action potential traces for the Control and +BDNF conditions demonstrate a change in latency and repolarization rate when BDNF is applied to NM neurons. Differences in potassium currents at various voltages show that low- and high-voltage activated potassium channels are affected by BDNF.

Late embryonic effects of BDNF

E20-21 Low Frequency NM

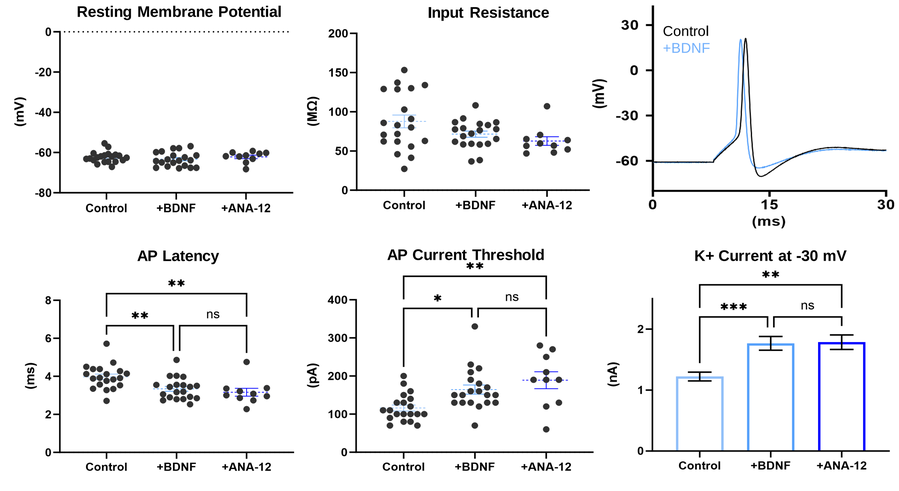


Figure 3: BDNF decreases neuronal excitability, and the effects are likely from BDNF-TrkC activation. BDNF application lowers the action potential latency and increases the current threshold. This is mediated by an increase in low-voltage activated potassium currents elicited in Voltage Clamp. However, the addition of ANA-12 does not reverse these effects, suggesting that BDNF binds to TrkC to induce these changes.

E20-21 High Frequency NM

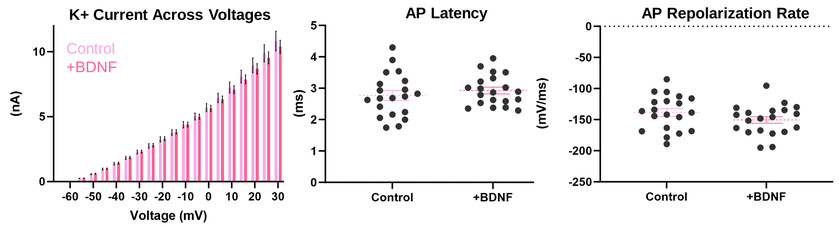
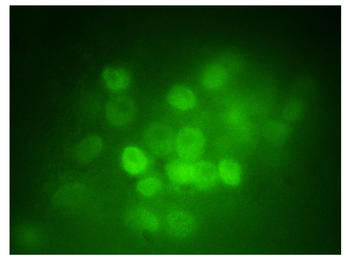
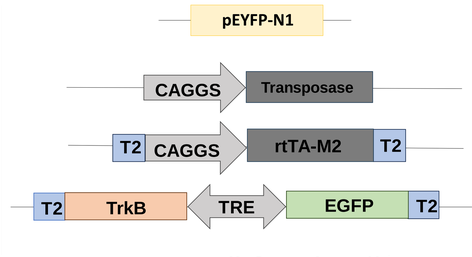


Figure 4: BDNF has no effect on late developing high frequency NM neurons. BDNF application did not significantly affect passive or active properties for high frequency late developing NM neurons. This is likely because TrkB is minimally expressed at this mature developmental stage.

Next steps: TrkB genetic manipulation





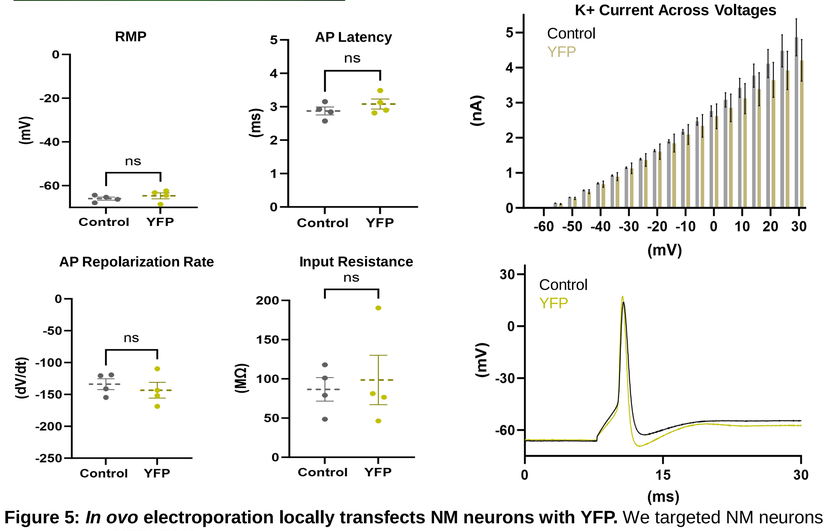


Figure 5: *In ovo* electroporation locally transfects NM neurons with YFP. We targeted NM neurons with control YFP DNA plasmids and found no differences to the neurons' electrophysiological properties. We aim to use this technique to prolong the expression of TrkB throughout development.

Conclusions

1. BDNF-TrkB signaling drastically alters the development of NM neurons, particularly in high frequency regions, by slowing neuronal maturation. This is consistent with changes seen to voltage gated potassium currents.
2. No effects are reported when BDNF is applied to low frequency neurons, suggesting tonotopic specificity within NM after BDNF application.
3. Later in development, BDNF does not affect the intrinsic properties of high frequency NM neurons. Effects of BDNF on low frequency neurons suggests non-preferential binding of BDNF to other Trk receptors.
4. Our next step is to genetically prolong the expression of TrkB in NM across development and investigate its electrophysiological and functional effects.

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