Neurotrophin Signaling Alters High-Frequency Neurons in the Avian Cochlear Nucleus

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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 applied BDNF on NM neurons *ex vivo* and studied neuronal properties using electrophysiology, immunohistochemistry, and modeling.

Methods

Brainstem slices were obtained from chicken embryos (*Gallus gallus domesticus*) at early (embryonic day 13) and late (embryonic days 20-21) 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 recordings to inhibit synaptic activity. Current was injected into NM somas using an Axon Multiclamp 700B amplifier. Neurons with a series resistance greater than 10 MΩ were excluded. Results were analyzed using Clampfit 11.0 software. Significance was determined using parametric t-tests or one-way ANOVA with multiple comparisons. * = <0.05, ** = <0.01, *** = < 0.001, **** = < 0.0001.

Early embryonic effects of BDNF

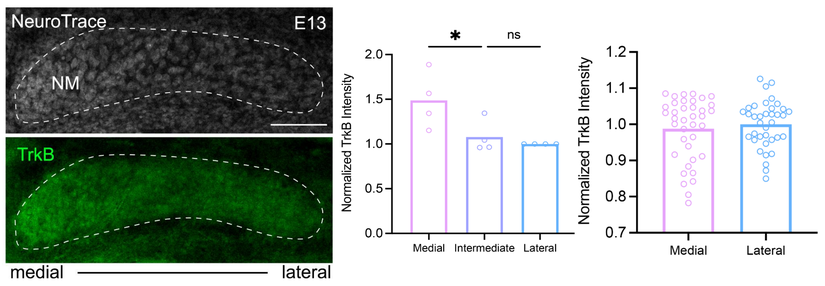

Figure 1: TrkB immunoreactivity in early-developing NM neurons. (Left) TrkB immunoreactivity from an E13 chicken embryo. Note the gradient of overall TrkB intensity in NM with higher staining in medial NM. Scale bar = 100 µm. (Middle) Quantification of mean TrkB intensity from the medial, intermediate, and lateral NM of 4 embryos. Data were normalized to the lateral NM of the same embryo. (Right) Quantification of somatic TrkB intensity of individual neurons from medial and lateral NM. Data were normalized to the mean of neurons measured from lateral NM of the same embryo.

E13 Low-Frequency

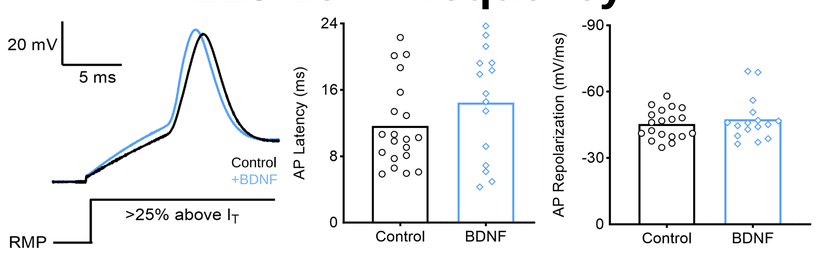

Figure 2: BDNF has no effect on early developing low-frequency NM neurons. BDNF did not significantly affect passive or active properties for low frequency early developing NM neurons.

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E13 High-Frequency

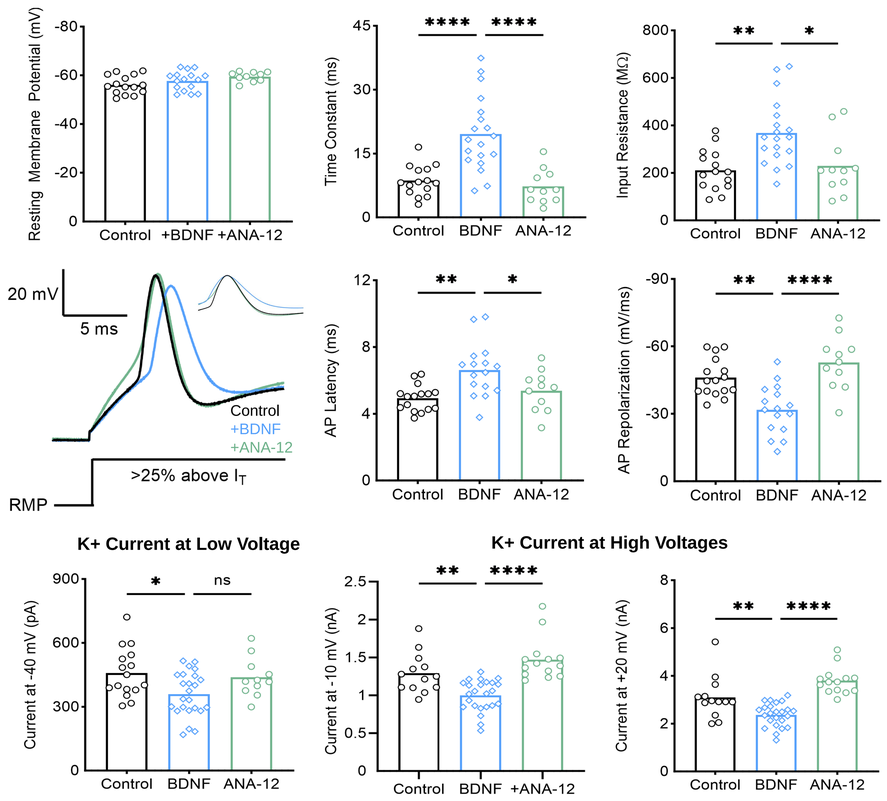

Figure 3: BDNF-TrkB activation alters the intrinsic properties of high frequency NM neurons earlier in development. Population data demonstrating active and passive neuronal properties for all conditions. Representative action potential traces for the control, +BDNF, and +ANA-12 conditions demonstrate a change in latency and repolarization rate when BDNF is applied to NM neurons. Decreases in potassium currents at various voltages show that BDNF affects low- and high-voltage activated potassium channels.

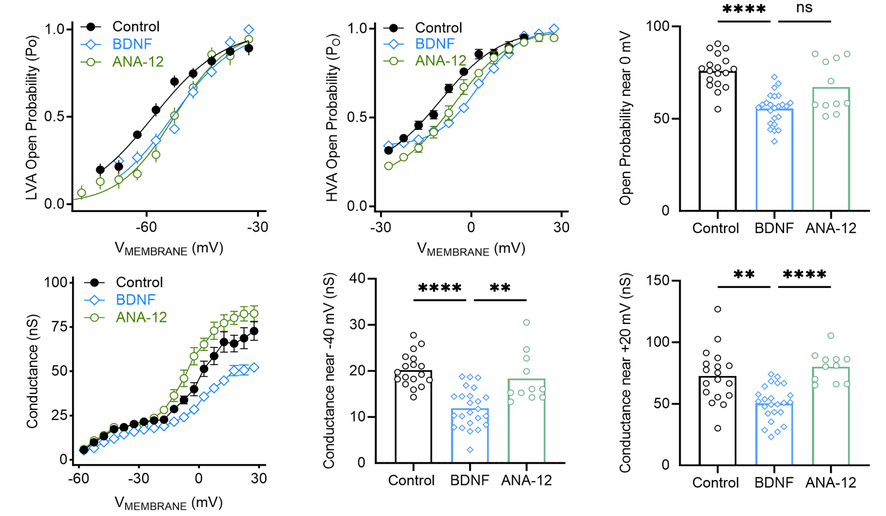

Figure 4: Biophysical modeling of E13 high-frequency neurons. Sigmoidal curves derived from a Boltzmann distribution are fitted to the open probability of low-voltage activated (LVA) and high-voltage activated (HVA) potassium channels. The open probability near 0 mV is highlighted to show that BDNF application decreases HVA open probability, and this is recovered with ANA-12. Below, differential conductance across all voltage commands is plotted and highlighted at a low depolarizing voltage (near -40 mV) and a high depolarizing voltage (near +20 mV). At both voltages, differential conductance decreases and is recovered by ANA-12. This suggests that BDNF alters potassium channel density and subtype.

Late embryonic effects of BDNF

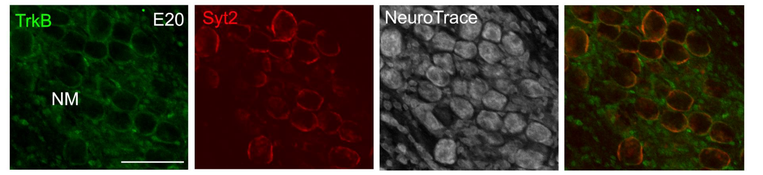

Figure 5:TrkB immunoreactivity in early-developing NM. TrkB signal overlapped with Syt2, indicating a predominant localization of TrkB in presynaptic endbulb terminals. Scale bar = 50 µm.

E20-21 High-Frequency

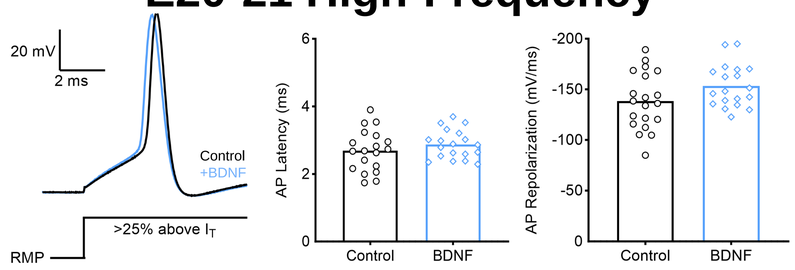

Figure 6: BDNF has no effect on late developing high-frequency NM neurons. BDNF application did not significantly affect passive or active properties for high frequency late developing NM neurons. This is likely because TrkB is minimally expressed at this mature developmental stage.

E20-21 Low-Frequency

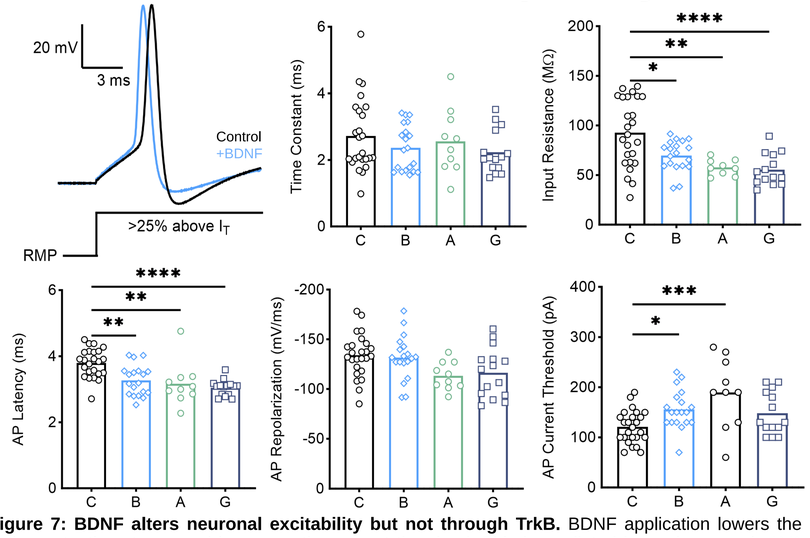

Figure 7: BDNF alters neuronal excitability but not through TrkB. BDNF application lowers the action potential latency and increases the current threshold. This is mediated by an increase in LVA potassium currents (not shown). However, the addition of ANA-12 or a pan-Trk blocker GNF 5837 does not reverse all effects, suggesting that BDNF is not binding to Trk receptors to induce changes.

Conclusions

1. BDNF-TrkB signaling alters the early 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 of BDNF-TrkB signaling. This is confirmed by tonotopic variation of TrkB expression across NM seen with IHC.
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 receptors.
4. Next step: genetically prolong the expression of TrkB in NM across development and investigate its neuronal and functional effects.