Exogenous BDNF application alters ionic currents in a spatiotemporal manner in the avian auditory brainstem

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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), an auditory region analogous to the mammalian anteroventral cochlear nucleus.
- TrkB expression in NM neurons is relatively high early in embryonic development (E9) and steadily decreases to negligible amounts by the time of neuronal maturation (~E18) (Cochran et al., 1999)
- It is currently 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 rostromedial tonotopic gradient.
- To investigate this, we exogenously applied BDNF on NM neurons *ex vivo* and studied the neurons' intrinsic properties using whole-cell patch clamp electrophysiology.

Methods

Brainstem slices were obtained from White Leghorn chicken embryos (*Gallus gallus domesticus*) at early (embryonic day 13) and late (embryonic days 19-21) developmental stages. In all experiments, slices were incubated in ACSF for at least two hours before patch clamp electrophysiology recordings began. In experimental conditions, coronal slices were bathed with BDNF and/or ANA-12 (BDNF receptor, TrkB, antagonist) for at least two hours before recording. Whole-cell patch clamp electrophysiology experiments were conducted using Current and Voltage Clamp protocols on neurons found in the most 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. Voltage commands and current injections of varying durations and strengths were induced in 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. Statistical significance was determined either using parametric or non-parametric t-tests or one-way ANOVA with multiple comparisons when ANA-12 data was involved. * = <0.05, ** = <0.01, *** = < 0.001, **** = < 0.00001.

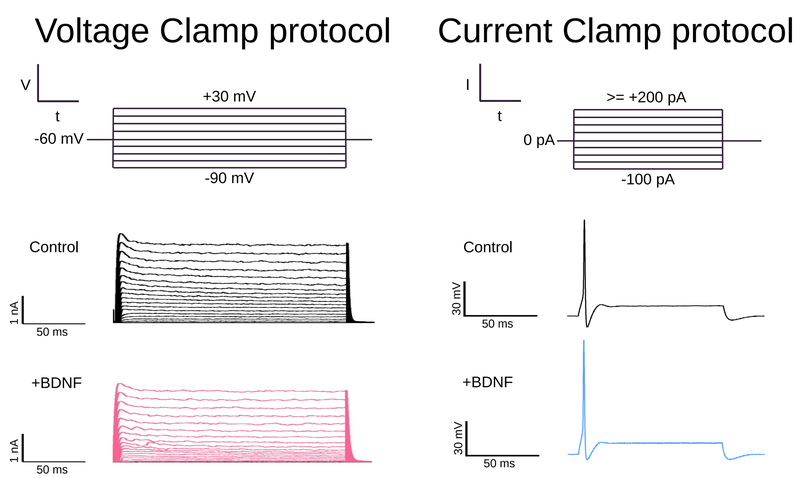

Figure 1. Voltage and Current clamp protocols and resulting representative traces from E13 high frequency NM neurons. In Voltage Clamp, NM neurons were held at -60 mV and then hyperpolarized or depolarized to varying potentials between -90 mV and +30 mV for 150 ms before returning to a holding command of -60 mV. Resulting current traces for E13 high frequency Control and +BDNF conditions are shown for each voltage command between -60 and +30 mV. In Current Clamp, current injections between -100 pA and +200 pA (or more, if action potentials are not induced) were injected into the soma of NM neurons. Resulting representative action potentials from E20-21 low frequency neurons are shown for Control and +BDNF conditions.

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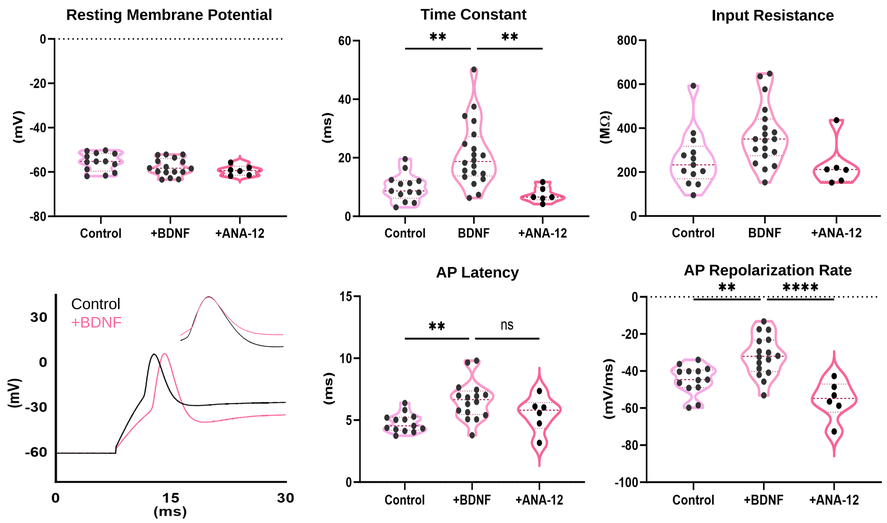
Early embryonic effects of BDNF

E13 High Frequency NM



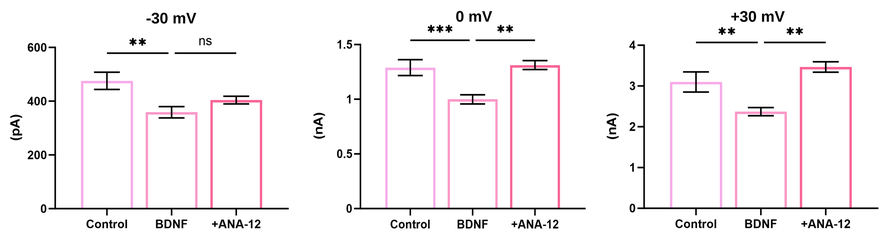


Figure 2: BDNF-TrkB activation alters the intrinsic properties of high frequency NM neurons earlier in development. Population data for Control, +BDNF, and +BDNF +ANA-12 conditions are shown depicting resting membrane potential, membrane time constant, and input resistance. Representative action potential traces for the Control and +BDNF conditions demonstrate a change in latency and repolarization rate when BDNF is applied to NM neurons. Representative potassium current traces in response to Voltage Clamp commands for the Control and +BDNF conditions show an overall decrease in outward potassium current when BDNF is applied. Both low- and high-voltage activated potassium channels are affected by BDNF.

E13 Low Frequency NM

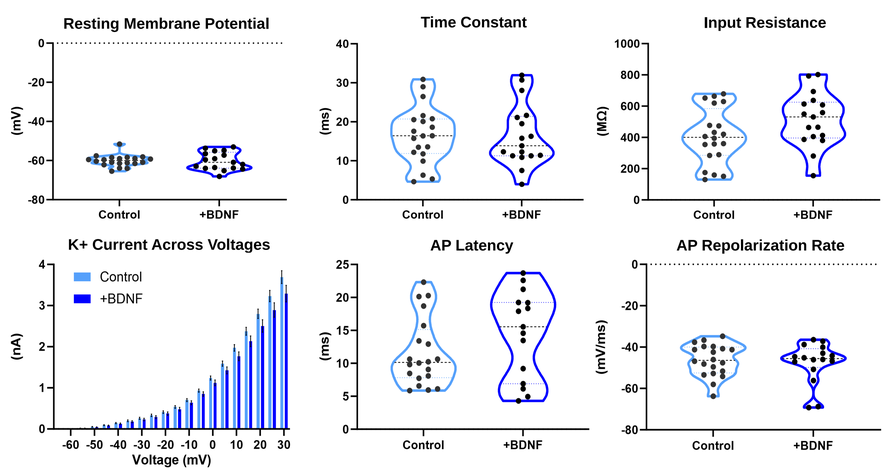


Figure 3: BDNF has little to no effect on early developing low frequency NM neurons. BDNF application did not significantly affect passive or active properties for low frequency early developing NM neurons, and potassium current magnitudes evoked at varying voltage commands also did not change.

Late embryonic effects of BDNF

E20-21 High Frequency NM

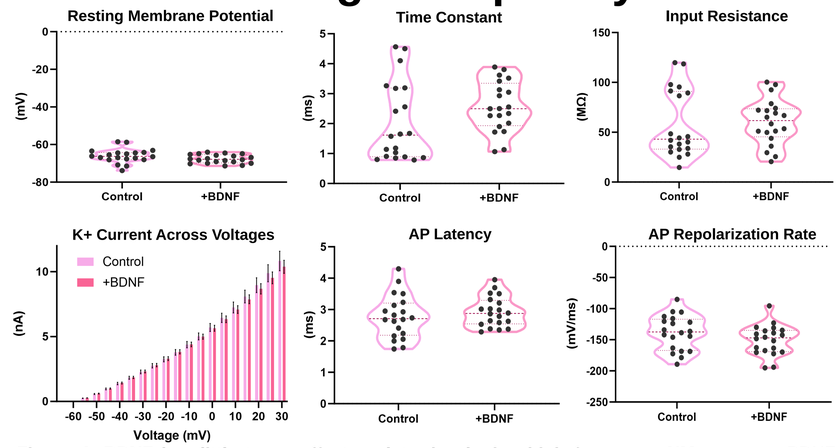


Figure 4: BDNF has little to no effect on late developing high frequency NM neurons. BDNF application did not significantly affect passive or active properties for high frequency late developing NM neurons, and potassium current magnitudes evoked at varying voltage commands also did not change. This is likely because TrkB is minimally expressed at this mature developmental stage.

E20-21 Low Frequency NM

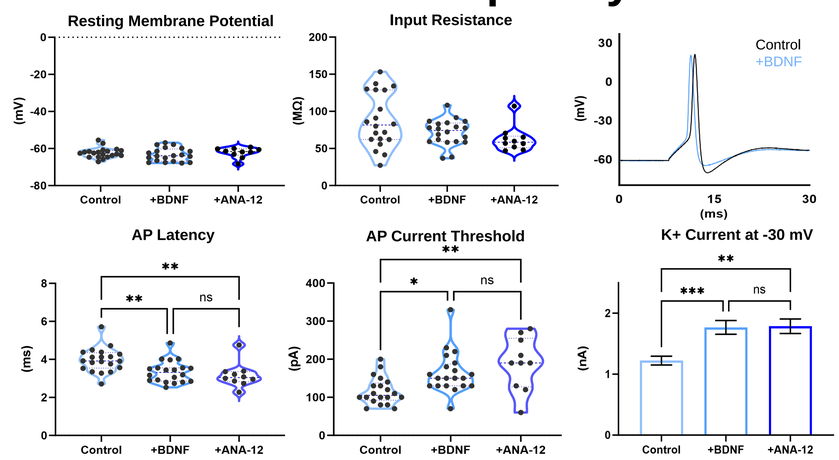


Figure 5: BDNF decreases neuronal excitability, but the effects are likely from BDNF-TrkC activation. While BDNF application does not affect low frequency NM passive properties, the action potential latency decreases, and the current threshold increases. This is mediated by an increase in low-voltage 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.

Conclusions

1. BDNF-TrkB signaling decreases low- and high-voltage activated potassium currents and consequently widens the shape of the action potential for early developing high frequency NM neurons.
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, when TrkB is minimally expressed, BDNF does not affect the intrinsic properties of high frequency NM neurons.
4. A marked decrease was seen in the overall excitability of late-developing low frequency neurons after BDNF application, but this is presumably caused by BDNF non-preferentially binding to TrkC, since the presence of the TrkB antagonist ANA-12 did not reverse these effects.
5. In all, BDNF drastically alters the development of NM neurons, particularly in high frequency regions, by inhibiting normal neuronal maturation.