In-ovo electroporation successfully targets neurons in the avian cochlear nucleus

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Background

The development of the avian and mammalian nervous system is regulated by signaling proteins called neurotrophins. Brain-derived neurotrophic factor (BDNF) is a neurotrophin that binds to tyrosine receptor kinase B (TrkB) and is known to influence the development of the avian cochlear nucleus: nucleus magnocellularis (NM). TrkB expression decreases over embryonic development so it must be expressed via genetic manipulation at the late stages of maturation. The question that we want to investigate is: **Does the late expression of TrkB on NM neurons change the intrinsic properties to be similar to immature NM neurons?** However, wild-type BDNF-TrkB signaling has not been fully electrophysiologically characterized and it is unknown if the method used to genetically manipulate the neurons, known as *in ovo* electroporation, affects the intrinsic properties of the neuron. Here, we establish that 1) exogenous application of BDNF results in BDNF-TrkB signaling that is consistent with what is expected in early and late developmental stages, 2) *in ovo* electroporation with a yellow fluorescent protein (YFP) successfully targets neurons in NM, 3) the electrophysiology of YFP-expressing neurons is similar to wild-type control neurons.

Methods

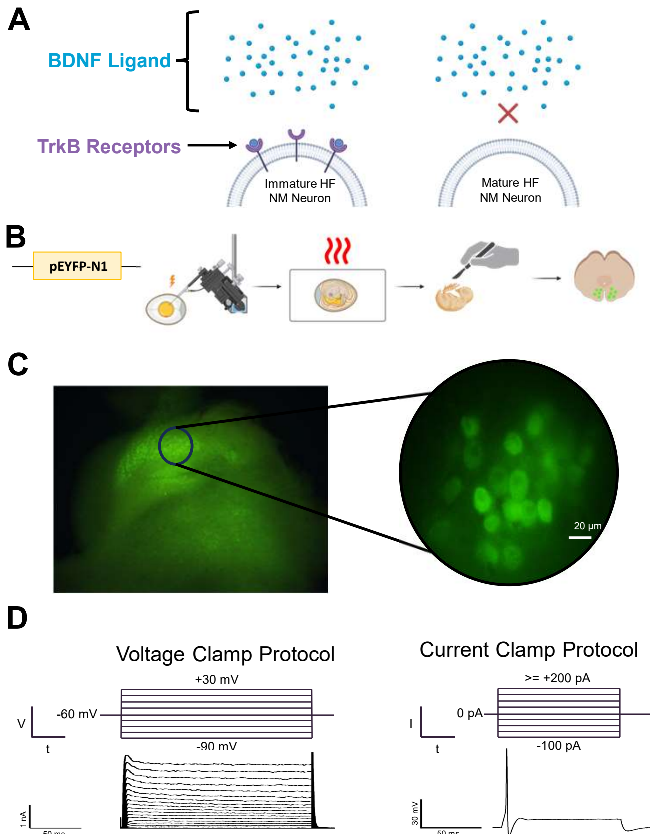


Figure 1. Experimental Methodology: A, TrkB expression decreases as the NM neurons mature according to immunohistochemistry experiments. Brainstem slices were incubated in BDNF for 2 hours before electrophysiological data was collected. **B**, *In-ovo* electroporation, was used to transfect YFP on embryos of White Leghorn chickens (Gallus domesticus) on an embryonic day two (E2). Brain stem slices were acquired from early (E13) and late-stage (E18-21) embryos. **C**, NM cells fluorescing after successful transfection of YFP via in-ovo electroporation. **D**, Whole-cell patch clamp electrophysiology was used to collect data. Synaptic transmission was pharmacologically blocked. Data analysis was conducted through Clampfit Analysis Software (Control n =4 and YFP n = 4). E13 Control, BDNF, E20-21 Control, BDNF n =20 and ANA-12 n =9. Error bars show standard error.

Exogenous BDNF Application

E13 NM Data

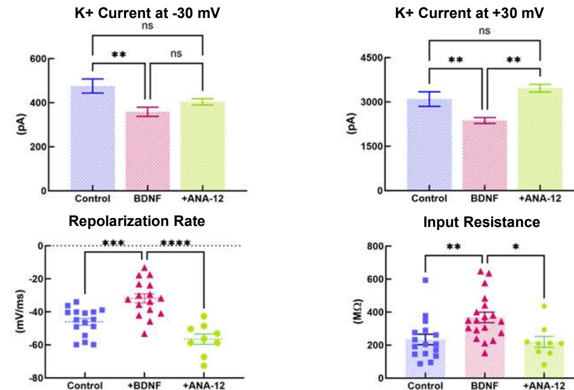


Figure 2. Application of BDNF changes the neuronal excitability of high-frequency NM neurons in the early stages of development. When TrkB receptors are blocked with ANA-12 the changes revert to control conditions establishing that TrkB-BDNF signaling is the source of these changes.

E20-21 NM Data

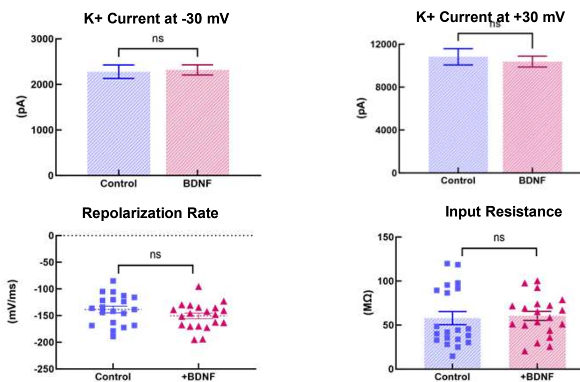


Figure 3. BDNF-TrkB signaling has no effect on high-frequency NM neurons at the late stages of development. We found no differences in the intrinsic properties of BDNF-bathed NM neurons in the late stages of development.

YFP vs. Control NM Neurons

Voltage Clamp Data

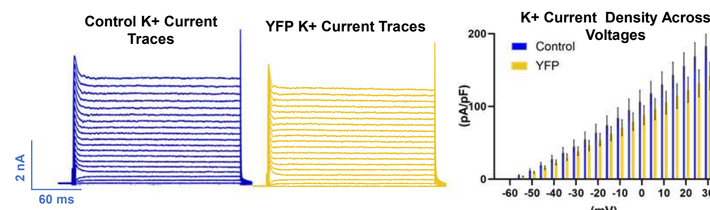


Figure 4. Potassium currents of NM neurons are preserved after YFP transfection. In the current density plot, the YFP neurons seemingly have less potassium current. This is likely due to the small sample size (n= 4). These findings suggest that the amount of potassium channels on the cell remains the same.

Current Clamp Data: Active Properties

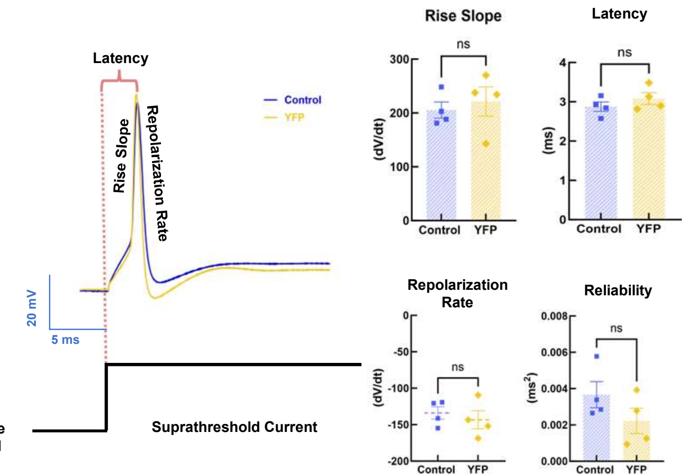


Figure 5. The control and YFP groups produce homogenous action potentials. There were no significant differences in the rise rate, latency, repolarization rate and latency of the action potentials. This suggests that the excitability properties of the YFP-expressing neurons were unaffected.

Current Clamp Data: Passive Properties

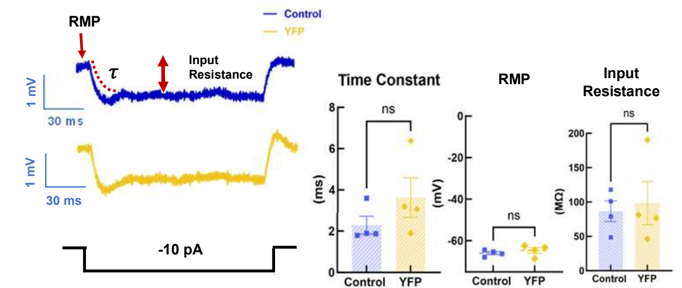
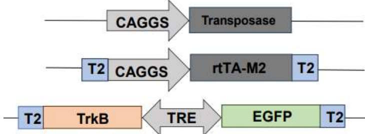


Figure 6. The passive properties are consistent between the YFP and Control groups. These findings further demonstrate that the neuronal excitability between the two groups is the same.

Conclusions

1. The successful transfection of YFP plasmids without affecting neuronal excitability demonstrates that *in-ovo* electroporation may be used in future experiments to test neuronal changes to TrkB transfection.
2. BDNF regulates the expression of potassium channels in the early stages of NM development.
3. BDNF has no significant impact on the neuronal properties in the late stages of embryonic development.
4. Future direction is to transfect TrkB using in-ovo electroporation to maintain its expression.



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