Apoptosis in Neurons: Transient and Reversible

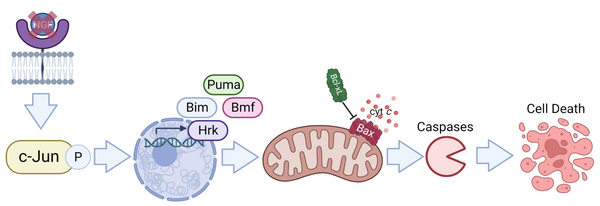
Ella Hammond[2], Keeley Spiess[1], Mohanish Deshmukh[1]
[1]University of North Carolina at Chapel Hill Neuroscience Center, [2]NSCI 395

Deshmukh Lab

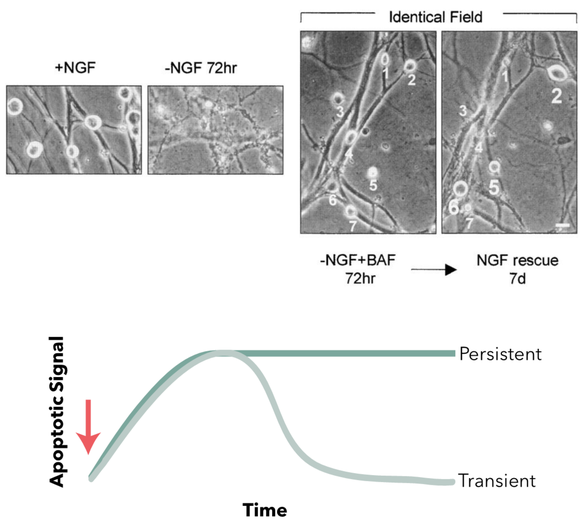
Introduction

- Programmed cell death, or apoptosis, is induced to eliminate excess or damaged cells during the development and maturation of the nervous system.
- The apoptotic pathway in superior cervical ganglia (SCG) neurons is commonly studied using a sympathetic neuron model of nerve growth factor (NGF) deprivation.
- An NGF deprivation and restoration model has shown neurons can reverse their commitment to death even after activating the apoptotic pathway.[1]
- Previous work has shown that the apoptotic pathway is activated transiently in SCG neurons.
- In this study, we analyzed (1) how SCGs downregulate their apoptotic signaling, (2) if other apoptotic insults can be reversed in neurons, and (3) if previous exposure to an apoptotic insult confers increased sensitivity or resistance to a second apoptotic insult.

Apoptosis Pathway



Neuronal Apoptosis Reversibility



Bax plays a role in regulating the transience of p-c-Jun

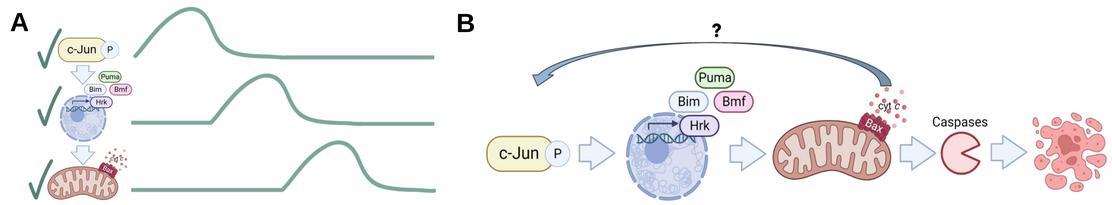


Figure 1: (A) The apoptotic pathway in neurons is activated transiently, including the phosphorylation of the transcription factor c-Jun, the induction of BH3-only proteins, and the activation of Bax. **(B)** Given the various transient processes in apoptosis, we questioned whether Bax modulates negative feedback on earlier steps of apoptosis, such as the phosphorylation of c-Jun.

Bax plays a role in regulating the transience of p-c-Jun

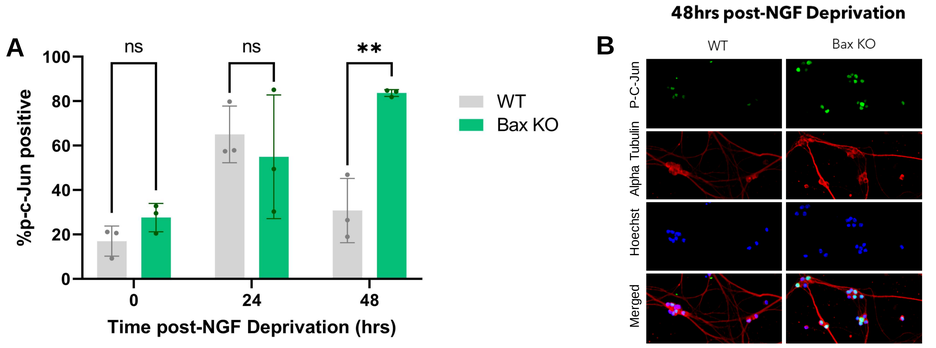


Figure 2: (A) Neurons were deprived of NGF over a 48-hour time period in the presence of QVD, with representative images taken at 0, 24, and 48 hours after deprivation. The number of p-c-Jun positive cells were counted at each time point in both wildtype and Bax KO plates, and multiple unpaired t-tests (alpha= 0.05) showed a significant difference in p-c-Jun levels at the 48-hour time point (p=0.0032). **(B)** Representative immunofluorescence of p-c-Jun in wildtype and Bax KO neurons co-stained with Hoechst to mark nuclei and alpha tubulin to show neuron morphology.

Are other Bax-dependent apoptosis cues reversible in SCG neurons?

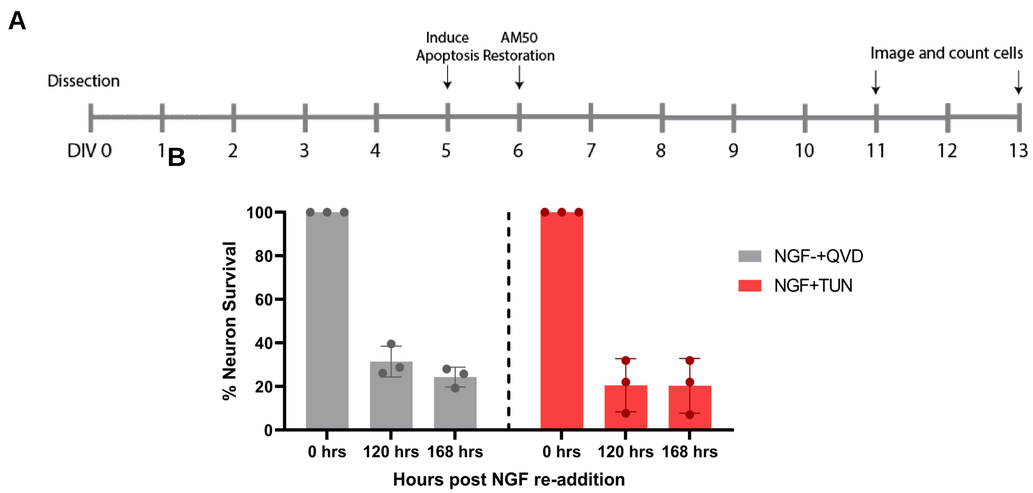


Figure 3: (A) Treatment timeline of "reversibility" experiment. On DIV5, apoptosis is induced either with NGF deprivation (grey) or 2.5uM TUN (red), and healthy conditions restored 24 hours later, with images taken at 0 hours, 120 hours and 168 hours after restoration. **(B)** The percent of neurons that survived and reversed their commitment to apoptosis after NGF deprivation (grey) or TUN (red). Both NGF deprivation and TUN treatment are reversible.

Reversing apoptosis modestly protects against future apoptotic insults

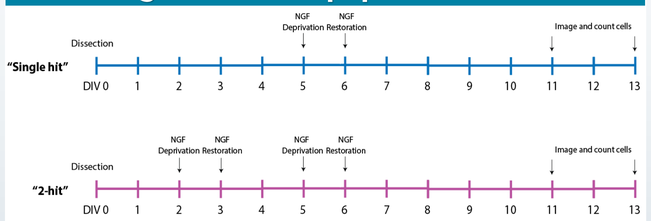


Figure 4: Timeline of treatment for a single NGF deprivation treatment (NGF-+QVD, blue) and two independent NGF deprivation treatments (NGF-+QVD 2-hit, purple).

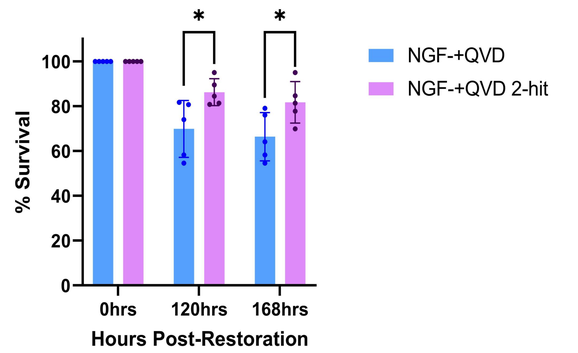


Figure 5: Quantification of neuron survival following a single NGF deprivation (blue) or two sequential NGF deprivations (purple) (120hrs p=0.031, 168hrs p=0.042). There is a modest but significant protective effect of previous exposure to an apoptotic insult.

Summary

- Bax KO neurons show elevated p-c-Jun levels compared to WT controls, indicating Bax may act as a negative feedback regulator on p-c-Jun signaling during apoptosis.
- Neurons were able to utilize apoptotic reversal mechanisms in response to Tunicamycin treatment, just as seen in the context of NGF deprivation.
- The 2-hit experiment showed neurons that have reversed their commitment to death are modestly protected from a subsequent apoptotic insult.
- Future Directions:
  - Given this data focuses on developing neurons that are dependent on NGF, we plan to test whether apoptotic stressors relevant to mature neurons (TUN or etoposide) confer protection or sensitivity to subsequent stress.

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References

1. Deshmukh, M., Kuida, K. & Johnson, E. M. Caspase Inhibition Extends the Commitment to Neuronal Death Beyond Cytochrome c Release to the Point of Mitochondrial Depolarization. *The Journal of Cell Biology* **150,** 131–144 (2000).