NCX-9 regulates calcium exchange at the mitochondrion and is required for neural circuit patterning in *Caenorhabditis elegans*

Vishal Sharma[1,2], Soumitra Roy[3], Israel Seckler[3], Damien O'Halloran[1,2]
Department of Biological Sciences[1] and Institute for Neuroscience[2], The George Washington University, Ben Guerin University[3]

Introduction

Developmental pathways that map anatomical asymmetry onto neural circuits are largely unknown. Previous work has shown that stereotyped patterning schemes in the DA/DB motor neuron circuit in *C. elegans* depend on specific Heparan Sulfate-modification patterns[3]. Heparan Sulfate Proteoglycans are well characterized regulators of circuit development, suggesting that left/right patterning of neural circuits may also be under the regulation of classic neurodevelopmental programs. Our research uses the asymmetrical commissural patterning scheme of the VD and DD GABAergic motor neuron circuit to understand how anatomical asymmetries are mapped onto neural circuits.

Methods

- Mutants for *ncx-9* were acquired through the Million Mutation Project. Each *ncx-9* mutant strain was crossed five times through a wildtype background before analysis
- We examine wildtype and mutant strains for defects in VD/DD anatomical patterning using the ^{prom}*unc-25::GFP* (juIs76) and ^{prom}*unc-47::GFP* (oxIs12) transgenes
- *In vitro* physiological assessment of NCX-9 cDNA was performed using HEK-293. Plasma membrane exchange was quantified using cells loaded with Fura-2, while mitochondrial exchange was measured using cells transfected with a mitochondrially targeted RP-mt protein pericam.

Results

Expression Pattern of *ncx-9*

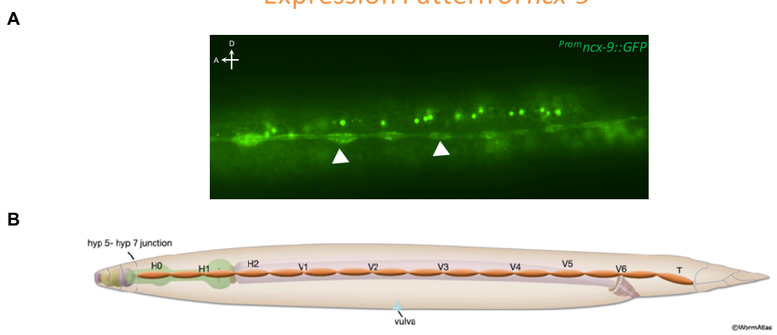
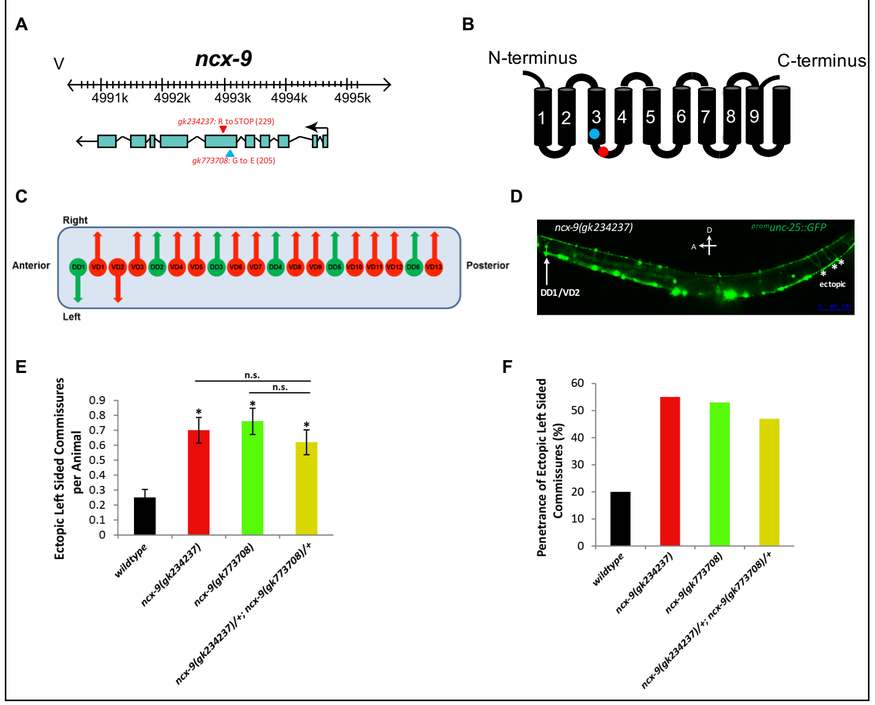


Figure 1 : Expression of *ncx-9* is detected in the hypodermal seam cells (white triangles) of the worm

Characterization of *ncx-9*$^{-/-}$ mutants



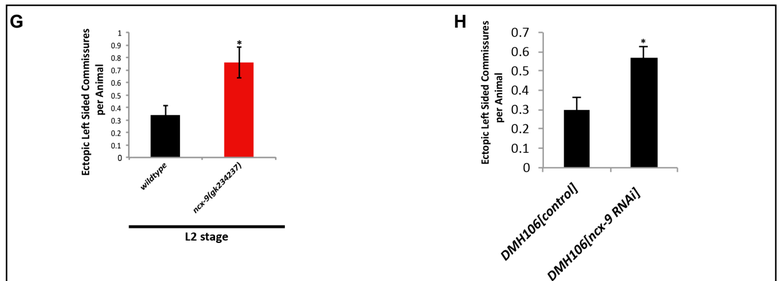


Figure 2 : Characterization of Left/Right Patterning Defects in *ncx-9*$^{-/-}$ mutants.
**p* <.001 for wildtype versus mutants (n.s. = not significant)

Signaling Pathway Analysis

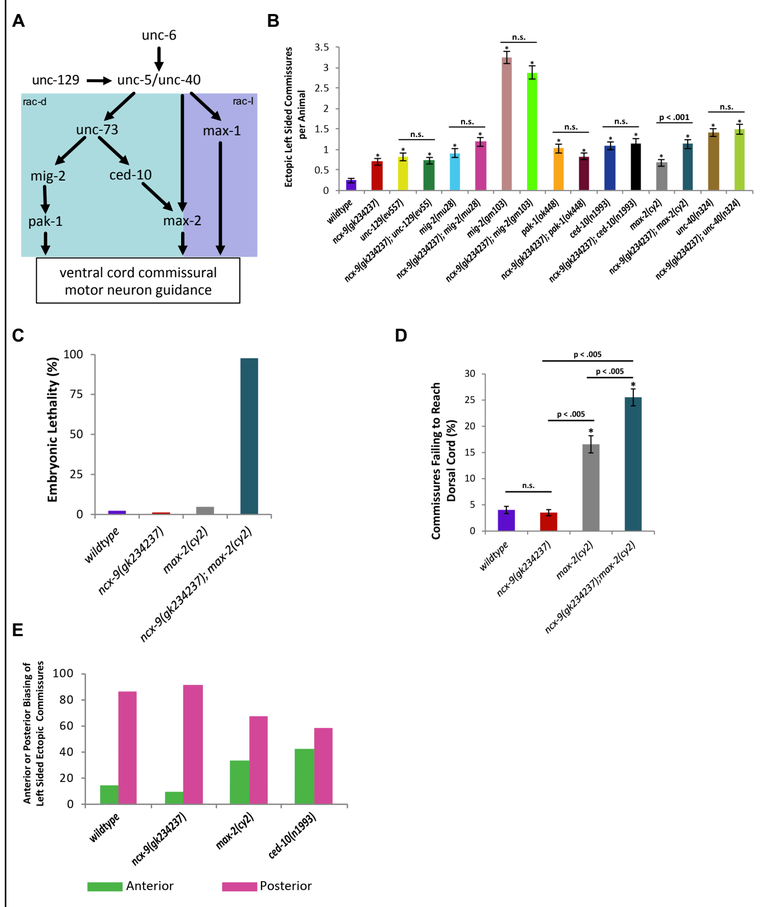
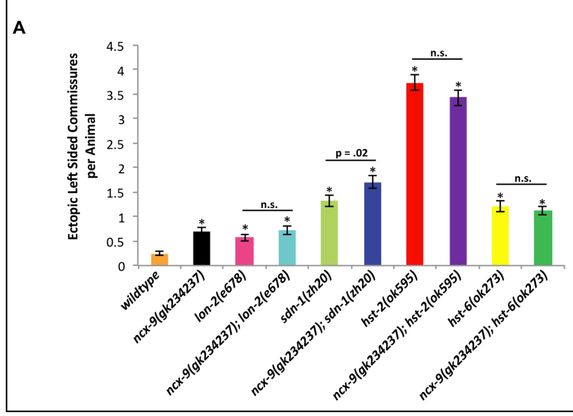


Figure 3 : NCX-9 regulates neural circuit patterning through a RAC-dependent signaling pathway.
**p* <.005 for wildtype versus mutants (n.s. = not significant)



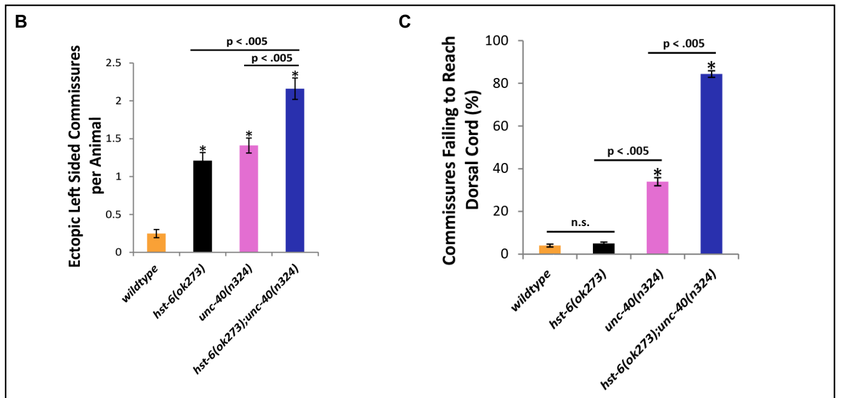


Figure 4: NCX-9 regulates neural circuit patterning through a LON-2/Glypican Heparan Sulfate Pathway. **p* <.005 for wildtype versus mutants (n.s. = not significant)

Physiology of NCX-9

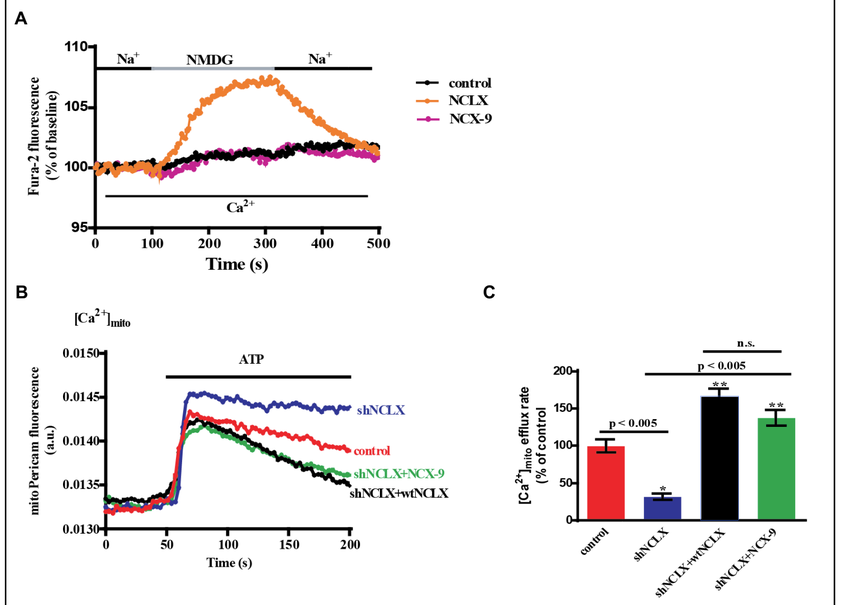


Figure 5: NCX-9 localizes to the mitochondria *in vitro* and facilitates Na^+/Ca^{2+} exchange. (*p<0.005 vs control and **p<0.005 vs shNCLX)

Conclusion

Mutant characterization of *ncx-9* represents the first description of a circuit development defect by a sodium calcium exchanger. Regulators of calcium homeostasis during neuronal development are still largely unknown and members of the sodium calcium exchanger family are exciting candidates for this function. We also show a novel role for Netrin signaling proteins in asymmetric circuit patterning and provide a mechanistic framework for how commissures within a single circuit are differentially patterned on a left/right basis. Finally, we also find that similar to its mammalian orthologue (NCLX), NCX-9 functions at the mitochondrion to exchange calcium, suggesting that mitochondria may play an important role in regulating calcium signaling during circuit patterning.

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