Investigating Anesthetic Neurotoxicity in the Nematode: worm movement and mitochondrial mutant effects

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INTRODUCTION

Anesthetic neurotoxicity has primarily been studied in rodents and non-human primates. In our laboratory, we have developed a model of anesthetic neurotoxicity in the nematode C. elegans. We have shown that anesthetic exposure during the first larval stage is associated with a neurobehavioral defect in adult worms, namely, that these exposed nematodes have difficulty with chemotaxis towards food. This defect was prevented by a mutation that blocked apoptosis from occurring, suggesting that the mechanism of anesthetic neurotoxicity in nematodes involves the apoptotic cascade, as in higher order species, and is thus highly conserved (1). Recently, we have refined the chemotaxis assay to produce tighter results; we have also developed a second end point using worm tracking software that shows an effect of anesthetic exposure on speed of worm movement. We are now screening C. elegans mutants for alterations in the neurotoxic response to movement. We are now screening C. elegans mutants for alterations in the neurotoxic response to movement. This figure suggests the defect may be in part due to a delay in worm movement and mitochondrial mutant effects.

C. elegans strains. Wild type N2, sod-2, and clk-1 mutants were obtained from the Caenorhabditis Genetics Center (CGC, Minneapolis, MN). Animals were grown on agar plates spread with OP50 E. coli and maintained at 20°C.

Synchrotron and Anesthetic Protocol: Adult nematodes were synchronized for three hours at 20 degrees and the eggs incubated overnight at 15 degrees. After hatching, L1 worms were anesthetized with ~7% isoflurane for four hours at 20 degrees. Anesthetic concentration was confirmed using gas chromatography. Control worms were incubated at 15 degrees during the anesthetic period. Worms were then incubated at 20 degrees until they reached young adulthood, at which point they were subjected to either chemotaxis or movement assays.

Chemotaxis Assays: In chemotaxis assays, the number of worms moving toward an attractant is compared to the number that go elsewhere on the plate. Adult worms were washed onto a plate containing one spot of an attractant (E. coli OP50). At 30 minute intervals, the chemotaxis index (# at attractant/total) was calculated. This protocol was adapted from Bargmann (3).

Movement Assays: For movement analysis, 60-second movies are taken of young adult worms moving on a standard NGM plate spread with OP50. An average speed of all the worms on the plate is calculated (mm/sec, Worm Tracker 2.0).

RESULTS

Fig. 1: Chemotaxis deficits caused by early anesthetic exposure

Fig. 2: Adult movement is slowed after a larval anesthetic exposure

Fig. 3: Mitochondrial mutants are susceptible to neurotoxicity

Mitochondrial Mutants:

sod-2 encodes an iron/manganese superoxide dismutase, predicted to be mitochondrial, that might defend against oxidative stress and promote normal lifespan.

clk-1 encodes a highly conserved demethoxyuniquinone hydroxylase necessary for ubiquinone biosynthesis. It is required for normal growth and development in C. elegans. (2)

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REFERENCES

2. Wombles B. www.wormbase.org