

BOS08
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 Room 144C

Anesthetics Interfere with Axon Guidance and Growth Cone Function in Developing Neocortical Neurons via a GABAA Receptor Mechanism

Cyrus D. Mintz, M.D., Ph.D., Kendall M. Barrett, Student, Sarah C. Smith, M.D., Deanna L. Benson, Ph.D., Neil L. Harrison, Ph.D.
 Columbia University Medical Center, New York, New York, United States

Introduction: Exposure to general anesthetics (GAs) in childhood may increase rates of learning disabilities (1). Previous work has focused on the potential for GAs to enhance neuronal apoptosis during development (2), but little attention has been directed towards the possibility that deficits may arise from dysfunction of the surviving neurons. Brain function is critically dependent on circuit formation, a complex process in which the growth cones of developing axons follow chemotropic guidance cues to the appropriate dendritic targets.

Methods: GA exposures were conducted in dissociated developing neocortical neurons in culture and in early postnatal neocortical slices overlaid with GFP labeled dissociated neurons. Axon targeting, growth cone collapse, and axon branching were measured using immunocytochemistry and quantitative fluorescence microscopy analyzed via NeuroLucida software.

Results: Axons projected from neurons overlaid on an early post-natal brain slice normally take a ventral trajectory under the influence of the repulsive guidance cue, Semaphorin 3A (Sema3A (A: example tracing of control axons on a single slice, C: summary of control axon trajectories). Eight hours of 1.2% isoflurane treatment results in almost randomly oriented trajectories, indicating a complete loss of axon guidance (B, D). Sema3A induces a switch from an extended (E) to a collapsed (F) morphology in axonal growth cones (AGCs), which can be measured in dissociated culture as an increase from 30-40% collapse at baseline to 60-70% collapsed after 20 minutes of Sema3A treatment (compare "Baseline" and "Con" in G-I). A one hour exposure to 2.4% isoflurane blocks Sema3A induced collapse (G). This effect is dose-dependent between 0.6% and 2.4% isoflurane (not shown), and it is restored after a three hour wash and recovery period (G), indicating that it is reversible. Similar results are obtained with propofol, midazolam, and thiopental, but not with fentanyl or dexmedetomidine (not shown). A nearly identical loss of the Sema3A collapse response is obtained with the GABA_A agonist Muscimol (Mus), and collapse is restored by co-treatment with Picrotoxin (PTX), a GABA_A channel blocker (H). Isoflurane also blocks collapse of AGCs grown on laminin and treated with Netrin-1, a guidance cue that employs receptors and signaling pathways distinct from Sema3A, and this effect is reversed with co-administration of PTX (I). Interestingly, treatment with isoflurane does not interfere with the formation of new axonal branches (examples in J-L, summary in M), an action of Netrin-1 that is distinct from its guidance function at the growth cone.

Conclusion: These data demonstrate that GAs with activity at GABA_A receptors interfere with axon guidance via inhibition of repulsive activity of guidance cues at the axonal growth cone. The finding that GAs interfere with axon guidance suggests that exposure to GAs during brain development may disrupt brain circuit formation, and thus it represents a novel form of anesthesia neurotoxicity relevant to pediatric and prenatal exposure.

1.

Flick RP et al., *Pediatrics* (2011), 128(5):1053-61

2.

Stratmann G, *Anesth Analg* (2011), 113(5):1170-9.

Figure 1