The Effect of Isoflurane on the Developing Brain: a Function of Age

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Introduction: In an *in vivo* postnatal day 7 (PND) rat pup model isoflurane elicited widespread neurodegeneration. (1) Using organotypic hippocampal slices, thus eliminating the physiologic variables inherent in an *in vivo* model, we studied the effect of isoflurane on neuronal cell death in rat pups of differing postnatal age. *We hypothesized that there are age related differences in isoflurane's effect on neurodegeneration in the developing brain.*

Methods: Organotypic hippocampal slices were prepared from Sprague-Dawley rat pups (PND 4,7,14 and 21) as described by Stoppini et al. with some modification and maintained in culture for 7-14 days. Isoflurane (1.5%) was administered using an agent specific vaporizer with air (21% O_2, 5% CO_2 and 70% N_2) for 1, 3 or 5 hrs. All conditions were maintained within 37 +/- 3 ^{0}C. Control conditions were exposed to fresh gas flow as per above in the absence of isoflurane. Neuronal cell death was assessed 1 and 3 days after exposure to isoflurane using Sytox staining and expressed as mean optical density.

Results: In slices from PND 4 and 14 pups, 10% of CA1 neurons (relative to controls exposed to air) were lost after a 5 hr exposure to 1.5% isoflurane (p= 0.08). The greatest effect on cell death was noted in PND 7 slices (>50% neuronal cell loss), exposed to 1.5% isoflurane for 5 hours (p <0.001). This effect was *absent*, however, in slices prepared from PND 21 pups otherwise treated the same (p=0.87). This age related effect of isoflurane on neuronal degeneration was *not* noted with exposure times less than 5 hours. *(Figure 1)*

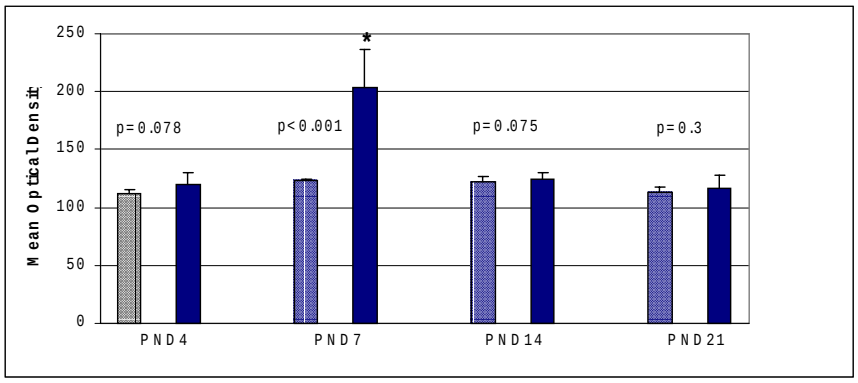

Figure 1: *Hippocampal slices from postnatal day 4,7,14 and 21. Rat pups were exposed to 1.5% isoflurane (solid) for 5 hrs. Data below show an age related effect on cell death. Control (stippled) represents exposure to fresh gas alone. Data are displayed as mean optical density +/- SD. * denotes statistical significance p<0.05*

Discussion: Neurogenesis in the brain during ontogeny, as distinguished from the mature state, may either diminish or enhance or enhance its vulnerability to anesthetic exposure. This is likely attributable to developmental differences in $GABA_A$ and NMDA receptor subunit composition.(2,3) Thus, the effect of 1.5% isoflurane on neuronal cell death seems dependent on the postnatal age of the rat pup used and may be related to the relative developmental differences in NMDA and $GABA_A$ receptor subtypes that occur during normal brain development. For example, agonism of the GABAA receptor causes depolarization in *immature* (PND < 21) rat brain, but inhibition in *mature* rat brain. (3) Similarly, agonism of the NMDA receptor causes a minimal depolarization response at PND 0 but normal adult function by PND 21.(2)As a result, conclusions drawn form adult brain brain regarding responses to anesthetics may be different from responses of developing neural tissue.

Conclusion: Isoflurane (1.5%), both an NMDA antagonist and $GABA_A$ agonist, induces neurodegeneration in the developing rat brain that is dependent on the postnatal age of the rat pup studied. This effect is hypothesized to be a function of the relative $GABA_A$ and/or NMDA receptor subunit expression that occurs during the ontogenetic development of the brain.

References:

1. Early exposure to common anesthetics causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. 2003. J Neuroscience, 23:876-882.
2. Ontogeny of the N-methyl-d-aspartate (NMDA) receptor system and susceptibility to neurotoxicity. 2002. Toxic Sci 68: 9-17.
3. Neuronal activity regulates $GABA_A$ receptor subunit expression in organotypic hippocampal slice cultures. 2003. Neuroscience 118: 967-974.