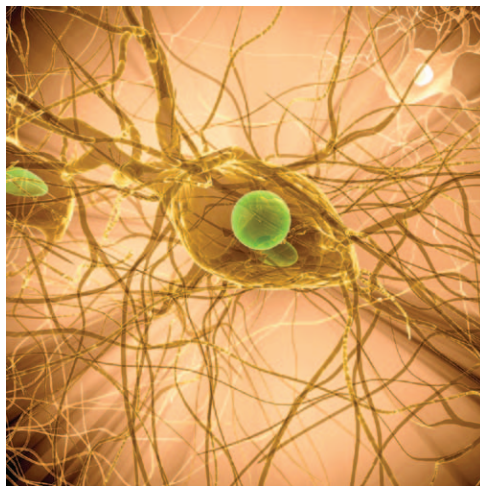


Dual Effects of Isoflurane on Neuronal Proliferation/Differentiation

A Substrate to Impaired Cognitive Function?

IS it conceivable to reconcile the view that opposite neurobehavioral effects (*i.e.*, neurotoxic *vs.* neuroprotective) of a single anesthetic drug may be accounted for by a differential regulation of a common cellular signaling? The study by Zhao *et al.*¹ supports this hypothesis. Impaired cognitive function after anesthesia and surgery represents a very important, still poorly understood, phenomenon occurring in surgical patients. Whether exposure to anesthetics *per se* contributes to impaired postoperative cognitive function remains a matter of debate. A tremendous amount of experimental work has shown that volatile anesthetics exhibit potent neuroprotective effects against ischemic/anoxic injury. The extrapolation of these findings to the clinical setting, has remained, however, disappointing. Since the initial demonstration that exposure to volatile anesthetics can induce widespread apoptosis and neuronal degeneration in the developing brain,² a large body of recent work has shown the susceptibility to anesthetic-induced neurotoxicity of both the developing and aging brain in experimental models. The mechanisms of these effects remain, however, poorly understood. Isoflurane causes cognitive hippocampal-dependent cognitive deficits in rodents, but the molecular mechanisms of these effects are unclear. Neuronal apoptosis, a major physiological process leading to delayed cell death, is certainly a major mechanism responsible for the progression of injury after a brain damage. Inhaled anesthetics have been shown to exhibit both pro- and antiapoptotic effects that are independent of γ -aminobutyric acid A receptor activation.³ However, less attention has been paid to the effects of volatile anesthetics on neurogenesis, and their possible contribution to anesthetic-induced cognitive deficits. For example, there are some clues suggesting that hippocampal neurogenesis is



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important for learning and memory formation in the adult.⁴ In adult rats, selective blockade of neurogenesis impairs long-term retention memory.⁵ It is therefore conceivable that neurogenesis may be a target for volatile anesthetic-induced neurotoxicity. Noteworthy, only a few experimental studies with often controversial results have tested this hypothesis.

In this issue of ANESTHESIOLOGY, Zhao *et al.* provide illuminating data highlighting the dose- and/or time-dependent effects of volatile anesthetics on neurogenesis.¹ Using an immortalized human neural progenitor cell line (ReNcell CX cells), the authors demonstrated for the first time a dual effect of isoflurane, affording enhanced proliferation of neural cells at a low concentration (0.6%), no effect at a clinically relevant concentration (1.2%), and decreased proliferation at a 2.4% concentration. They were also able to show that neuronal cell damage and glial proliferation induced by isoflurane depend both on the concentration and duration of anesthetic exposure. Further, they showed that the differential (neuroprotective *vs.* neurotoxic) effects depending on the concentration and duration of exposure to isoflurane correlated with a differential regulation of intracellular calcium concentrations. Pretreatment with inositol triphosphate or ryanodine receptor antagonists mostly prevented isoflurane-mediated effects on survival, proliferation, and differentiation. The authors also found that preconditioning the progenitor cell cultures with a short exposure to isoflurane markedly attenuated cell damage induced by prolonged exposure to isoflurane. These cultures exhibited less isoflurane-evoked changes in calcium concentrations than controls. Taken together, these results confirm and extend the dual, dose- and time-dependent effect of isoflurane exposure on neural tissue, and suggest a pivotal role

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played by a differential regulation of intracellular calcium in these effects.

Does this mean that we now have a unique, universal, explanation that both reconciles the neuroprotective and neurotoxic effects of inhaled anesthetics on the brain and accounts for anesthetic-induced impairment in cognitive function? Certainly not. A number of limiting factors to the findings by Zhao *et al.* findings have to be highlighted. First, the results obtained from an immortalized neuronal progenitor cell line may not necessarily be extrapolable to normal human neurons, given the paucity of data on the biochemical and electrophysiological characterization of these cells. In agreement with Zhao *et al.*'s findings, other recent studies using cultured neural stem cells found inhibition of cell proliferation at high (1.4–3.7%) isoflurane concentrations and no cytotoxic effect when isoflurane was administered for 4–6 h.^{6,7} In contrast to the study by Zhao *et al.*'s, however, there was no effect on proliferation at a low isoflurane concentration (0.7%). Stratmann *et al.* found that one minimum-alveolar concentration isoflurane for 4 h did not affect brain cell death, hippocampal neurogenesis, or long-term cognitive outcome in aged rats.⁸ Conversely, isoflurane caused progressive memory impairment associated with loss of neural stem cells and reduced neurogenesis in postnatal day 14, but not adult, rats and mice.⁹ Therefore, the effect of isoflurane on neurogenesis may be more relevant to the immature than the mature brain. It is also tempting to speculate that using isoflurane concentrations at a low concentration range should not affect neuronal viability. Second, isoflurane is no longer the first-choice inhaled anesthetic in the modern era of our practice. From a clinical point of view, it would have probably been more relevant to have examined either sevoflurane or desflurane effects on the neurogenesis process. Third, Zhao *et al.* related their findings to an effect of isoflurane on the inositol 1,4,5-triphosphate receptor (InsP3R) and ryanodine receptor. These receptors are located on the endoplasmic reticulum of the cell and are the two main receptors involved in calcium (Ca²⁺) movement within the cell. Ca²⁺ signaling is essential for almost every cellular activity, and disruption of intracellular Ca²⁺ homeostasis can induce neuroapoptosis. In addition to Ca²⁺ regulation, there is a clear role of InsP3 in brain development. Deficiency of type 1 InsP3 in mouse, which is widely expressed in brain and spinal tissue, is associated with abnormal development of the central nervous system, and dysfunction of several electrophysiologic processes necessary for motor learning and synaptic plasticity.¹⁰ Interestingly, isoflurane reduced cell death and apoptosis in hypoxic neurons through Ca²⁺- and InsP3 receptor-dependent mechanisms.¹¹ Although the effect of isoflurane on Ca²⁺ release has been clearly demonstrated in this study, it is not possible to make a causal link between Ca²⁺ regulation and isoflurane-mediated effects on survival, proliferation, and differentiation of neural stem cells. Ca²⁺ release is the

final mechanism of a large number of metabolic pathways in the cell. Activation of various cell-surface receptors may cause the release of Ca²⁺ from endogenous stores. The antagonists used in this study are not 100% selective and it cannot be ruled out that other metabolic pathways not explored in this study may contribute as well to the reported effects on cell differentiation or proliferation. For example, stimulation of γ -aminobutyric acid A receptors induces Ca²⁺ release and might account for some of the reported effects.¹² The link between γ -aminobutyric acid A receptor activation and neuroprotective or neurotoxic effects has not been properly investigated in Zhao's study.

Zhao *et al.* have to be commended for having shown so nicely in their model that both concentration and duration of administration of an inhaled anesthetic have direct influence on the balance between neuroprotective and neurotoxic effects on neural cells. The knowledge gap between the present findings and impaired cognitive function related to anesthesia remains to be explored. However, based on these data, anesthesiologists should keep in mind the (at least) theoretical hazards for the immature brain of high concentrations of inhaled anesthetics delivered during prolonged periods of time.

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References

1. Zhao X, Yang Z, Liang G, Wu Z, Peng Y, Joseph DJ, Inan S, Wei H: Dual effects of isoflurane on proliferation, differentiation, and survival in human neuroprogenitor cells. *ANESTHESIOLOGY* 2013; 118:537–49
2. Jevtovic-Todorovic V, Hartman RE, Izumi Y, Benshoff ND, Dikranian K, Zorumski CF, Olney JW, Wozniak DF: Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. *J Neurosci* 2003; 23:876–82
3. Sanders RD, Xu J, Shu Y, Januszewski A, Halder S, Fidalgo A, Sun P, Hossain M, Ma D, Maze M: Dexmedetomidine attenuates isoflurane-induced neurocognitive impairment in neonatal rats. *ANESTHESIOLOGY* 2009; 110:1077–85
4. Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E: Neurogenesis in the adult is involved in the formation of trace memories. *Nature* 2001; 410:372–6
5. Jessberger S, Clark RE, Broadbent NJ, Clemenson GD Jr, Consiglio A, Lie DC, Squire LR, Gage FH: Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. *Learn Mem* 2009; 16:147–54
6. Sall JW, Stratmann G, Leong J, McKleroy W, Mason D, Shenoy S, Pleasure SJ, Bickler PE: Isoflurane inhibits growth but does not cause cell death in hippocampal neural precursor cells grown in culture. *ANESTHESIOLOGY* 2009; 110:826–33
7. Culley DJ, Boyd JD, Palanisamy A, Xie Z, Kojima K, Vacanti CA, Tanzi RE, Crosby G: Isoflurane decreases self-renewal capacity of rat cultured neural stem cells. *ANESTHESIOLOGY* 2011; 115:754–63

8. Stratmann G, Sall JW, Bell JS, Alvi RS, May Ld, Ku B, Dowlatshahi M, Dai R, Bickler PE, Russell I, Lee MT, Hrubos MW, Chiu C: Isoflurane does not affect brain cell death, hippocampal neurogenesis, or long-term neurocognitive outcome in aged rats. *ANESTHESIOLOGY* 2010; 112:305–15
9. Zhu C, Gao J, Karlsson N, Li Q, Zhang Y, Huang Z, Li H, Kuhn HG, Blomgren K: Isoflurane anesthesia induced persistent, progressive memory impairment, caused a loss of neural stem cells, and reduced neurogenesis in young, but not adult, rodents. *J Cereb Blood Flow Metab* 2010; 30:1017–30
10. Mikoshiba K: Role of IP3 receptor in development. *Cell Calcium* 2011; 49:331–40
11. Bickler PE, Fahlman CS: The inhaled anesthetic, isoflurane, enhances Ca²⁺-dependent survival signaling in cortical neurons and modulates MAP kinases, apoptosis proteins and transcription factors during hypoxia. *Anesth Analg* 2006; 103:419–29
12. Zhao YL, Xiang Q, Shi QY, Li SY, Tan L, Wang JT, Jin XG, Luo AL: GABAergic excitotoxicity injury of the immature hippocampal pyramidal neurons' exposure to isoflurane. *Anesth Analg* 2011; 113:1152–60