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Dual effects of neuroprotection and neurotoxicity by general anesthetics: Role of intracellular calcium homeostasis

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Abstract

Although general anesthetics have long been considered neuroprotective, there are growing concerns about neurotoxicity. Preclinical studies clearly demonstrated that commonly used general anesthetics are both neuroprotective and neurotoxic, with unclear mechanisms. Recent studies suggest that differential activation of inositol 1,4,5-trisphosphate receptors, a calcium release channel located on the membrane of endoplasmic reticulum (ER), play important role on determining the fate of neuroprotection or neurotoxicity by general anesthetics. General anesthetics at low concentrations for short duration are sublethal stress factors which induce endogenous neuroprotective mechanisms and provide neuroprotection via adequate activation of InsP_3R and moderate calcium release from ER. On the other hand, general anesthetics at high concentrations for prolonged duration are lethal stress factors which induce neuronal damage by over activation of InsP_3R and excessive and abnormal Ca^{2+} release from ER. This review emphasizes the dual effects of both neuroprotection and neurotoxicity via differential regulation of intracellular Ca^{2+} homeostasis by commonly used general anesthetics and recommends strategy to maximize neuroprotective but minimize neurotoxic effects of general anesthetics.

Ca^{2+} is an important second messenger in cell growth and differentiation, membrane excitability, exocytosis, synaptic activity, apoptosis and autophagy¹⁻³. Cytosolic Ca^{2+} concentrations in neurons are usually maintained around 100 nM, 10,000-fold lower than extracellular space, or about at least 1000-folds lower than inside lumen of the endoplasmic reticulum (ER), a primary intracellular Ca^{2+} store in various types of neurons^{1,4}. Cytosolic Ca^{2+} concentrations can be increased by Ca^{2+} influx through different calcium channels, such as NMDA and other glutamate receptors or L-type voltage dependent Ca^{2+} channels (VDCC), or by Ca^{2+} release from the ER via either inositol-1,4,5-trisphosphate receptors (InsP_3R , Figure 1)⁵ or ryanodine receptors (RYRs) Ca^{2+} channels⁴ located on the ER membrane. Activation of InsP_3Rs by both InsP_3 (which is generated through the phospholipase C pathway and RYRs triggers Ca^{2+} release from the ER). Another important organelle in the regulation of intracellular Ca^{2+} concentration is the mitochondria⁶. The mitochondrial ATP-sensitive K (mitoK_{ATP}) channels and mitochondrial permeability transition pores (mPTP) are essential for Ca^{2+} regulation, apoptosis, and cell death.

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Role of Ca²⁺ in anesthetics-mediated neuroprotection

A brief subclinical application of anesthetics just before or after neurotoxic stimuli increases tolerance and decreases neuronal cell damage in various models of tissue cultures and animal studies. General anesthetics are protective against cell damage and cell death induced by glutamate, hypoxia, ischemia and reperfusion as well as oxygen-glucose deprivation (OGD) in cell culture and in animal studies. For example, isoflurane, sevoflurane and halothane in clinically used concentrations reduce lactate dehydrogenase (LDH) release (informs about plasma membrane integrity) and Ca²⁺ uptake in primary cortical neuronal-glial cells exposed to NMDA or glutamate⁷. Short term application of isoflurane decreases cell death in rat hippocampal slices exposed to OGD⁸ and increases survival rates of Purkinje neurons in rat cerebella slices exposed to glutamate⁹.

An adequate Ca²⁺ release from ER via InsP₃R and moderate increase in Ca²⁺ concentration in cytosolic space is thought to be the mechanism of hypoxia and isoflurane preconditioning-induced cytoprotection (Figure 1, left side). Gray et al.¹⁰ proposed that moderate increases in cytosolic Ca²⁺ concentration in the presence of isoflurane stimulates mitogen-activated protein kinase (MAPK) signaling cascade, including: extracellular signal-regulated kinases (ERK), c-Jun N-terminal protein kinases (JNK), p38, and protein kinase B (Akt), which are survival-signaling proteins. Isoflurane¹⁰ applied during hypoxia reduced neuronal cell death in rat hippocampal slices. In isoflurane-exposed slices, increased cytosolic Ca²⁺ concentration due to hypoxia was decreased and ERK as well as phosphorylated Akt signaling (pro-apoptotic) were increased. Also, Bickler and Fahlman¹¹ reported a Ca²⁺-dependent increase in survival signaling proteins in mouse cortical neurons by isoflurane during hypoxia. All effects induced by isoflurane were inhibited by Xestospongine C (Xc), an InsP₃R antagonist^{10–12}. Because other inhalational anesthetics (sevoflurane and desflurane) and intravenous anesthetic propofol can also activate InsP₃R^{13–15}, these general anesthetics at low concentrations for short duration may also provide neuroprotection through preconditioning by causing moderate Ca²⁺ release from ER via moderate activation of InsP₃R.

Mitochondria also play an important role in anesthetics mediated neuroprotection. Ca²⁺ released from the ER via InsP₃R can be uptake into mitochondria due to the close contact between two organelles¹⁶. Physiological Ca²⁺ uptake into mitochondria is essential for ATP production¹⁷, thus may protect neurons from damage and provide preconditioning protection (Figure 1, left side). In addition, the mitoK_{ATP} channel stays open during ischemia and reperfusion to conserve mitochondrial ATP production rate and to reduce structural damage and excessive Ca²⁺ increase¹⁸. Korge et al.¹⁹ reported that diazoxide, a selective mitoK_{ATP} channel agonist, reduces mitochondrial injury by inhibiting the motivating force for Ca²⁺ through mPTP and decreasing CytC loss from the inter membrane space. Both effects were blocked in the presence of the selective mitoK_{ATP} antagonist, 5-hydroxydecanoate (5-HD). Both pre-and postconditioning with sevoflurane reduced cerebral infarct size and neurologic deficit score and provided better motor coordination in rats subjected to focal cerebral ischemia^{20;21}. Pretreating rats with 5-HD abolish sevoflurane-induced neuroprotection²¹. These results indicate that sevoflurane provides neuroprotection via opening mitoK_{ATP} channels in rats²¹. Feng and Zuo²² showed that preconditioning with isoflurane also increases endothelial cell tolerance against ischemia partly via mitoK_{ATP} channels.

Similar to its neuroprotective effects in tissue cultures^{10–12}, Zhou et al.²³ studied the neuroprotective effect of isoflurane postconditioning by activation of endogenous AKT neuroprotective pathway on neonatal hypoxic-ischemic brains in rats. At 48 h, isoflurane significantly reduced infarct size and caspase-3 activation and preserved phosphorylated Akt. At 4 weeks, isoflurane attenuated brain atrophy and improved neurologic function.

Glycogen synthase kinase 3 (GSK3 β), which is one of the downstream kinases in the Akt pathway, was found to be involved in neuronal protective effects of short term applications of isoflurane, sevoflurane, or desflurane after OGD in neurons²⁴. All these results from cell and animal studies clearly indicate that cell survival signaling pathways are activated and increases in Ca²⁺ level during excitotoxicity is brought to moderate levels in anesthetic-induced neuroprotection (Figure 1, left side).

General anesthetics may affect memory and learning ability by regulating hippocampal LTP via modulating activation of NMDA receptors. NMDA activation and Ca²⁺ influx into cytosolic space plays important roles on formation of long term potentiation (LTP) in hippocampus and memory and learning ability²⁵. Mice anesthetized with isoflurane presented better cognitive function compared to control animals²⁶. NR2B, a subunit of the NMDA receptor, up-regulation was found in the hippocampus of mice, and pretreatment with NR2B selective antagonists abolished the cognitive function improvement induced by isoflurane. Also, long-term potentiation (LTP) was enhanced in the CA1 region with isoflurane²⁶. Culley et al.²⁷ reported that isoflurane anesthesia improves spatial memory in young rats but causes impairment in aged rats. It seems that age is an influential factor in anesthetic-induced cognitive function changes.

Sevoflurane anesthesia prevented impairment in cognitive and motor function and hippocampal cell damage induced by cerebral ischemia in rats²⁰. Also, postconditioning with sevoflurane induced neuroprotection against cerebral ischemia. It reduced apoptotic cells, infarct size, neurologic deficit score and brain edema; upregulated Bcl-2 and phosphorylated Akt; down-regulated p53 and Bax; and improved spatial learning and memory²⁸. When isoflurane administered to rats 24 h before ischemia, both infarct size and incidence of cell death were decreased. Isoflurane induced an increase in phosphorylated p38 MAPK in the cortex and neuroprotective effects were abolished by pretreating rats with a p38 MAPK inhibitor²⁹. Long term protection is provided by isoflurane in rats^{30;31}. Decrease in infarct volume and improvement in neurologic functions continued 4 weeks after focal cerebral ischemia in rats pretreated with isoflurane, but not with desflurane³¹. Propofol has also been demonstrated to be neuroprotective against neuronal damage induced by multiple insults in various tissue cultures or animal models^{32–34}, although with an unclear mechanism. Our recent study suggest that propofol alone can activate InsP₃R³⁵ and therefore may provide neuroprotection by causing adequate Ca²⁺ release from ER via InsP₃R and moderate elevation of cytosolic Ca²⁺ concentrations, similar to the effects of isoflurane^{10–12} (Figure 1, left side), albeit more studies are needed.

Role of Ca²⁺ in anesthetics-induced neurotoxicity

In the immature neurons in the developing brains, NMDA activation and subsequent Ca²⁺ influx is required for neuronal survival and maturation^{36;37}. Inhibition of NMDA receptors by general anesthetics (e.g. isoflurane, ketamine) may therefore inhibit normal Ca²⁺ influx via NMDA receptors and suppress normal intracellular Ca²⁺ oscillation, resulting in the inhibition of neuronal survival and maturation^{36;38}. Dickinson et al.³⁹ reported that both xenon and isoflurane bind to the glycine site of the NR1 subunit of NMDA receptors. Interestingly, the commonly used sedative drug midazolam also induce neuronal apoptosis on differentiating neurons by suppressing the intracellular calcium oscillation⁴⁰.

Increasing studies suggest that abnormal and excessive calcium release from the ER via either InsP₃R and/or RYR plays important role in anesthesia mediated neurotoxicity (Figure 1, right side). Neurotoxic effects of inhalational anesthetics have been studied in different cell lines and primary neurons^{41–44}. Wei et al.⁴⁴ compared cytotoxic effects of equipotent concentrations of isoflurane and sevoflurane on rat PC 12 pheochromocytoma cells and

primary cortical neurons. Administration of isoflurane (2.4%) for 24 h significantly increased LDH release and decreased MTT levels; however, sevoflurane (4%) for 24 h had no effect on both cell lines. Both caspase 3 and 9 activities (apoptotic) were increased by isoflurane, but not by sevoflurane. Wei et al.⁴⁴ hypothesized that isoflurane induces apoptosis by increasing Ca^{2+} release from the ER through activation of RYRs in these cells. Pretreating cells with dantrolene, a RYR antagonist, significantly decreased isoflurane-induced apoptosis. Xiang et al.⁴⁵ showed that isoflurane enhanced spontaneous Ca^{2+} oscillations dose dependently in developing rat hippocampal neurons. Spontaneous synchronized cytoplasmic Ca^{2+} oscillations play an important role in cell proliferation, neuronal, axonal, and dendritic migration, and glutaminergic synapse maturation⁴⁶. Hence, Ca^{2+} -mediated signal transduction, and neuronal plasticity would be affected in developing brains by general anesthetics, a NMDA receptor antagonist⁴⁷. Also, Xiang et al.⁴⁵ showed that isoflurane-induced changes in Ca^{2+} oscillations are reversed by bicuculline, a GABA_A receptor antagonist.

We investigated the role of InsP_3Rs in general anesthetics-induced cytotoxicity in various tissue cultures including B lymphocyte (DT40) and its total InsP_3R knock-out DT40 cells (DT40 InsP_3R TKO), rat pheochromocytoma PC 12 cells transfected with wild type presenilin-1 (PS1), point mutated PS1 (L286V, a cell model of Alzheimer's disease), Striatal cells wild type, and knock-in of Q111 Huntingtin (a cell model of Huntington disease) and primary cortical neurons^{13;14;42;43;48}. When DT-40 cells were exposed to different concentrations of isoflurane (0.6–2.4%) for 24 h, DT40 TKO cells were resistant to cytotoxic effects, which were detected by annexin V (early cell damage) and propidium iodide (late cell damage) staining, and caspase-3 activity of isoflurane^{13;43}. Additionally, isoflurane increased mitochondrial and decrease ER Ca^{2+} concentrations in wild type cells, but not in TKO cells¹³. On the other hand, neurons with elevated InsP_3R activity by mutated Alzheimer's presenilin-1 mutation in L286V PC12 cells or mutated huntingtin protein in striatum neurons with over expansion of polyglutamine seems to be more sensitive to anesthetics induced Ca^{2+} release from ER and subsequent cell death^{14;43;49}. Consistently, InsP_3R antagonist xestospongine C inhibits abnormal Ca^{2+} release from the ER and cell death induced by various inhalational anesthetics not only in tissue cultures but also in rat developing brains^{14;42;43;49}. Furthermore, our recent studies using single channel patch clamp techniques on nucleus membrane indicate that isoflurane or propofol alone can activate type 1 or 3 InsP_3R , especially in cell with elevated baseline InsP_3R activity such as L286V Alzheimer's cells^{15;35}, providing direct evidence at molecular level that anesthetics may induce cell death by over activation of InsP_3R , especially in neurons features with Alzheimer's disease or Huntington's disease.

It seems that three commonly used inhalational general anesthetics (isoflurane, sevoflurane and desflurane) have different potency to activate InsP_3R and to induce Ca^{2+} release from ER, with isoflurane more potent than sevoflurane or desflurane¹³. Consistently, isoflurane is more potent to induce cell death by apoptosis and neurodegeneration not only various tissue cultures^{14;44;49;50}, but also in rat developing brains⁵¹. Additionally, inhalational general anesthetics may increase mutated huntingtin protein aggregation and aggregate neurodegeneration via activation of InsP_3 receptors in a cell model of Huntington's disease, and that isoflurane is more potent than sevoflurane or desflurane. Previous studies have shown that hypercapnia during general anesthesia may cause neurodegeneration in developing brains^{52;53}. It should be noted isoflurane is also more potent than sevoflurane in providing neuroprotection via preconditioning mechanisms when anesthetics are used at low concentrations for a short time; a sublethal stress factor to neurons⁵⁴. It should be pointed out that there is not sufficient evidence to confirm that isoflurane is more potent than other general anesthetics including propofol to activate InsP_3R and cause calcium release from the

ER and subsequent neuroprotection or neurotoxicity currently. More studies are needed to investigate this phenomenon especially in clinical patients.

Isoflurane is not only an NMDA glutamate receptor antagonist, but also a GABA_A receptor agonist^{7,47}. Recent study suggests that isoflurane can also cause excitotoxicity by abnormally elevating cytosolic Ca²⁺ concentrations via activation of GABA_A receptors in immature neurons⁵⁵. Activation of GABA_A receptor in immature neurons cause chloride efflux rather than influx, resulting in plasma membrane depolarization⁵⁶ and subsequent activation of L type voltage dependent Ca²⁺ channels (VDCC) and Ca²⁺ influx into cytosolic space from extracellular space. In immature hippocampal neurons, isoflurane has been shown to activate GABA_A and then L-type VDCC, causing neuronal excitotoxicity by excessive calcium influx and abnormal elevation of cytosolic Ca²⁺ concentrations^{55,57}. This is an important finding in that not only the downstream mechanisms of anesthetics mediated neurotoxicity after activation of GABA_A in the developing brains has been proposed, but also a possible therapeutic approach by L-type VDCC blockers such as nicardipine or nimodipine may be used to ameliorate anesthetics mediated neurotoxicity.

Ketamine, also an NMDA receptor antagonist like isoflurane, has been shown to induce neurotoxicity in various tissue cultures and animal studies including primates^{38,57-61}. The inhibition of NMDA receptor by ketamine in immature neurons suppresses Ca²⁺ influx and normal intracellular calcium oscillation, which will then impair the normal survival of immature neurons. In our recent study, we also demonstrated that the most commonly used intravenous general anesthetic propofol can activate InsP₃R³⁵. Similar to our previous finding on isoflurane⁴³, our unpublished data also indicates that propofol only induce cell death in DT-40 wild type but not DT-40 with triple knock out of InsP₃R (TKO), suggesting that over activation of InsP₃R plays important roles on propofol-induced neurotoxicity. Overall, activation of GABA_A, inhibition of NMDA receptor and over activation of InsP₃R and/or RYRs all can cause cell death in immature neurons by disruption of intracellular calcium homeostasis.

Besides effects of general anesthetics to induce neuronal apoptosis via disruption of intracellular calcium homeostasis, many other mechanisms have been proposed, such as interaction with NMDA receptors or alpha 2 adrenoceptor receptor etc.⁶²⁻⁶⁴. It will be interesting to know if other mechanisms will eventually use calcium regulation as a downstream pathway to exert the proapoptotic effects.

Although many studies have consistently demonstrated that general anesthetics, especially isoflurane, induce neuronal apoptosis in the developing brains in rodents or monkeys as discussed above, it should be pointed out that these studies cannot be directly used to draw suggestion in human considering the frequently observed disturbance in physiological status, short life span in rodent or other animal species. It is important and urgent to have well-designed clinical studies for confirmation of possible neurotoxic effects of general anesthetics in human developing brains. On the other hands, the patients almost always receive surgeries when they received general anesthesia, while these surgical stimulation may have significant effects on neuronal apoptosis in the developing brain induced by general anesthetics^{65,66}.

Although recent study suggest a link between anesthetic exposure and impairment of memory and learning ability in pediatric patients^{67,68}, it is far from a conclusion if general anesthetics may harm brain in humans. Lack of established biomarkers with high sensitivity and specificity for neurodegeneration is an obvious hurdle to the study of anesthetic-induced neurodegeneration in human. Our recent studies in the rodent developing brains suggest that S100 may be a sensitive and effective biomarker to detect anesthesia mediated brain

damage^{51;69}. S100 is an intracellular calcium binding protein and expressed and secreted by astrocytes in the vertebrate brain. Increased level of S100 in serum and cerebrospinal fluid (CSF) is one of the biochemical responses of glia to acute and chronic conditions of brain damage⁷⁰. It is not clear if serum S100 can be used a neurodegenerative biomarker to detect anesthetics mediated neurodegeneration in human brains and more studies are needed.

Role of differential activation of InsP₃R in anesthetics mediated dual effects of neuroprotection and neurotoxicity

General anesthetics have been shown to be both neuroprotective and neurotoxic, but the mechanisms are unclear. Particularly in our recent studies, 2.4% isoflurane for short duration (1 hr) did not induce cell damage by itself but nearly abolished the neuronal damage induced by prolonged exposure of 2.4% isoflurane for 24 hr in different types of neurons⁵⁴.

Similarly, 1.5% isoflurane for 0.5 hr did not cause neurodegeneration in 7-day-old rat developing brains, but significantly inhibited neuronal apoptosis induced by 1.5% isoflurane for 6 hr⁷¹. Furthermore, our recent study⁷² also demonstrated that isoflurane induce cell damage dose- and time-dependently via over activation of InsP₃R and/or RYR in ReNcell CX human neural stem cells. Short exposure of isoflurane did not cause neuronal damage by itself but abolished neuronal damage induced by prolonged exposure of isoflurane at same concentration. Also, it seems that isoflurane exert both its neuroprotective and neurotoxic effects via activation of InsP₃R and/or RYR at different degrees. In addition, isoflurane at low concentration for short duration stimulate but at high concentration for long duration inhibits human stem cells proliferation via activation of InsP₃R and/or RYR at different degrees. Similarly, isoflurane for short duration did not affect human stem cells differentiation into neurons, but isoflurane for prolonged duration cause significantly inhibition of stem cell differentiation via over activation of InsP₃R and/or RYR. Therefore, differential activation of InsP₃R and/or RYR by general anesthetics at different concentrations for various durations plays important roles on anesthetics mediated dual effects of pro and anti-survival, stem cell proliferations and differentiation. In 7-day-old newborn developing brains, 1.5% isoflurane for short duration (0.5 hr) did not induce brain damage but significantly inhibited brain infarction induced by hypoxia and cerebral ischemia⁷³, while 1.5% isoflurane for long duration (6 hr) induces widespread neuroapoptosis in different brain regions³⁶, suggesting duration of anesthetics plays important roles in anesthetics mediated neuroprotection or neurotoxicity. Maternal exposure of 1.3% isoflurane (6 hr) during gestational day 21 inhibit neuroapoptosis in fetal developing brains⁷⁴. However when isoflurane concentration is increased to 3% (1 h) in gestational day 21, it induced significant neuroapoptosis in both hippocampus and cortex of fetus developing brains, suggesting that anesthetic concentrations also play important roles in determining anesthetics mediated neuroprotection or neurotoxicity⁶⁹.

Based on preclinical studies involving various tissue cultures and animal studies from ours and others discussed above^{10–14;35;36;43;49;54;71;73;74}, we have hypothesized that moderate activation of InsP₃R by general anesthetics at low concentration for short duration cause adequate Ca²⁺ release from ER and moderate elevation of cytosolic Ca²⁺ concentrations, which in turn activate endogenous neuroprotective pathways, such as AKT pathway^{10–12} and increased ATP production¹⁷ (Figure 1, Left side). On the other hand, General anesthetics at high concentrations for prolonged use will over activate InsP₃R and cause excessive and abnormal Ca²⁺ release from ER, depletion of ER Ca²⁺ and abnormal elevation of cytosolic Ca²⁺ concentrations. All of which may cause cell death by apoptosis via intrinsic pathway, activation of Ca²⁺ dependent enzyme such as calpain and inhibition of protein synthesis and trafficking (Figure 1, Right side)⁷⁵. Obviously, this hypothesis needs to be validated in future clinical studies.

It should be noted that general anesthetics may exert their dual effects of neuroprotection and neurotoxicity not solely by its regulatory effects on intracellular calcium homeostasis via differential activation of InsP₃R^{13;14;42;49;54;72}. Other mechanisms may contribute their dual effects of neuroprotection and neurotoxicity, such as through differential regulation of Bcl-2/Bax ratio⁷⁶ and the aspartyl protease beta-site amyloid precursor protein-cleaved enzyme⁶⁵. Regardless of mechanisms, it is important to emphasize the idea that general anesthetics may be both neuroprotective and neurotoxic, depending on the dose and duration used.

Conclusions

General anesthetics can be both neuroprotective and neurotoxic through differential activation of InsP₃R and Ca²⁺ release from the ER. General anesthetics at low concentrations for short duration, like short time cerebral ischemia, are sublethal stress factors, which will not cause neuronal death but induce endogenous neuroprotective mechanisms and provide neuroprotection via adequate activation of InsP₃R, moderate Ca²⁺ release from ER (Figure 1, Left side). On the other hand, general anesthetics at high concentrations for prolonged duration, like prolonged cerebral ischemia, become lethal stress factors, which induce neuronal damage by over activation of InsP₃R and excessive and abnormal calcium release from ER (Figure 1, right side). It is important to figure out the adequate concentrations and duration of general anesthetics, so that their beneficial effects can be maximized and harmful effects can be minimized, especially in human patients. Future studies are urgently needed to search for biomarkers for neurodegeneration in CNS with high sensitivity and specificity, so that the possible neurotoxicity of general anesthetics can be confirmed in pediatric patients. Also, the methods or agents, such as those that ameliorate the anesthetics mediated calcium dysregulation, can be developed to minimize the possible toxic effects of general anesthetics in the developing brain.

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References

1. Orrenius S, Zhivotovsky B, Nicotera P. Regulation of cell death: The calcium-apoptosis link. *Nature Reviews Molecular Cell Biology*. 2003; 4:552–565.
2. Hajnoczky G, Davies E, Madesh M. Calcium signaling and apoptosis. [Review] [93 refs]. *Biochemical & Biophysical Research Communications*. 2003; 304(3):445–454. [PubMed: 12729578]
3. Decuyper JP, Monaco G, Bultynck G, Missiaen L, De SH, Parys JB. The IP(3) receptor-mitochondria connection in apoptosis and autophagy. *Biochimica et Biophysica Acta*. 2011; 1813:1003–1013. [PubMed: 21146562]
4. Inan S, Wei H. The cytoprotective effects of dantrolene: a ryanodine receptor antagonist. *Anesthesia and Analgesia*. 2010; 111:1400–1410. [PubMed: 20861418]
5. Berridge MJ. Inositol trisphosphate and calcium signalling. *Nature*. 1993; 361:315–325. [PubMed: 8381210]
6. Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. *Science*. 2004; 305:626–629. [PubMed: 15286356]

7. Kudo M, Aono M, Lee Y, Massey G, Pearlstein RD, Warner DS. Effects of volatile anesthetics on N-methyl-D-aspartate excitotoxicity in primary rat neuronal-glial cultures. *Anesthesiology*. 2001; 95:756–765. [PubMed: 11575551]
8. Popovic R, Liniger R, Bickler PE. Anesthetics and mild hypothermia similarly prevent hippocampal neuron death in an in vitro model of cerebral ischemia. *Anesthesiology*. 2000; 92:1343–1349. [PubMed: 10781280]
9. Zheng S, Zuo Z. Isoflurane preconditioning decreases glutamate receptor overactivation-induced Purkinje neuronal injury in rat cerebellar slices. *Brain Research*. 2005; 1054(2):143–151. [PubMed: 16081051]
10. Gray JJ, Bickler PE, Fahlman CS, Zhan X, Schuyler JA. Isoflurane neuroprotection in hypoxic hippocampal slice cultures involves increases in intracellular Ca²⁺ and mitogen-activated protein kinases. *Anesthesiology*. 2005; 102(3):606–615. [PubMed: 15731600]
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; 103:419–429. [PubMed: 16861427]
12. Bickler PE, Zhan X, Fahlman CS. Isoflurane preconditions hippocampal neurons against oxygen-glucose deprivation: role of intracellular Ca²⁺ and mitogen-activated protein kinase signaling. *Anesthesiology*. 2005; 103:532–539. [PubMed: 16129978]
13. Yang H, Liang G, Hawkins BJ, Madesh M, Pierwola A, Wei HF. Inhalational anesthetics induce cell damage by disruption of intracellular calcium homeostasis with different potencies. *Anesthesiology*. 2008; 109:243–250. [PubMed: 18648233]
14. Wang Q, Liang G, Yang H, Wang S, Eckenhoff MF, Wei H. The common inhaled anesthetic isoflurane increases aggregation of huntingtin and alters calcium homeostasis in a cell model of Huntington's disease. *Toxicology and Applied Pharmacology*. 2011; 250:291–298. [PubMed: 21059370]
15. Peng, Y.; Cheung, KH.; Foscett, J.; Li, J.; Wei, H. Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience; 2009. Isoflurane activates inositol 1,4,5-trisphosphate receptors. Program # 818.11/C4. 2009. [2009 Online](#).
16. Rutter GA. Moving Ca²⁺ from the endoplasmic reticulum to mitochondria: is spatial intimacy enough? *Biochemical Society Transactions*. 2006; 34:351–355. [PubMed: 16709159]
17. Cardenas C, Miller RA, Smith I, Bui T, Molgo J, Muller M, Vais H, Cheung KH, Yang J, Parker I, Thompson CB, Birnbaum MJ, Hallows KR, Foscett JK. Essential regulation of cell bioenergetics by constitutive InsP₃ receptor Ca²⁺ transfer to mitochondria. *Cell*. 2010; 142:270–283. [PubMed: 20655468]
18. Tanaka K, Ludwig LM, Kersten JR, Pagel PS, Warltier DC. Mechanisms of cardioprotection by volatile anesthetics. *Anesthesiology*. 2004; 100:707–721. [PubMed: 15108989]
19. Korge P, Honda HM, Weiss JN. Protection of cardiac mitochondria by diazoxide and protein kinase C: implications for ischemic preconditioning. *Proc. Natl. Acad. Sci. U.S.A.* 2002; 99:3312–3317. [PubMed: 11867760]
20. Eberspacher E, Eckel B, Engelhard K, Muller K, Hoffman WE, Blobner M, Werner C. Effects of sevoflurane on cognitive deficit, motor function, and histopathology after cerebral ischemia in rats. *Acta Anaesthesiologica Scandinavica*. 2009; 53:774–782. [PubMed: 19388889]
21. Adamczyk S, Robin E, Simerabet M, Kipnis E, Tavernier B, Vallet B, Bordet R, Lebuffe G. Sevoflurane pre- and post-conditioning protect the brain via the mitochondrial K ATP channel. *British Journal of Anaesthesia*. 2010; 104:191–200. [PubMed: 20086064]
22. Feng J, Zuo Z. Isoflurane preconditioning increases endothelial cell tolerance to in-vitro simulated ischaemia. *Journal of Pharmacy and Pharmacology*. 2011; 63:106–110. [PubMed: 21155822]
23. Zhou Y, Lekic T, Fathali N, Ostrowski RP, Martin RD, Tang J, Zhang JH. Isoflurane posttreatment reduces neonatal hypoxic-ischemic brain injury in rats by the sphingosine-1-phosphate/phosphatidylinositol-3-kinase/Akt pathway. *Stroke*. 2010; 41:1521–1527. [PubMed: 20508187]
24. Lin D, Li G, Zuo Z. Volatile anesthetic post-treatment induces protection via inhibition of glycogen synthase kinase 3 β in human neuron-like cells. *Neuroscience*. 2011; 179:73–79. [PubMed: 21277352]

25. Chakraborty S, Goussakov I, Miller MB, Stutzmann GE. Deviant ryanodine receptor-mediated calcium release resets synaptic homeostasis in presymptomatic 3×Tg-AD mice. *Journal of Neuroscience*. 2009; 29:9458–9470. [PubMed: 19641109]
26. Rammes G, Starker LK, Haseneder R, Berkmann J, Plack A, Zieglgansberger W, Ohl F, Kochs EF, Blobner M. Isoflurane anaesthesia reversibly improves cognitive function and long-term potentiation (LTP) via an up-regulation in NMDA receptor 2B subunit expression. *Neuropharmacology*. 2009; 56:626–636. [PubMed: 19059421]
27. Culley DJ, Baxter M, Yukhananov R, Crosby G. The memory effects of general anesthesia persist for weeks in young and aged rats. *Anesth Analg*. 2003; 96:1004–1009. [PubMed: 12651650]
28. Wang JK, Yu LN, Zhang FJ, Yang MJ, Yu J, Yan M, Chen G. Postconditioning with sevoflurane protects against focal cerebral ischemia and reperfusion injury via PI3K/Akt pathway. *Brain Research*. 2010; 1357:142–151. [PubMed: 20705063]
29. Zheng S, Zuo Z. Isoflurane preconditioning induces neuroprotection against ischemia via activation of P38 mitogen-activated protein kinases. *Molecular Pharmacology*. 2004; 65(5):1172–1180. [PubMed: 15102945]
30. Sakai H, Sheng H, Yates RB, Ishida K, Pearlstein RD, Warner DS. Isoflurane provides long-term protection against focal cerebral ischemia in the rat. *Anesthesiology*. 2007; 106:92–99. [PubMed: 17197850]
31. Li L, Zuo Z. Isoflurane preconditioning improves short-term and long-term neurological outcome after focal brain ischemia in adult rats. *Neuroscience*. 2009; 164:497–506. [PubMed: 19679170]
32. Kawaguchi M, Furuya H, Patel PM. Neuroprotective effects of anesthetic agents. *J. Anesth*. 2005; 19:150–156. [PubMed: 15875133]
33. Cai J, Hu Y, Li W, Li L, Li S, Zhang M, Li Q. The neuroprotective effect of propofol against brain ischemia mediated by the glutamatergic signaling pathway in rats. *Neurochemical Research*. 2011; 36:1724–1731. [PubMed: 21556843]
34. Wu GJ, Chen WF, Hung HC, Jean YH, Sung CS, Chakraborty C, Lee HP, Chen NF, Wen ZH. Effects of propofol on proliferation and anti-apoptosis of neuroblastoma SH-SY5Y cell line: new insights into neuroprotection. *Brain Research*. 2011; 1384:42–50. [PubMed: 21315692]
35. Peng, Y.; Fosket, K.; Vais, H.; Joseph, D.; Liang, G.; Wei, H. Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience; 2011. The general anesthetics facilitate activation of inositol 1,4,5-trisphosphate (InsP3) receptors. 2012. Online
36. 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. *Journal of Neuroscience*. 2003; 23:876–882. [PubMed: 12574416]
37. Ikonomidou C, Bittigau P, Koch C, Genz K, Hoerster F, Felderhoff-Mueser U, Tenkova T, Dikranian K, Olney JW. Neurotransmitters and apoptosis in the developing brain. *Biochemical Pharmacology*. 2001; 62:401–405. [PubMed: 11448448]
38. Sinner B, Friedrich O, Zink W, Zausig Y, Graf BM. The toxic effects of s(+)-ketamine on differentiating neurons in vitro as a consequence of suppressed neuronal Ca²⁺ oscillations. *Anesthesia and Analgesia*. 2011; 113:1161–1169. [PubMed: 21788311]
39. Dickinson R, Peterson BK, Banks P, Simillis C, Martin JC, Valenzuela CA, Maze M, Franks NP. Competitive inhibition at the glycine site of the N-methyl-D-aspartate receptor by the anesthetics xenon and isoflurane: evidence from molecular modeling and electrophysiology. *Anesthesiology*. 2007; 107:756–767. [PubMed: 18073551]
40. Sinner B, Friedrich O, Zausig Y, Bein T, Graf BM. Toxic effects of midazolam on differentiating neurons in vitro as a consequence of suppressed neuronal Ca²⁺-oscillations. *Toxicology*. 2011; 290:96–101. [PubMed: 21920406]
41. Kvolik S, Glavas-Obrovac L, Bares V, Karner I. Effects of inhalation anesthetics halothane, sevoflurane, and isoflurane on human cell lines. *Life Sciences*. 2005; 77:2369–2383. [PubMed: 15993426]
42. Zhao Y, Liang G, Chen Q, Joseph DJ, Meng Q, Eckenhoff RG, Eckenhoff MF, Wei H. Anesthetic-induced neurodegeneration mediated via inositol 1,4,5-trisphosphate receptors. *Journal of Pharmacology and Experimental Therapeutics*. 2010; 333:14–22. [PubMed: 20086058]

43. Wei HF, Liang G, Yang H, Wang QJ, Hawkins B, Madesh M, Wang SP, Eckenhoff RG. The common inhalational anesthetic isoflurane induces apoptosis via activation of inositol 1,4,5-trisphosphate receptors. *Anesthesiology*. 2008; 108:251–260. [PubMed: 18212570]
44. Wei H, Kang B, Wei W, Liang G, Meng QC, Li Y, Eckenhoff RG. Isoflurane and sevoflurane affect cell survival and BCL-2/BAX ratio differently. *Brain Research*. 2005; 1037:139–147. [PubMed: 15777762]
45. Xiang Q, Tan L, Zhao YL, Wang JT, Jin XG, Luo AL. Isoflurane enhances spontaneous Ca(2+) oscillations in developing rat hippocampal neurons in vitro. *Acta Anaesthesiologica Scandinavica*. 2009; 53:765–773. [PubMed: 19388897]
46. Garaschuk O, Hanse E, Konnerth A. Developmental profile and synaptic origin of early network oscillations in the CA1 region of rat neonatal hippocampus. *J. Physiol.* 1998; 507(Pt 1):219–236. [PubMed: 9490842]
47. Franks NP. General anaesthesia: From molecular targets to neuronal pathways of sleep and arousal. *Nature Reviews Neuroscience*. 2008; 9:370–386.
48. Wei H, Xie Z. Anesthesia, calcium homeostasis and Alzheimer's disease. *Curr. Alzheimer Res.* 2009; 6:30–35. [PubMed: 19199872]
49. Liang G, Wang QJ, Li Y, Kang B, Eckenhoff MF, Eckenhoff RG, Wei HF. A presenilin-1 mutation renders neurons vulnerable to isoflurane toxicity. *Anesthesia and Analgesia*. 2008; 106:492–500. [PubMed: 18227305]
50. Slow EJ, van Raamsdonk J, Rogers D, Coleman SH, Graham RK, Deng Y, Oh R, Bissada N, Hossain SM, Yang YZ, Li XJ, Simpson EM, Gutekunst CA, Leavitt BR, Hayden MR. Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. *Human Molecular Genetics*. 2003; 12:1555–1567. [PubMed: 12812983]
51. Liang G, Ward C, Peng J, Zhao Y, Huang B, Wei H. Isoflurane causes greater neurodegeneration than an equivalent exposure of sevoflurane in the developing brain of neonatal mice. *Anesthesiology*. 2010; 112:1325–1334. [PubMed: 20460994]
52. Istaphanous GK, Howard J, Nan X, Hughes EA, Mccann JC, McAuliffe JJ, Danzer SC, Loepke AW. Comparison of the neuroapoptotic properties of equipotent anesthetic concentrations of desflurane, isoflurane, or sevoflurane in neonatal mice. *Anesthesiology*. 2011; 114:578–587. [PubMed: 21293251]
53. Stratmann G, May LDV, Sall JW, Alvi RS, Bell JS, Ormerod BK, Rau V, Hilton JF, Dai R, Lee MT, Visrodia KH, Ku B, Zusmer EJ, Guggenheim J, Firouzian A. Effect of hypercarbia and isoflurane on brain cell death and neurocognitive dysfunction in 7-day-old rats. *Anesthesiology*. 2009; 110:849–861. [PubMed: 19293696]
54. Wei H, Liang G, Yang H. Isoflurane preconditioning inhibited isoflurane-induced neurotoxicity. *Neuroscience Letters*. 2007; 425:59–62. [PubMed: 17723266]
55. 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. *Anesthesia and Analgesia*. 2011; 113:1152–1160. [PubMed: 21918167]
56. Ben Ari Y. Excitatory actions of GABA during development: The nature of the nurture. *Nature Reviews Neuroscience*. 2002; 3:728–739.
457. Wei H. The role of calcium dysregulation in anesthetic-mediated neurotoxicity. *Anesthesia and Analgesia*. 2011; 113:972–974. [PubMed: 22021793]
58. Jevtovic-Todorovic V, Carter LB. The anesthetics nitrous oxide and ketamine are more neurotoxic to old than to young rat brain. *Neurobiology of Disease*. 2005; 26:947–956.
59. Slikker W, Zou XJ, Hotchkiss CE, Divine RL, Sadovova N, Twaddle NC, Doerge DR, Scallet AC, Patterson TA, Hanig JP, Paule MG, Wang C. Ketamine-induced neuronal cell death in the perinatal rhesus monkey. *Toxicological Sciences*. 2007; 98:145–158. [PubMed: 17426105]
60. Soriano SG, Liu Q, Li J, Liu JR, Han XH, Kanter JL, Bajic D, Ibla JC. Ketamine activates cell cycle signaling and apoptosis in the neonatal rat brain. *Anesthesiology*. 2010; 112:1155–1163. [PubMed: 20418696]
61. Takadera T, Ishida A, Ohyashiki T. Ketamine-induced apoptosis in cultured rat cortical neurons. *Toxicology & Applied Pharmacology*. 2006; 210(1–2):100–107. [PubMed: 16307766]

62. Ma D, Williamson P, Januszewski A, Nogaro MC, Hossain M, Ong LP, Shu Y, Franks NP, Maze M. Xenon mitigates isoflurane-induced neuronal apoptosis in the developing rodent brain. *Anesthesiology*. 2007; 106:746–753. [PubMed: 17413912]
63. 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; 110:1077–1085. [PubMed: 19352168]
64. Shu Y, Patel SM, Pac-Soo C, Fidalgo AR, Wan Y, Maze M, Ma D. Xenon pretreatment attenuates anesthetic-induced apoptosis in the developing brain in comparison with nitrous oxide and hypoxia. *Anesthesiology*. 2010; 113:360–368. [PubMed: 20613483]
65. Pan C, Xu Z, Dong Y, Zhang Y, Zhang J, McAuliffe S, Yue Y, Li T, Xie Z. The potential dual effects of anesthetic isoflurane on hypoxia-induced caspase-3 activation and increases in beta-site amyloid precursor protein-cleaving enzyme levels. *Anesthesia and Analgesia*. 2011; 113:145–152. [PubMed: 21519046]
66. Shu Y, Zhou Z, Wan Y, Sanders RD, Li M, Pac-Soo CK, Maze M, Ma D. Nociceptive stimuli enhance anesthetic-induced neuroapoptosis in the rat developing brain. *Neurobiology of Disease*. 2012; 45:743–750. [PubMed: 22075165]
67. Wilder RT, Flick RP, Sprung J, Katusic SK, Barbaresi WJ, Mickelson C, Gleich SJ, Schroeder DR, Weaver AL, Warner DO. Early exposure to anesthesia and learning disabilities in a population-based birth cohort. *Anesthesiology*. 2009; 110:796–804. [PubMed: 19293700]
68. Kalkman CJ, Peelen L, Moons KG, Veenhuizen M, Bruens M, Sinnema G, de Jong TP. Behavior and development in children and age at the time of first anesthetic exposure. *Anesthesiology*. 2009; 110:805–812. [PubMed: 19293699]
69. Wang S, Peretich K, Zhao Y, Liang G, Meng Q, Wei H. Anesthesia-induced neurodegeneration in fetal rat brains. *Pediatric Research*. 2009; 66:435–440. [PubMed: 20016413]
70. Bloomfield SM, McKinney J, Smith L, Brisman J. Reliability of S100B in predicting severity of central nervous system injury. *Neurocritical Care*. 2007; 6:121–138. [PubMed: 17522796]
71. Peng, J.; Inan, S.; Liang, G.; Wei, H. Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience; 2010. Preconditioning with isoflurane prevents prolonged exposure of isoflurane-induced apoptosis in developing rat brains. Program No. 157.6/Q5. 2010 Online. 2010.
72. 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; 118:537–549. [PubMed: 23314167]
73. Zhao P, Zuo Z. Isoflurane preconditioning induces neuroprotection that is inducible nitric oxide synthase-dependent in neonatal rats. *Anesthesiology*. 2004; 101(3):695–703. [PubMed: 15329594]
74. Li Y, Liang G, Wang S, Meng Q, Wang Q, Wei H. Effect of fetal exposure to isoflurane on postnatal memory and learning in rats. *Neuropharmacology*. 2007; 53:942–950. [PubMed: 17959201]
75. Hanson CJ, Bootman MD, Roderick HL. Cell signalling: IP3 receptors channel calcium into cell death. *Current Biology*. 2004; 14:R933–R935. [PubMed: 15530388]
76. Xu Z, Dong Y, Wu X, Zhang J, McAuliffe S, Pan C, Zhang Y, Ichinose F, Yue Y, Xie Z. The potential dual effects of anesthetic isoflurane on Abeta-induced apoptosis. *Curr. Alzheimer Res*. 2011; 8:741–752. [PubMed: 21244349]

Figure 1. Summary of hypothetical pathways involved in neuroprotective and neurotoxic effects of commonly used general anesthetics

The left side of figure represents neuroprotection and the right side represents neurotoxicity. Short term and low concentration of anesthetics (left side) for pre- or post-conditioning induces moderate Ca²⁺ release from endoplasmic reticulum (ER) through InsP₃Rs. Adequate Ca²⁺ transfer into mitochondria stimulates ATP production and other mitochondria functions to provide neuroprotection. Similarly, modest increase in cytosolic Ca²⁺ concentration activates pro-survival pathways such as phosphotydyl inositol 3 kinase (PI3K) and the mitogen-activated protein kinase (MAPK) signaling cascades [extracellular signal-regulated kinases (ERK), c-Jun N-terminal protein kinases (JNK) and p38 as well as

protein kinase B (Akt)]. Activation of cell-survival signaling decreases apoptosis and cell death. By contrast, prolonged exposure to high anesthetic concentrations (right side), increases cytosolic $[Ca^{2+}]$ to toxic levels due excessive Ca^{2+} release from ER. This results in excessive transfer of Ca^{2+} from ER into mitochondria and increased mitochondrial permeability transition pore (mPTP) activity, resulting in mitochondrial swelling and rupture, and release of pro-apoptotic molecules. Abnormally elevated cytosolic Ca^{2+} concentrations can also activate calpain and then caspases and induce cell death by apoptosis, resulting in neurodegeneration.