

## Neuroprotective and Neurotoxic Properties of General Anesthetics: A Double-Edged Sword

Huafeng Wei, Alexander T. King, and Saadet Inan

### ABSTRACT

From Department of Anaesthesiology and Critical Care, University of Pennsylvania, Philadelphia, USA.

**Correspondence** to Dr. Huafeng Wei at [weih@uphs.upenn.edu](mailto:weih@uphs.upenn.edu).

**Citation:** Hua-Feng Wei, Alexander T. King, Saadet Inan. Neuroprotective and neurotoxic properties of general anesthetics: a double-edged sword. *J Anesth Perioper Med* 2015; 2: 158-66.

**Aim of review:** Summarize current and past studies on the neuroprotective and neurotoxic properties of general anesthetics.

**Method:** We have reviewed general anesthetic-induced dual effects of neuroprotection and neurotoxicity in cell culture, animal, and human studies.

**Recent findings:** We have analyzed the contributing factors to the dual effects of neuroprotection and neurotoxicity by general anesthetics. Particularly, the concentration of intracellular calcium may moderate such dual effects of general anesthetics. A modest increase of intracellular calcium by general anesthetics provides neuroprotection, while an excessive or abnormal increase of intracellular calcium may result in neurotoxicity. Future studies are needed to identify the demarcation point upon which the use of general anesthetics becomes neurotoxic. By highlighting this issue, the article fills a gap in the general anesthetic literature.

**Summary:** The variables governing the neuroprotective and neurotoxic effects of general anesthetics appear to be patient age, concentration of general anesthetic used, and length of anesthetic exposure. Preclinical studies suggested that preconditioning with inhalational anesthetics, especially isoflurane at low concentrations, provides neuroprotection. However, inhalational anesthetics can also cause neurotoxicity when used at high concentrations for prolonged periods. Elderly subjects with preexisting neurodegenerative conditions and subjects in their prenatal or postnatal periods seem sensitive to the neurotoxic effects of general anesthesia.

General anesthetics, especially volatile anesthetics, are commonly used to maintain general anesthesia for surgeries in patients across a spectrum of ages. Preconditioning and postconditioning with general anesthetics have been shown to provide neuroprotection against reperfusion injury and hypoxia (1, 2). However, there is growing apprehension that general anesthetics may cause permanent cognitive dysfunction following peri- and post-operative periods. Particularly, the neonatal and elderly populations seem most vulnerable to the neurotoxic risk of anes-

thetics. Results from our laboratory and others suggested that the dual effects of general anesthetics depend on the concentration and the duration of anesthetic exposure (3-5).

Determining the neurotoxicity of general anesthetics in humans from the results of cellular and animal studies poses several challenges. Firstly, the timing of neuro- developmental stages varies among different animal species. For example, while periods of rapid human brain development occur from the third trimester through age two, neuro-anatomical and neuro-physiologic evidence

suggests that similar development occurs in rodents from birth to postnatal day 14 (6, 7). Hence, it is difficult to infer specific intervals of patient susceptibility. Secondly, the lack of surgical stimuli, inflammatory responses, and comparable diseases within many animal models complicates their adaptation to humans. Because most preclinical studies have focused on cellular and animal studies, research evaluating general anesthetic neurotoxicity in humans is sparse. Knowing how much the exposure time of anesthetics should be adjusted from animals to humans is one of the questions of animal-human translational research. Therefore, an understanding of the mechanisms behind general anesthetics' dual effects can only help to optimize neuroprotection and minimize neurotoxicity in anesthetized patients.

### General Anesthetic-Induced Neuroprotection

Pre- and postconditioning with anesthetics are utilized for neuroprotection. A brief subclinical application of anesthetics just before or after neurotoxic stimuli increases tolerance and decreases neuronal cell damage. General anesthetics have been shown to be protective against cell death induced by glutamate, hypoxia, ischemia, and oxygen-glucose deprivation (OGD) in cell culture and animal studies. For example, use of isoflurane, sevoflurane, and halothane in clinically-used concentrations reduces lactate dehydrogenase (LDH) release, a marker for tissue damage (8). Short-term administration of isoflurane also decreases apoptosis in OGD-exposed rat hippocampal slices (9) and increases the survival rate of Purkinje neurons exposed to glutamate (10). The possible mechanisms of general anesthetic-induced neuroprotection are discussed below.

### Role of Calcium and Cell Survival Signaling Pathways

Previous research indicated that intracellular calcium ( $Ca^{2+}$ ) concentration is a defining factor for the neuroprotective properties of general anesthetics. As an important second messenger in cell growth and differentiation, calcium is maintained at 100 nM between the intra- and extracellular space under normal conditions (11-14). Sarcoplasmic reticulum  $Ca^{2+}$ -ATPase (SERCA)

pumps, inositol-1,4,5-triphosphate receptors (InsP<sub>3</sub>R), ryanodine receptors (RYRs), and  $Ca^{2+}$ -binding proteins all regulate  $Ca^{2+}$  within the endoplasmic reticulum (ER). When RYRs and InsP<sub>3</sub>, a component of the phospholipase C pathway (15), activate InsP<sub>3</sub>Rs,  $Ca^{2+}$  is released from the ER.

Besides the ER, the mitochondria are another significant organelle involved in the regulation of intracellular  $Ca^{2+}$  concentration. The mitochondrial permeability transition pores (mPTP) and the mitochondrial ATP-sensitive K (mitoK<sub>ATP</sub>) channels both play crucial roles in  $Ca^{2+}$  regulation and apoptosis. Overall activation of mPTP increases mitochondrial permeability inside the mitochondria and results in mitochondrial swelling, rupture, and the release of cytochrome c (CytC), an activator of pro-apoptotic factors. To achieve mitochondrial protection, the mitoK<sub>ATP</sub> channel stays open during ischemia and reperfusion to conserve the mitochondrial ATP production rate and to mitigate  $Ca^{2+}$  over-release (16).

A moderate increase in  $Ca^{2+}$  concentration is thought to be the mechanism of hypoxia and isoflurane preconditioning-induced cytoprotection. Gray et al. (17) reported that slight increases in  $Ca^{2+}$  concentration in the presence of isoflurane stimulated the mitogen-activated protein kinase (MAPK) signaling cascade, a cell-survival pathway. Likewise, Bickler and Fahlman (18) compared the expression of signal transduction genes following hypoxic or isoflurane preconditioning in rat hippocampal slices. In both cases, preconditioning initiated an increase in intracellular  $Ca^{2+}$  levels and established similar cell survival rates. When hypoxic and isoflurane preconditionings were applied together, the neuroprotective effect was higher than when they were administered alone and neuronal death decreased following the combination pretreatment (19).

Researchers have also examined the effect of postconditioning with general anesthetics. Zhou et al. (20) examined the effect of isoflurane postconditioning on neonatal hypoxic-ischemic brains in rats. These authors reported reduced infarct size at 48 hours and attenuated brain atrophy at four weeks. Pretreating the rats with the sphingosine-1-phosphate (S1P) antagonist before the postconditioning treatment antago-

nized isoflurane-induced neuroprotection. Therefore, the data indicate that isoflurane-induced neuroprotection occurs via activation of S1P/PI3K/Akt through modest increases in cytosolic  $Ca^{2+}$  concentration.

Overall, these studies demonstrated that pre- and postconditioning with general anesthetics moderate the  $Ca^{2+}$  level within cells and activate cell survival signal pathways in anesthetic-induced neuroprotection.

### Role of Receptor Interactions

Volatile anesthetics mainly act on ligand-gated ion channels and two-pore domain  $K^+$  channels. The three ligand-gated ion channels typically targeted by most anesthetics are the  $\gamma$ -aminobutyric acid type A ( $GABA_A$ ) receptor, the inhibitory glycine receptor, and the N-methyl-D-aspartate (NMDA) receptor (21-24). With the exception of xenon and cyclopropane, almost all anesthetics potentiate GABA-induced  $Cl^-$  currents and serve as agonists on  $GABA_A$  receptors (21). The antagonistic properties of general anesthetics on NMDA receptors are thought to be responsible for the analgesic, neuroprotective, and immobilizing effects of anesthetics (8, 10, 25).

The activation of inhibitory synaptic  $K^+$  current by anesthetics has also been well researched. The three types of  $K^+$  channels are the voltage-gated ( $K_v$ ), inwardly rectifying ( $K_{ir}$ ), and two-pore ( $K_{2p}$ ) domain channels (26-29).  $K_{ATP}$  is one of the members of  $K_{ir}$  and, as previously mentioned, plays an essential role in  $Ca^{2+}$  regulation and cell death. The  $K^+$  channels TREK-1 and TASK are two of the most important members of the  $K_{2p}$  family. While chloroform, diethyl ether, halothane and isoflurane activate TREK-1, only halothane and isoflurane stimulate TASK (30). The activation of GABA receptors and inhibitory  $K^+$  channels and the inhibition of NMDA receptors by anesthetics cause hyperpolarization and reduced glutamate release. As a result, these receptor interactions have an absolute effect on decreasing excitotoxicity during ischemia, hypoxia, and reperfusion.

Another possible mechanism for the neuroprotective effects of volatile anesthetics is the enhancement or preservation of excitatory amino acid transporters (EAAT), especially that of Type 3 (EAAT3). EAATs are responsible for glutamate

re-uptake from the extracellular space into cells. The major glutamate transporter in neurons is EAAT3. Previous studies have shown that volatile anesthetics enhance EAAT3 activity and gene expression in cells via protein kinase C (PKC) (31, 32). EAAT3 also transports cysteine, a rate-limiting substrate for the synthesis of a major intracellular antioxidant, glutathione. Lee et al. (33) reported that volatile anesthetics attenuate the tert-Butyl hydroperoxide-induced decrease in EAAT3 activity, thus reducing the transport L-cysteine and L-glutamate in rat EAAT3 expressed in xenopus oocytes. These authors concluded that volatile anesthetics increase EAAT3 activity and the neuroprotective effect of volatile anesthetics may stem from the preservation of EAAT3 function under oxidative stress.

Both NMDA and GABA receptors take part in memory and learning formation in the hippocampus. Compared to a control group, mice anesthetized with isoflurane presented better cognitive function (34). Up-regulation of NR2B, a subunit of the NMDA receptor, was found in the hippocampus of mice, and pretreatment with NR2B selective antagonists mitigated the improved cognitive function induced by isoflurane. Also, isoflurane enhanced long-term potentiation in the CA1 region of the hippocampus (34).

### Role of Nitric Oxide Synthase and Anti-Inflammatory Properties of General Anesthetics

Nitric oxide (NO) is produced endogenously from the amino acid L-arginine by nitric oxide synthase (NOS). Four different isoforms of NOS have been classified: endothelial (eNOS), neuronal (nNOS), inducible (iNOS) and mitochondrial (mtNOS) (35-37). All forms of NOS are expressed in the brain (38, 39). The authors concluded that eNOS, nNOS, and mtNOS activities are constitutive and calcium- and calmodulin-dependent. However, iNOS activity is independent of calcium or calmodulin; instead, cytokines, infection, inflammation, or cardiac stress induce its response.

The effects of NO are highly dependent upon concentration. At low levels, NO can act as an intracellular and intercellular regulatory messenger. At high levels, NO can be cytotoxic by producing free oxygen radicals, activating post-

translational peroxynitrite and inducing apoptosis (14, 39). The isoform of NOS that produces NO is functionally important. For example, both overproduction of NO by iNOS and hypoproduction of NO by mtNOS are involved in the pathogenesis of aging (39). Different sites of activity may be important in determining whether NO effects are neuroprotective or neurotoxic.

Isoflurane or halothane preconditioning provided prolonged neuroprotection against ischemia in rats induced by middle cerebral artery occlusion (MCAO) and in primary cortical neurons induced by OGD (40). The preconditioning increased iNOS protein levels and the administration of an iNOS inhibitor prevented anesthetic-induced infarct volume reduction in rats. In OGD-exposed hippocampal cells, preconditioning with isoflurane increased iNOS mRNA levels (41). Isoflurane preconditioning in neonatal rats also induced neuroprotection in a perinatal stroke model (42). Isoflurane increased iNOS levels and the pretreatment with an iNOS inhibitor antagonized isoflurane-induced neuroprotection. Thirty minutes of inhalation of 2.8% isoflurane 24 hours before spinal cord ischemia in rats significantly increased iNOS expression in the spinal cord motor neurons (43). Also, isoflurane induced ischemic preconditioning in ischemic rat hearts by increasing expression and activity of iNOS (44).

Inflammation is common in central nervous system (CNS) diseases involving brain trauma, stroke, brain infection, and neurodegeneration. Microglial cells play a critical role in the brain inflammatory response. During neuroinflammation, microglial cells are activated and pro-inflammatory cytokines and reactive oxygen species are produced. Xu et al. (45) tested how isoflurane preconditioning affects microglial function in mouse microglial cultures. One hour exposure of isoflurane before application of lipopolysaccharide (LPS) and interferon- $\gamma$  (IFN $\gamma$ ) to microglial cells decreased cell death, iNOS expression, and glutamate release. These results indicate that isoflurane reduces microglial activity induced by LPS and IFN $\gamma$  and consequently inhibits the iNOS-NO-glutamate pathway activated by microglia. Anti-inflammatory activity was not only reported with isoflurane but also with desflurane (46) and sevoflurane (47).

### Differential Effects of Inhalational Anesthetics in Neuroprotection

Inhalational anesthetics protect the brain during ischemia and hypoxia by reducing cerebral metabolic rate, cortical electrical activity, cerebral oxygen consumption, and lactate accumulation through sustaining ATP stores and phosphocreatine depletion (48, 49). Positron emission tomography studies in humans demonstrate that inhalational anesthetics globally and regionally diminish cerebral metabolism (50, 51). Among the inhalational anesthetics, isoflurane provides better cerebral protection than halothane and enflurane. Michenfelder et al. (52) investigated the records of 2,223 patients who underwent carotid endarterectomy at the Mayo Clinic between 1972 and 1985 to compare the effects of isoflurane, halothane, and enflurane on cerebral blood flow (CBF), incidence of electroencephalographic (EEG) ischemic changes, and neurologic outcome. CBF was lower in the isoflurane group, compared to the halothane and enflurane groups. There was no difference among groups in neurologic outcome, but ischemic EEG changes were significantly less in the isoflurane group than those in the halothane and enflurane groups. Another study reported that desflurane lowered plasma endothelin levels after anesthesia in patients undergoing intracranial aneurysm clipping (54). This effect of desflurane may contribute to the prevention of unwanted acute cerebral vasospasm.

### General Anesthetic-Induced Neurotoxicity

Since most anesthetics are NMDA antagonists and GABA<sub>A</sub> agonists, both of these receptors have been extensively studied in neurotoxic research. It has been shown that the blockage of NMDA receptors and stimulation of GABA<sub>A</sub> receptors caused apoptotic neurodegeneration in animals when given in the early neonatal period (6, 54-57). Ethanol, an NMDA receptor antagonist and a GABA<sub>A</sub> receptor agonist, induced apoptotic neurodegeneration in animals (6, 56). Fetal alcohol syndrome, a consequence of intrauterine exposure of the fetus to ethanol in humans, causes neurobehavioral abnormalities ranging from learning disabilities to psychosis, mirroring the effect of NMDA and GABA<sub>A</sub> re-

ceptors. Other factors involved in anesthetic-induced neurotoxicity are discussed below.

### Role of Calcium

Abnormal calcium release from the ER via either  $\text{InsP}_3\text{R}$  and/or RYR plays important role in anesthesia-mediated neurotoxicity. Wei et al. (58) used PC 12 pheochromocytoma cells and primary cortical neurons to compare the cytotoxic effects of equipotent concentrations of isoflurane and sevoflurane. The application of isoflurane (2.4%) for 24 hours increased LDH release but the application of sevoflurane (4%) for the same timeframe had no effect on both cell lines. Isoflurane increased the pro-apoptotic activities of caspase 3 and 9, but sevoflurane affected neither of the signaling proteins. The authors hypothesized that isoflurane induces apoptosis by increasing calcium release from the ER through the activation of RYRs. Cells that had a pretreatment of dantrolene, an antagonist of RYR, exhibited significantly decreased isoflurane-induced apoptosis.

Eckenhoff et al. (59) demonstrated that both halothane and isoflurane enhance amyloid  $\beta$  ( $\text{A}\beta$ ) oligomerization and  $\text{A}\beta$ -induced cell toxicity in rat pheochromocytoma cells.  $\text{A}\beta$  is produced from amyloid  $\beta$  precursor protein (APP) by aspartyl protease  $\beta$ -site APP-cleaving enzyme (BACE) or  $\beta$ -secretase and  $\gamma$ -secretase (60). Similar studies showed that BACE and  $\gamma$ -secretase levels were increased in primary cortical and hippocampal neurons and in H4 human neuroglioma cells following treatments of isoflurane and sevoflurane (61-64). In these studies, when the isoflurane-induced increase in cytosolic calcium level was reduced using calcium chelators, the isoflurane-induced cytotoxic effects were attenuated. For example, memantine, a NMDA partial antagonist and USA Food and Drug Administration (FDA)-approved Alzheimer's disease (AD) medication, prevented isoflurane-elicited caspase-3 activity and apoptosis in cell lines (64).

While all inhalational anesthetics have the capacity to induce cell damage, the three commonly used inhalational anesthetics do not appear to have the same potency. Our results showed that isoflurane is more potent than sevoflurane or desflurane in causing cell damage in the rodent developing brain (65). Other studies confirmed

that isoflurane seems to be more potent in causing calcium release from the ER and cell death, as well as huntingtin protein aggregation, than equipotent concentrations of sevoflurane or desflurane (61, 65-68). It should be noted that isoflurane is also more potent than sevoflurane in providing neuroprotection via preconditioning mechanisms.

### Effect of Age on Neuroprotection and Neurotoxicity

Various studies showed that neurologic outcome after inhalational anesthetic exposure depends on age of the subject. In one study, maternal exposure of isoflurane during gestational day 21 of rats had no marked effect on fetal brains (4). Apoptosis was similar in both exposed and control fetal brains and spatial memory and learning were not impaired. However, when isoflurane concentration was increased to 3% (1 hour) in gestational day 21, caspase-3 positive cells in both the hippocampal CA1 region and the retrosplenial cortex of neonatal rats were significantly increased (5). In another study (69), rats were exposed to isoflurane during gestational day 14 and studied behaviorally at the age of 8 weeks. Deficits in spatial memory and anxiety were found in rats receiving isoflurane. We conclude that isoflurane may inhibit or promote apoptosis in fetal brains, depending not only on the time of exposure but also the concentration used.

Combined midazolam, nitrous oxide and isoflurane exposure to 7-day-old rats led to apoptotic neurodegeneration, synaptic dysfunction, and long-term impairment in spatial learning and memory (70). In 14-day-old rats, similar results were not found, suggesting that the developing brain is more vulnerable during the peak time of synaptogenesis (71). Exposure of 7-day-old mice to only isoflurane did not influence locomotion, learning, or memory at 2 months. Yet, isoflurane (0.75%) combined with nitrous oxide (6 hours) increased caspase-3 activity in spinal cords of 7-day-old rats (72). Likewise, a subclinical concentration of sevoflurane increased caspase-3 activity in 7-day-old mice (73, 74). When the concentration is increased, sevoflurane not only increased apoptosis but also caused persistent learning and social interaction

deficits during adulthood (75).

Istaphanous et al. (76) found that apoptotic and neuronal cell death activities of equipotent concentrations of isoflurane, sevoflurane, and desflurane were similar in 7-day-old mice. Low concentrations of isoflurane combined with nitrous oxide elicited apoptosis (70-72). On the other hand, higher concentrations of isoflurane were needed when given with just oxygen in the 7-8 days old rodents (5, 76). It seems that the combination of administered anesthetics influences neurotoxicity. Again, the peak of synaptogenesis seems to be the most vulnerable time for anesthetics exposure.

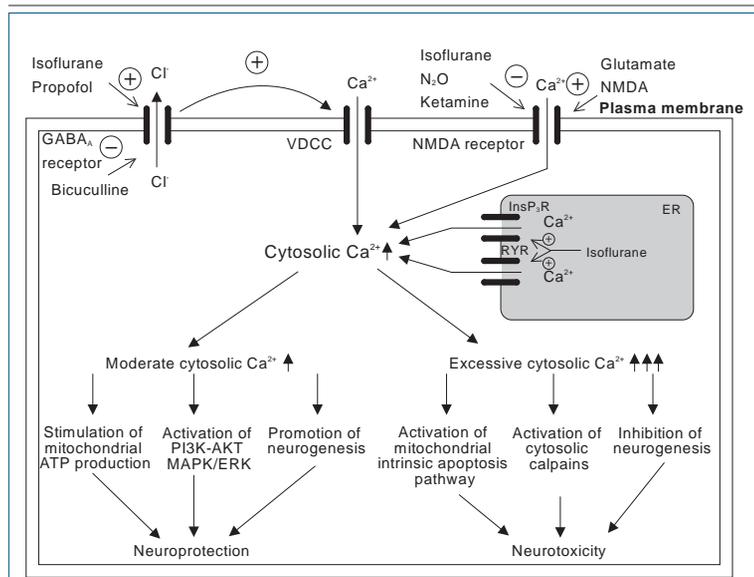
Stratmann et al. (77) investigated the effects of 1 minimum alveolar concentration (MAC) isoflurane exposure on neurogenesis and neurocognitive function in 7-day- and 60-day-old rats. In 60-day-old rats, isoflurane increased neuronal differentiation and progenitor proliferation and improved neurocognitive functions. However, in newborn rats, isoflurane decreased progenitor proliferation and caused persistent and progressive neurocognitive deficit. Thus, the authors suggested that anesthetic-induced cognitive dysfunction seems to occur during specific periods of brain development, as the 60-day old rats, although young, displayed limited impairment. Similarly, repeated isoflurane exposure (1.7% solution applied for 35 minutes over 4 consecutive days) was studied in PN14 rats and mice as well as PN60 rats (78). Persistent and progressive learning and memory deficits were detected in the younger group but not the older group. Undifferentiated hippocampal neural stem cells were reduced in the PN14 rats, indicating that young brains are more vulnerable to isoflurane. In aged rats, isoflurane-nitrous oxide anesthesia has been shown to cause long-term spatial memory impairment (79-81). These results showed a trend of newborn and aged rats exhibiting the greatest effects of neurotoxicity.

Despite evidence of neurodegeneration induced by anesthetics in cell culture and neonatal animal brain slices, human studies remain limited. The lack of established biomarkers for human neurodegeneration complicates proper analysis. Further, human studies would require decades of follow-up in very large cohorts of patients to evaluate behavioral outcome after in utero or neona-

tal anesthesia exposure. Kalkman et al. (82) calculated that a sample size of 2,268 children would be needed to perform a large-scale, retrospective study to investigate disturbed neurobehavioral development in children exposed to anesthetics at ages 0-6 years and reach a statistically valid conclusion. Although it was not statistically significant, their pilot study showed that children undergoing surgery before month 24 had more behavioral disturbance than children having surgery after age two. Learning disabilities were detected in children receiving multiple anesthetic exposures prior to age four, but not in children exposed to a single anesthetic exposure (83).

In elderly humans, long-term cognitive problems can occur following anesthesia and previous surgeries may increase the risk of neurodegenerative diseases (84, 85). As a result, postoperative delirium and cognitive decline (POCD) are important issues in the geriatric population. POCD is characterized by disturbances in memory, concentration, language comprehension, and social interactions. It can persist for weeks and months after surgery, and may even become permanent. Besides advanced age, other risk factors for POCD are limited education, diabetes, atherosclerotic heart disease, hypotension, hypoxia, and cerebral emboli during surgery (86-88). Delirium mostly occurs on postoperative days one and three and can be associated with disorientation, temporary memory dysfunction, and sleep-wake cycle alterations. POCD occurs in 10-26% of patients over 65 years of age and significantly increases morbidity and mortality. The interaction of anesthetics with the central muscarinic cholinergic system is thought to be the underlying mechanism of POCD since the system's reduced activity has been found in the pathogenesis of diseases involving mood and memory (89, 90).

Cognitive function decline in patients examined at 7 days and at 3 months postoperatively were similar after both regional and general anesthesia (91). For patients at day 3 only, POCD incidence was higher in the general anesthesia group than in the regional anesthesia group (92). Following coronary artery bypass graft surgery, neurocognitive function was better in patients who received isoflurane compared to patients who received desflurane and sevoflurane. The incidence of POCD was similar in elderly patients



**Figure 1. An Overview of the Role of Calcium in the Dual Effects of General Anesthetic-Mediated Neuroprotection and Neurotoxicity in Immature Neurons.**

The cytosolic concentration of calcium can be increased either by calcium influx through the voltage-dependent calcium channel (VDC) or the N-methyl-D-aspartate (NMDA) receptor or by calcium release from the endoplasmic reticulum (ER) through the 1,4,5-trisphosphate receptor (InsP<sub>3</sub>R) or ryanodine receptor (RYR). Short-term exposure to low-concentrations of a general anesthetic like isoflurane induces a moderate cytosolic calcium increase. An adequate calcium elevation acts as a pre- or postconditioning mechanism and promotes pro-survival pathways like ATP production, mitogen-activated protein kinase (MAPK) signaling cascade activation, and neurogenesis. However, extended exposure to high-concentrations of general anesthesia disrupts calcium homeostasis and increases cytosolic calcium to toxic levels, provoking intrinsic pathway apoptosis, activating pro-apoptotic molecules calpains and inhibiting neurogenesis.

receiving sevoflurane or desflurane for non-cardiac surgery (93). Two years after non-cardiac surgery, POCD incidence among elderly patients was 1% (94). After minor surgery, POCD incidence was 6.8% at 7 days and 6.6% at 3 months (95). Significant risk factors for POCD were determined to be an age greater than 70 years and being an in-patient rather than an out-patient, suggesting that long-term hospitalization should be avoided in elderly patients.

The nature and severity of cognitive decline were evaluated at discharge and 3 months after non-cardiac surgery (96). At discharge, 56% of patients had cognitive decline with equal type and severity. But at 3 months, 13% of patients had only memory dysfunction, 8% had only executive function decline, and 9% of patients had

both. POCD also occurred in middle-aged patients, but it was resolved in a shorter time period (97). Long-term consequences of POCD were studied for 8.5 years following non-cardiac surgery (98). Mortality rates were higher in patients who had POCD at 3 months than in patients who had POCD at 7 days.

An important question in neurotoxic studies concerns whether the degree of anesthetic-induced neurodegeneration in the developing brain is correlated to the level of anesthetic-induced memory and learning impairments. Although one previous study suggested an association between anesthetic-mediated neurodegeneration and memory and learning disabilities in rodent developing brains (70), our recent studies did not support this statement (61, 74), consistent with recent findings from other labs (99). Recent studies suggested that some additional mechanisms other than anesthesia-mediated neurodegeneration may underlie the anesthesia-mediated cognitive dysfunction, such as the reduction of synapses in the developing brain (99). However, further studies are needed to understand the effects of general anesthetics on synapse structures and their contribution to anesthesia-mediated cognitive dysfunction.

**Conclusions**

Preclinical studies suggested that general anesthetics may be both neuroprotective and neurotoxic, depending on concentration and duration (Figure 1). Anesthetics used at low concentration for a short time may provide neuroprotection via induction of moderate elevation of cytosolic calcium concentration. However, anesthetics used at a high concentration for prolonged times may cause cell damage by excessive and abnormal elevation of cytosolic calcium concentration. Compounding the problem, specific subsets of the human population are more vulnerable to the effects of general anesthetics than others.

Although human clinical practice typically involves a combination of anesthetics, intubation, and controlled respiration, most animal studies fail to replicate these clinical conditions. Many experimenters use just one type of anesthetic combined with oxygen and permit spontaneous respiration. In cellular studies, it is uncertain if

the response of cells treated directly with anesthetics mirrors that of human cells during general anesthesia. Human cells, with circulation and physiological barriers, may need different concentrations of anesthetics than what has been used with cultured cells. Finally, research restrictions imposed on human studies provide yet another problem for data translation. The necessity for long-term follow-up of subjects to study brain development and the difficulty of establishing a homogenous study group make human studies costly.

From the studies of both neuroprotection and neurotoxicity in this review, we suggest that general anesthetics act as stress factors, such as ischemia. Short cerebral ischemia or adequate exposure of general anesthetics at low concentrations precondition different neurons of the CNS and

provide neuroprotection by inducing endogenous neuroprotective mechanisms. However, prolonged cerebral ischemia or excessive exposure to general anesthetics will cause neuronal damage. Future clinical studies are needed to determine the dividing point of the general anesthetic exposure level at which the effects of general anesthetics will be converted from neuroprotection to neurotoxicity.

This work was supported by National Institutes of Health awards GM084979, GM084979-02S1 and 2R01GM084979-06A1 to Huafeng Wei, and K08-GM073224 to Hua-Feng Wei; March of Dimes Birth Defects Foundation Research Grant 12-FY08-167 to Huafeng Wei, and the Research Fund at the Department of Anesthesiology, Perelman School of Medicine, University of Pennsylvania. All authors declare no other competing financial interest.

We appreciate the valuable discussion and support from Roderic G. Eckenhoff, MD, Maryellen F. Eckenhoff, PhD, and Lee A. Fleisher, MD, Department of Anesthesiology and Critical Care, University of Pennsylvania, Philadelphia, PA 19104, USA.

## References

1. Tsutsumi YM, Patel HH, Lai NC, Takahashi T, Head BP, Roth DM. Isoflurane produces sustained cardiac protection after ischemia-reperfusion injury in mice. *Anesthesiology* 2006; 104: 495-502.
2. Chi OZ, Hunter C, Liu X, Weiss HR. The effects of isoflurane pretreatment on cerebral blood flow, capillary permeability, and oxygen consumption in focal cerebral ischemia in rats. *Anesth Analg* 2010; 110: 1412-8.
3. Wei H, Liang G, Yang H. Isoflurane preconditioning inhibited isoflurane-induced neurotoxicity. *Neurosci Lett* 2007; 425: 59-62.
4. 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: 942-50.
5. Wang S, Peretich K, Zhao Y, Liang G, Meng Q, Wei H. Anesthesia-induced neurodegeneration in fetal rat brains. *Pediatr Res* 2009; 66: 435-40.
6. Olney JW, Tenkova T, Dikranian K, Qin YQ, Labruyere J, Ikonomidou C. Ethanol-induced apoptotic neurodegeneration in the developing C57BL/6 mouse brain. *Dev Brain Res* 2002; 133: 115-26.
7. Clancy B, Finlay BL, Darlington RB, Anand KJ. Extrapolating brain development from experimental species to humans. *Neurotoxicology* 2007; 28: 931-7.
8. 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: 756-65.
9. 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: 1343-9.
10. Zheng S, Zuo Z. Isoflurane preconditioning decreases glutamate receptor overactivation-induced purkinje neuronal injury in rat cerebellar slices. *Brain Res* 2005; 1054: 143-51.
11. Orrenius S, Zhivotovskiy B, Nicotera P. Regulation of cell death. The calcium-apoptosis link. *Nat Rev Mol Cell Biol* 2003; 4: 552-65.
12. Hoyer-Hansen M, Jäättelä M. Connecting endoplasmic reticulum stress to autophagy by unfolded protein response and calcium. *Cell Death Differ* 2007; 14: 1576-82.
13. Hoyer-Hansen M, Bastholm L, Sznajderowski P, Campanella M, Szabadkai G, Farkas T, et al. Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-beta, and Bcl-2. *Mol Cell* 2007; 25: 193-205.
14. Inan S, Wei H. The cytoprotective effects of dantrolene: a ryanodine receptor antagonist. *Anesth Analg* 2010; 111: 1400-10.
15. Berridge MJ. Inositol trisphosphate and calcium signalling. *Nature* 1993; 361: 315-25.
16. Bienengraeber MW, Wehrauch D, Kersten JR, Pangel PS, Wartler DC. Cardioprotection by volatile anesthetics. *Vascu Pharmacol* 2005; 42: 243-52.
17. Gray JJ, Bickler PE, Fahlman CS, Zhan X, Schuyler JA. Isoflurane neuroprotection in hypoxic hippocampal slice cultures involves increases in intracellular Ca<sup>2+</sup> and mitogen-activated protein kinases. *Anesthesiology* 2005; 102: 606-15.
18. Bickler PE, Fahlman CS. Expression of signal transduction genes differs after hypoxic or isoflurane preconditioning of rat hippocampal slice cultures. *Anesthesiology* 2009; 111: 258-66.
19. Bickler PE, Fahlman CS. Enhanced hypoxic preconditioning by isoflurane: signaling gene expression and requirement of intracellular Ca<sup>2+</sup> and inositol triphosphate receptors. *Brain Res* 2010; 1340: 86-95.
20. Zhou Y, Lekic T, Fathali N, Ostrowski RP, Martin RD, Tang J, et al. Isoflurane posttreatment reduces neonatal hypoxic-ischemic brain injury in rats by the sphingosine-1-phosphate/phosphatidylinositol-3-kinase/Akt pathway. *Stroke* 2010; 41: 1521-7.
21. de Sousa SL, Dickinson R, Lieb WR, Franks NP. Contrasting synaptic actions of the inhalational general anesthetics isoflurane and xenon. *Anesthesiology* 2000; 92: 1055-66.
22. Dickinson R, Peterson BK, Banks P, Simillis C, Martin JC, Valenzuela CA, et al. 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: 756-67.
23. Franks NP. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. *Nat Rev Neurosci* 2008; 9: 370-86.
24. Olsen RW, Li GD. GABA(A) receptors as molecular targets of general anesthetics: identification of binding sites provides clues to allosteric modulation. *Can J Anaesth* 2011; 58: 206-15.
25. Sewell JC, Raines DE, Eger EI 2nd, Laster MJ, Sear JW. A comparison of the molecular bases for N-methyl-D-aspartate-receptor inhibition versus immobilizing activities of volatile aromatic anesthetics. *Anesth Analg* 2009; 108: 168-75.
26. Gutman GA, Chandy KG, Grissmer S, Lazdunski M, McKinnon D, Pardo LA, et al. International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacol Rev* 2005; 57: 473-508.
27. Wei AD, Gutman GA, Aldrich R, Chandy KG, Grissmer S, Wulff H. International Union of Pharmacology. LII. Nomenclature and molecular relationships of calcium-activated potassium channels. *Pharmacol Rev* 2005; 57: 463-72.
28. Kubo Y, Adelman JP, Clapham DE, Jan LY, Karschin A, Kurachi Y, et al. International Union of Pharmacology. LIV. Nomenclature and molecular relationships of inwardly rectifying potassium channels. *Pharmacol Rev* 2005; 57: 509-26.
29. Goldstein SA, Bayliss DA, Kim D, Lesage F, Plant LD, Rajan S. International Union of Pharmacology. LV. Nomenclature and molecular relationships of two-potassium channels. *Pharmacol Rev* 2005; 57: 527-40.
30. Patel AJ, Honore E, Lesage F, Fink M, Romey G, Lazdunski M. Inhalational anesthetics activate two-pore-domain background K<sup>+</sup> channels. *Nat Neurosci* 1999; 2: 422-6.
31. Do SH, Kamatchi GL, Washington JM, Zuo Z. Effects of volatile anesthetics on glutamate transporter, excitatory amino acid transporter type 3. *Anesthesiology* 2002; 96: 1492-7.
32. Huang Y, Zuo Z. Isoflurane enhances the expression and activity of glutamate transporter type 3 in C6 glioma cells. *Anesthesiology* 2003; 99: 1346-53.
33. Lee SA, Choi JG, Zuo Z. Volatile anesthetics attenuate oxidative stress-reduced activity of glutamate transporter type 3. *Anesth Analg* 2009; 109: 1506-10.
34. Rammes G, Starker LK, Haseneder R, Berkmann J, Plack A, Zieglgansberger W, et al. Isoflurane anaesthesia reversibly improves cognitive function and long-term potentiation (LTP) via an up-regulation in NMDA receptor 2B subunit expression. *Neuropharmacology* 2009; 56: 626-36.
35. Nathan C, Xie QW. Nitric oxide synthases: roles, tolls, and controls. *Cell* 1994; 78: 915-8.
36. Haynes V, Elfering S, Traaseth N, Giulivi C. Mitochondrial nitric-oxide synthase: enzyme expression, characterization, and regulation. *J Bioenerg Biomembr* 2004; 36: 341-6.
37. Lavandera J, Batlle A, Buzaleh AM. Mice brain nitric oxide synthase response induced by anesthetics and other porphyrinogenic drugs. *Drug Metab Lett* 2011; 5: 25-9.
38. Paakkari I, Lindsberg P. Nitric oxide in the central nervous system. *Ann Med* 1995; 27: 369-77.
39. Navarro A, Boveris A. Mitochondrial nitric oxide synthase, mitochondrial brain dysfunction in aging, and mitochondria-targeted antioxidants. *Adv Drug Deliv Rev* 2008; 60: 1534-44.
40. Kapinya KJ, Lowl D, Futterer C, Maurer M, Waschke KF, Isave NK, et al. Tolerance against ischemic neuronal injury can be induced by volatile anesthetics and is inducible NO synthase dependent. *Stroke* 2002; 33: 1889-98.
41. Li QF, Zhu YS, Jiang H. Isoflurane preconditioning activates HIF-1alpha, iNOS and Erk1/2 and protects against oxygen-glucose deprivation neuronal in-

- jury. *Brain Res* 2008; 1245: 26-35.
42. Zhao P, Zuo Z. Isoflurane preconditioning induces neuroprotection that is inducible nitric oxide synthase-dependent in neonatal rats. *Anesthesiology* 2004; 101: 695-703.
43. Sung YH, Lee SH, Sung JK, Han JH, Kim H, Kim CJ, et al. Preconditioning of isoflurane on spinal cord ischemia can increase the number of inducible nitric oxide synthase-expressing motor neurons in rat. *Korean J Anesthesiol* 2010; 58: 70-5.
44. Wakeno-Takahashi M, Otani H, Nakao S, Imamura H, Shingu K. Isoflurane induces second window of preconditioning through upregulation of inducible nitric oxide synthase in rat heart. *Am J Physiol Heart Circ Physiol* 2005; 289: H2585-91.
45. Xu X, Kim JA, Zuo Z. Isoflurane preconditioning reduces mouse microglial activation and injury induced by lipopolysaccharide and interferon-gamma. *Neuroscience* 2008; 154: 1002-8.
46. Boost KA, Hofstetter C, Flondor M, Betz C, Homann M, Pfeilschifter J, et al. Desflurane differentially affects the release of proinflammatory cytokines in plasma and bronchoalveolar fluid of endotoxemic rats. *Int J Mol Med* 2006; 17: 1139-44.
47. Wang H, Lu S, Yu Q, Liang W, Gao H, Li P, et al. Sevoflurane preconditioning confers neuroprotection via anti-inflammatory effects. *Front Biosci (Elite Ed)* 2011; 3: 604-15.
48. Newberg LA, Michenfelder JD. Cerebral protection by isoflurane during hypoxemia or ischemia. *Anesthesiology* 1983; 59: 29-35.
49. Baughman VL, Hoffman WE, Milechik DJ, Albrecht RF, Thomas C. Neurologic outcome in rats following incomplete cerebral ischemia during halothane, isoflurane, or N<sub>2</sub>O. *Anesthesiology* 1988; 69: 192-8.
50. Alkire MT, Haier RJ, Shah NK, Anderson CT. Positron emission tomography study of regional cerebral metabolism in humans during isoflurane anesthesia. *Anesthesiology* 1997; 86: 549-57.
51. Alkire MT, Pomfret CJ, Haier RJ, Gianzero MV, Chan CM, Jacobsen BP, et al. Functional brain imaging during anesthesia in humans: effects of halothane on global and regional cerebral glucose metabolism. *Anesthesiology* 1999; 90: 701-9.
52. Michenfelder JD, Sundt TM, Fode N, Sharbrough FW. Isoflurane when compared to enflurane and halothane decreases the frequency of cerebral ischemia during carotid endarterectomy. *Anesthesiology* 1987; 67: 336-40.
53. Wang T, Luo F, Shan R, Zhen Y, Zhao J, Zhang S. Changes of endothelin and calcitonin gene-related peptide during desflurane anesthesia in patients undergoing intracranial aneurysm clipping. *J Neurosurg Anesthesiol* 2004; 16: 236-9.
54. Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vockler J, Dikranian K, et al. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. *Science* 1999; 283: 70-4.
55. Ishimaru MJ, Ikonomidou C, Tenkova TI, Der TC, Dikranian K, Sesma MA, et al. Distinguishing excitotoxic from apoptotic neurodegeneration in the developing rat brain. *J Comp Neurol* 1999; 408: 461-76.
56. Ikonomidou C, Bittigau P, Ishimaru MJ, Wozniak DF, Koch C, Genz K, et al. Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. *Science* 2000; 287: 1056-60.
57. Ikonomidou C, Bittigau P, Koch C, Genz K, Hoerster F, Felderhoff-Mueser U, et al. Neurotransmitters and apoptosis in the developing brain. *Biochem Pharmacol* 2001; 62: 401-5.
58. Wei HF, Kang B, Wei W, Liang G, Meng QC, Li Y, et al. Isoflurane and sevoflurane affect cell survival and BCL-2/BAX ratio differently. *Brain Res* 2005; 1037: 139-47.
59. Eckenhoff RG, Johansson JS, Wei H, Carnini A, Kang B, Wei W, et al. Inhaled anesthetic enhancement of amyloid-beta oligomerization and cytotoxicity. *Anesthesiology* 2004; 101: 703-9.
60. Gu Y, Misonou H, Sato T, Dohmae N, Takio K, Ihara Y. Distinct intramembrane cleavage of the beta-amyloid precursor protein family resembling gamma-secretase-like cleavage of Notch. *J Biol Chem* 2001; 276: 35235-8.
61. Zhao Y, Liang G, Chen Q, Joseph DJ, Meng Q, Eckenhoff RG, et al. Anesthetic induced neurodegeneration mediated via inositol 1,4,5-Trisphosphate receptors. *J Pharmacol Exp Ther* 2010; 333: 1-9.
62. Xie Z, Dong Y, Maeda U, Moir RD, Xia W, Culley DJ, et al. The inhalation anesthetic isoflurane induces a vicious cycle of apoptosis and amyloid beta-protein accumulation. *J Neurosci* 2007; 27: 1247-54.
63. Dong Y, Zhang G, Zhang B, Moir RD, Xia W, Marcantonio ER, et al. The common inhalational anesthetic sevoflurane induces apoptosis and increases beta-amyloid protein levels. *Arch Neurol* 2009; 66: 620-31.
64. Zhang GH, Dong YL, Zhang B, Ichinose F, Wu X, Culley DJ, et al. Isoflurane-induced caspase-3 activation is dependent on cytosolic calcium and can be attenuated by memantine. *J Neurosci* 2008; 28: 4551-60.
65. 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: 243-50.
66. Wei HF, Liang G, Yang H, Wang QJ, Hawkins B, Madesh M, et al. The common inhalational anesthetic isoflurane induces apoptosis via activation of inositol 1,4,5-trisphosphate receptors. *Anesthesiology* 2008; 108: 251-60.
67. Liang G, Wang QJ, Li Y, Kang B, Eckenhoff MF, Eckenhoff RG, et al. A presenilin-1 mutation renders neurons vulnerable to isoflurane toxicity. *Anesth Analg* 2008; 106: 492-500.
68. 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. *Toxicol Appl Pharmacol* 2011; 250: 291-8.
69. Palanisamy A, Baxter MG, Keel PK, Xie Z, Crosby G, Culley DJ. Rats exposed to isoflurane in utero during early gestation are behaviorally abnormal as adults. *Anesthesiology* 2011; 114: 521-8.
70. Jevtic-Todorovic V, Hartman RE, Izumi Y, Benschhoff ND, Dikranian K, Zorumski CF, et al. Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. *J Neurosci* 2003; 23: 876-82.
71. Yon HY, Daniel-Johnson J, Carter LB, Jevtic-Todorovic V. Anesthesia induces neuronal cell death in the developing rat brain via the intrinsic and extrinsic apoptotic pathways. *Neuroscience* 2005; 135: 815-27.
72. Sanders RD, Xu J, Shu Y, Fidalgo A, Ma D, Maze M. General anesthetics induce apoptotic neurodegeneration in the neonatal rat spinal cord. *Anesth Analg* 2008; 106: 1708-11.
73. Zhang X, Xue Z, Sun A. Subclinical concentration of sevoflurane potentiates neuronal apoptosis in the developing C57BL/6 mouse brain. *Neurosci Lett* 2008; 447: 109-14.
74. 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: 1325-34.
75. Satomoto M, Satoh Y, Terui K, Miyao H, Takishima K, Ito M, et al. Neonatal exposure to sevoflurane induces abnormal social behaviors and deficits in fear conditioning in mice. *Anesthesiology* 2009; 110: 628-37.
76. Istaphanous GK, Howard J, Nan X, Hughes EA, Mccann JC, McAuliffe JJ, et al. Comparison of the neuroapoptotic properties of equipotent anesthetic concentrations of desflurane, isoflurane, or sevoflurane in neonatal mice. *Anesthesiology* 2011; 114: 578-87.
77. Stratmann G, Sall JW, May LDV, Bell JS, Magnusson KR, Rau V, et al. Isoflurane differentially affects neurogenesis and long-term neurocognitive function in 60-day-old and 7-day-old rats. *Anesthesiology* 2009; 110: 834-48.
78. Zhu C, Gao J, Karlsson N, Li Q, Zhang Y, Huang Z, et al. 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.
79. 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: 1004-9.
80. Culley DJ, Baxter MG, Crosby CA, Yukhananov R, Crosby G. Impaired acquisition of spatial memory 2 weeks after isoflurane and isoflurane-nitrous oxide anesthesia in aged rats. *Anesth Analg* 2004; 99: 1393-7.
81. Culley DJ, Baxter MG, Yukhananov R, Crosby G. Long-term impairment of acquisition of a spatial memory task following isoflurane-nitrous oxide anesthesia in rats. *Anesthesiology* 2004; 100: 309-14.
82. Kalkman CJ, Peelen L, Moons KG, Veenhuizen M, Bruens M, Sinnema G, et al. Behavior and development in children and age at the time of first anesthetic exposure. *Anesthesiology* 2009; 110: 805-12.
83. Wilder RT, Flick RP, Sprung J, Katusic SK, Barbari WJ, Mickelson C, et al. Early exposure to anesthesia and learning disabilities in a population-based birth cohort. *Anesthesiology* 2009; 110: 796-804.
84. Bohnen N, Warner MA, Kokmen E, Kurland LT. Early and midlife exposure to anesthesia and age of onset of Alzheimer's disease. *Int J Neurosci* 1994; 77: 181-5.
85. Muravchick S, Smith DS. Parkinsonian symptoms during emergence from general anesthesia. *Anesthesiology* 1995; 82: 305-7.
86. Deiner S, Silverstein JH. Postoperative delirium and cognitive dysfunction. *Br J Anaesth* 2009; 103 Suppl 1: i41-i46.
87. Gao L, Taha R, Gauvin D, Othman LB, Wang Y, Blaise G. Postoperative cognitive dysfunction after cardiac surgery. *Chest* 2005; 128: 3664-70.
88. Moller JT, Cluitmans R, Rasmussen LS, Houx P, Rasmussen H, Canet J, et al. Long-term postoperative cognitive dysfunction in the elderly ISPOCD1 study. ISPOCD investigators. *International Study of Post-Operative Cognitive Dysfunction*. *Lancet* 1998; 351: 857-61.
89. Pratico C, Quattrone D, Lucanto T, Amato A, Penna O, Roscicano C, et al. Drugs of anesthesia acting on central cholinergic system may cause post-operative cognitive dysfunction and delirium. *Med Hypotheses* 2005; 65: 972-82.
90. Perry E, Walker M, Grace J, Perry R. Acetylcholine in mind: a neurotransmitter correlate of consciousness? *Trends Neurosci* 1999; 22: 273-80.
91. Rasmussen LS, Johnson T, Kuipers HM, Kristensen D, Siersma VD, Vila P, et al. Does anaesthesia cause postoperative cognitive dysfunction? A randomised study of regional versus general anaesthesia in 438 elderly patients. *Acta Anaesthesiol Scand* 2003; 47: 260-6.
92. Papaioannou A, Fraidakis O, Michaloudis D, Balaalis C, Askitopoulou H. The impact of the type of anaesthesia on cognitive status and delirium during the first postoperative days in elderly patients. *Eur J Anaesthesiol* 2005; 22: 492-9.
93. Rortgen D, Kloos J, Fries M, Grotte O, Rex S, Rossaint R, et al. Comparison of early cognitive function and recovery after desflurane or sevoflurane anaesthesia in the elderly: a double-blinded randomized controlled trial. *Br J Anaesth* 2010; 104: 167-74.
94. Abildstrom H, Rasmussen LS, Rentowl P, Hanning CD, Rasmussen H, Kristensen PA, et al. Cognitive dysfunction 1-2 years after non-cardiac surgery in the elderly. ISPOCD group. *International Study of Post-Operative Cognitive Dysfunction*. *Acta Anaesthesiol Scand* 2000; 44: 1246-51.
95. Canet J, Raeder J, Rasmussen LS, Enlund M, Kuipers HM, Hanning CD, et al. Cognitive dysfunction after minor surgery in the elderly. *Acta Anaesthesiol Scand* 2003; 47: 1204-10.
96. Price CC, Garvan CW, Monk TG. Type and severity of cognitive decline in older adults after noncardiac surgery. *Anesthesiology* 2008; 108: 8-17.
97. Johnson T, Monk T, Rasmussen LS, Abildstrom H, Houx P, Korttila K, et al. Postoperative cognitive dysfunction in middle-aged patients. *Anesthesiology* 2002; 96: 1351-7.
98. Steinmetz J, Christensen KB, Lund T, Lohse N, Rasmussen LS. Long-term consequences of postoperative cognitive dysfunction. *Anesthesiology* 2009; 110: 548-55.
99. Head BP, Patel HH, Niesman IR, Drummond JC, Roth DM, Patel PM. Inhibition of p75 neurotrophin receptor attenuates isoflurane-mediated neuronal apoptosis in the neonatal central nervous system. *Anesthesiology* 2009; 110: 813-25.