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Review Article

Inhaled anesthetic sevoflurane: neurotoxicity or neuroprotection in the developing brain

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Abstract: The practice of pediatric anesthesia has changed during the last 30 years with a noticeable reduction in mortality and serious morbidity. This improvement resulted, in part, from the introduction of a new anesthetic agent called sevoflurane. Recently, data from animal and human studies have shown that sevoflurane may have neurotoxic effects on the developing brain, and these effects can cause subsequent neurofunctional impairments and induce learning and memory deficiencies. However, in other studies, sevoflurane has also shown protective effects in the central nervous system (CNS) by inhibiting inflammation and oxidative stress. The present review attempts to resolve this discrepancy and identify the possible underlying mechanism by reviewing the existing preclinical and clinical studies. Although it may not be easy to determine the exact role of sevoflurane, we concluded the variation is not only based on the dose, frequency, duration and developmental stage of exposure but also on the intensity of the noxious stimuli and body conditions. Moreover, the repeated and long-term usage of sevoflurane may be neurotoxic to immature brains, and such procedures should be delayed as much as possible unless the situation is urgent or potentially harmful if not attended to.

Keywords: Anesthesia, sevoflurane, animal research, clinical research, infant, neurotoxicity, neuroprotection

Introduction

Volatile anesthetics are used in millions of young children every year during surgical procedures and imaging studies [1]. During the past two decades, growing data from studies on rodents and nonhuman primates have raised concerns that general anesthetics may cause neurotoxic changes in the developing brain that lead to adverse neurodevelopmental outcomes later in life [2-6]. The safety of anesthesia, which anesthesiologists were once confident about, has now become uncertain. Anesthesiologists have to console the worried parents, who are anxious about the potential risks of anesthetic-induced brain damage, by suggesting that any detrimental effects would be "mild". Nevertheless, the accuracy of this conclusion remains elusive. Sevoflurane is a commonly used volatile anesthetic in pediatric anesthesia, particularly to induce anesthesia, because of its lower blood-gas partition coefficient compared to that of other anesthetics [7]. However, the molecular mechanisms underlying its anesthetic effects remain unclear. It is a γ-aminobutyric acid A ($GABA_A$) receptoragonist and/or N-methyl-D-aspartate (NMDA) receptor antagonist [8]. By depressing synaptic transmission, sevoflurane may lead to an anesthetic state, such as analgesia, sedation, amnesia and unconsciousness. Since the first animal study reported in 1999 that the use of NMDA receptor antagonists during late fetal or early neonatal life triggered widespread apoptotic neurodegeneration and caused neurotoxicity [9], many concerns have been raised about the relationship between anesthetics and neurodevelopment. Drugs that act on $GABA_A$ receptors, including sevoflurane, were confirmed to have a similar negative effect on the developing brain in some animal studies [6, 10, 11]. With extensive laboratory data, anesthesiologists have come to realize that the administration of volatile anesthetics in their routine practice during critical stages of central nervous system development may lead to cell death, impaired neuro-

genesis and synaptic growth and cognitive deficits. However, these studies cannot separate the effects of the anesthesia from other factors that can cause neurological damage, such as the children's underlying medical conditions and the stress of the surgery itself. Known as species variation, animal studies may not be fully applicable to humans, and most observational studies in children who underwent anesthesia early in life were retrospective studies. Therefore, whether the inhaled anesthetic sevoflurane can cause long-term impairment of neural function remains controversial. The specific neurofunctional effects of sevoflurane on the developing brain may differ under unique conditions.

To address these concerns, it seems timely to carefully review the currently available evidence for sevoflurane-induced changes in the central nervous system and its pharmacodynamics and pharmacokinetics.

Pharmacodynamics and pharmacokinetics

Sevoflurane is a colorless, volatile, and nonflammable liquid with a characteristic smell. It is stable at room temperature and has a boiling point of 58.6°C [12]. Because of its pleasant odor and the absence of irritation to the airways, sevoflurane can be used for inhalational induction and anesthesia maintenance both in children and adults [13]. MAC values of sevoflurane decrease obviously with age, from 3.3% in neonates and 2.5% in infants and young adults to 1.58%~2.05% in middle-aged adults and approximately 1.45% in adults who are more than 70 years old [14-16]. Sevoflurane as a volatile anesthetic of the ether group has various advantages over other intravenous and inhalation anesthetics. It has a low blood-gas partition coefficient, resulting in a quicker equilibration of the alveolar-to-inspiratory fraction (F_A/F_I ratio) than with enflurane and isoflurane as there is an inverse relationship exists between the blood-gas partition coefficient of a volatile anesthetic and the time required for the inspired and alveolar concentrations to reach equilibrium [17]. The maximum inspired concentration that was permitted in the design of the sevoflurane vaporizer was 8%. This was based on the pharmacokinetics of sevoflurane, its MAC, and its flammability in oxygen (i.e., under extreme laboratory conditions, 11% sevoflurane could support combustion). In humans, 95% to 98% of sevoflurane is eliminated through the lungs, while 2% to 5% of sevoflurane is metabolized by the liver, resulting in the formation of inorganic fluoride and the organic fluoride metabolite hexafluoroisopropanol [18].

Preclinical studies of sevoflurane-induced neurotoxicity in the developing brain

Experimental studies in animals (rats, mice, and nonhuman primates) have shown that exposure of the developing mammalian brain to the commonly used anesthetic agent sevoflurane during critical developmental periods can lead to neuronal apoptosis or neurodegeneration *in vitro* and measurable neurofunctional impairment and learning and memory deficiencies *in vivo* [11, 19-21]. The sevoflurane-induced neurotoxicity is dose, frequency and time dependent. As the CNS requires the proper formation of exquisitely precise neuronal circuits to function correctly, the window of vulnerability to neurotoxic drugs in rodents occurs primarily during the first 2-3 postnatal weeks when the most intense phase of synaptogenesis takes place in the cerebral cortex [22, 23]. Qiu et al. made the observation that the viability of neural stem cells obviously decreased and cytotoxicity increased in a time-dependent manner after exposure to 1 MAC sevoflurane. The protein level of $GABA_A$ receptor, pro-apoptotic protein Bax and apoptosis protein Caspase-3 increased while anti-apoptotic protein Bcl-2 decreased [20]. This anesthesia-induced neurodegeneration is not caused by cellular energy failure and necrosis, similar to what is observed during ischemic stroke, but rather by a cellular suicide program, termed apoptosis. Shen et al. first reported that anesthesia with 3% sevoflurane two hours daily for three days in postnatal day 6 (PND6) mice induced cognitive impairment detected at a later time (PND30 to PND36); however, anesthesia with 3% sevoflurane for two hours for only one day in young (PND6) mice did not induce cognitive impairment in the mice [24]. This study strongly demonstrated that multiple exposures (e.g., three times), but not a single exposure to anesthesia at an early age increased the risk of cognitive impairment in the mice. The hippocampus, part of a brain system responsible for learning and memory, has

been shown to be an important target of general anesthetics. It has been suggested that neuronal plasticity impairment could underlie the cognitive abnormalities. In a recent animal study, Xiao et al. found that neonatal rats exposed to 3% sevoflurane for 6 hours resulted in reduced spine density of apical dendrites along with elevated expression of synaptic vesicle-associated proteins (SNAP-25 and syntaxin), and synaptic ultrastructure damage in the hippocampus. The electrophysiological evidence indicated that hippocampal long-term potentiation, but not long-term depression, was inhibited and that learning and memory performances were impaired in two behavioral tasks (i.e., Morris water maze task and novel-object recognition task) in the sevoflurane 6 h group. In contrast, lesser structural and functional damage in the hippocampus was observed in the sevoflurane 1 h group [8]. At the same time, Amrock et al. showed repeated exposure (three times for 2 h) to sevoflurane resulted in greater synaptic loss relative to a single 2 h exposure and the magnitude of synaptic loss induced by three 2 h exposures was more profound than that of a single 6 h exposure [25]. The notion of a "threshold effect" may explain the functional differences of exposure duration on synaptic plasticity [24, 26]. All these results provide further evidence that different durations of anesthesia exposure could have differential effects on neuronal plasticity and neurocognitive performance. Physiological disturbances during anesthetic exposure can also contribute to potential neurotoxicity [27]. In a recent study, postnatal 14 day rats in spontaneously breathing groups treated with either 1.7% isoflurane or 2.4% sevoflurane for 4 hours were found to have a higher internal environment disturbance (e.g., hypercapnia, acidosis and hyperglycemia) than the control group. Meanwhile, behavioral observations and neurocognitive tests were indicative of their potential neurotoxicity.

Data from studies in primates, which more closely reflect humans, have also been collected. In rhesus monkeys, Raper et al. found the frequency of anxiety-related behaviors was significantly higher in those monkeys with multiple exposures to sevoflurane in infancy, and this may reflect the long-term adverse effects of anesthesia [28]. In a subsequent study, the analysis of maternal behavior in their study population reinforced their conclusions that altered emotional behavior was attributable to a long-term effect of exposure to anesthesia, rather than some other difference between anesthesia and control conditions related to the logistics of the different treatments [29]. In addition to behavioral studies, the potential adverse effects of sevoflurane on the developing rhesus monkey brain were also evaluated at transcriptional, molecular, and cellular levels, using DNA microarrays, lipidomic analysis, Luminex protein assays, and histological observations. The results demonstrated that a clinically relevant concentration of sevoflurane (2.5%) was capable of inducing and maintaining an effective surgical plane of anesthesia in the developing nonhuman primate and that a prolonged exposure of 9h resulted in profound changes in gene expression, cytokine levels, lipid metabolism and, subsequently, neuronal damage [11].

However, in another study, it seemed that neonatal exposure to sevoflurane for 5 h may not cause learning and memory deficits and behavioral abnormalities in the childhood of Cynomolgus monkeys [30]. Different species of monkeys and different frequency and exposure times may contribute to the different findings in these studies.

Preclinical studies of sevoflurane-induced neuroprotection in the developing brain

Inhaled anesthetics including sevoflurane, isoflurane, desflurane, halothane, and enflurane have been demonstrated to provide protective effects on various diseased states such as renal ischemia/reperfusion injury, acute lung injury, glucose-induced oxidative stress and cardiac injury in animal models [31-34]. In addition, inhaled anesthetics have also shown neuroprotection in several stroke models including neonatal hypoxic ischemic brain damage (HIBD), middle cerebral artery occlusion (MCAO), subarachnoid hemorrhage (SAH), intracerebral hemorrhage and traumatic brain injury [35-38]. Neonatal HIBD may be characterized as an injury that occurs in the immature brain, resulting in neonatal death and neurobehavioral impairments. HIBD may cause apoptosis and necrosis of hippocampal neurons by a cascade of damaging reactions, thus impairing learning and memory. Recently, sevoflurane postconditioning has been identified to signifi-

cantly improve the long-term learning and memory of neonatal HIBD rats and increase the number of surviving neurons in the CA1 and CA3 hippocampal regions [39]. Ren et al. also confirmed that sevoflurane postconditioning dose-dependently reduced brain tissue loss observed 7 days after neonatal HIBD [40].

Nociceptive stimulation, which is inevitable during pediatric surgery, must also be considered in anesthesia-induced neurofunctional outcomes [41]. In neonatal rodents, brief inflammatory pain leads to increased neuronal activation and cell death, particularly in immature animals [42]. Severe pain and tissue injury in the postnatal period were also verified to cause neuroplastic changes to the CNS in the animal model [43]. Fetuses and neonates subjected to pain and stress at an early stage were reported to have a high risk of adverse outcomes later in life, such as abnormalities in cognitive function and changes in emotional and psychosocial functions [25, 28]. Therefore, the application of sevoflurane in pediatric surgery, such as other anesthetic agents, may alleviate nociceptive stimulation and therefore partial neurofunctional impairment can be reduced. The relative protective effect of sevoflurane might depend on the balance between the depth of anesthesia and the extent of the injury.

Mechanism of sevoflurane-induced neurotoxicity or neuroprotection in the developing brain

While it has been accepted by an increasing number of anesthesiologists that long-term and multiple exposures to high doses of sevoflurane may cause neurotoxicity in the developing brain, the underlying mechanism by which it takes effect remains controversial. As different cell and animal studies through different experimental approaches may lead to different results, it is hard to discern the exact mechanism of sevoflurane-induced neurotoxicity. Currently, several mechanisms are thought to be responsible for the deleterious effects of sevoflurane in the developing brain.

First of all, sevoflurane has been shown to block NMDA receptors and enhance $GABA_A$ receptors [8, 44]. These two neurotransmitter receptors play a pivotal role in neural development and participate in various processes, including the development and differentiation of the nervous system, learning and memory, and synaptic plasticity [45]. The interaction of the glutamatergic and GABAergic systems is essential for many cognitive behaviors. NMDA receptor activation can obviously improve hippocampal-dependent spatial learning and memory. Thus, sevoflurane and other noncompetitive NMDA receptor antagonists induce deficits in learning and cognitive performances [24]. Previous studies have also shown that volatile anesthetics, such as isoflurane and sevoflurane, enhance $GABA_A$ receptor-mediated inhibition, suggesting that general anesthesia is produced, at least in part, by enhancing neural inhibition mediated by $GABA_A$ receptors.

Secondly, sevoflurane exposure appears to accelerate the physiological apoptosis of neurons, which may naturally occur in the developing brain. Anti-apoptotic protein Bcl-2 was inhibited and pro-apoptotic members such as Caspase-3 and Bax were enhanced in some sevoflurane-treated models [20]. The abnormal increase of apoptotic neurons, especially hippocampal neurons in the developing brain, caused cognitive dysfunction at later developmental stages. The FASL-FAS signaling pathway, as a major regulator of apoptosis may be involved in the process of neurofunctional impairment. Young FASL-knockout mice exhibited attenuated sevoflurane-induced neuron apoptosis, neurogenesis inhibition, and learning and memory impairment in a recent animal study [46].

And then, sevoflurane induces a transient hyperphosphorylation of tau protein that is associated with neurotoxicity in neonatal rats. Tau proteins are members of a family of microtubule-associated proteins, which are important in the assembly of microtubules, contribute to axonal integrity in mature neurons, and perform functions at the dendritic and nuclear level in neurons. At the early stage of neural development, sevoflurane exposure could induce an increase in the levels of tau mRNA as well as excessive phosphorylation of tau protein at Ser396 and Ser404 in the hippocampus of neonatal rats [19]. These hippocampal neuron microtubules were significantly changed and became disorganized following sevoflurane exposure. The hippocampus is involved in learning and the consolidation of explicit memories from short-term memory to cortical memory storage for the long term. Excessive phos-

phorylation of tau protein and microtubular disarray in the hippocampus are suggestive of a novel mechanism of sevoflurane-induced neurotoxicity in neurodevelopment.

Next, the dysregulation of intracellular Ca^{2+} homeostasis caused by sevoflurane should also be mentioned. The endoplasmic reticulum (ER) is a major Ca^{2+} pool in most cells, including neurons. Cytosolic Ca^{2+} plays an important role as an intracellular messenger and excessive cytosolic Ca^{2+} activates calpain and caspases, resulting in cell death [47]. Exposure to sevoflurane induces a release of Ca^{2+} from the ER into the cytosol. Aberrant Ca^{2+} mobilization may alter the protein-folding environment in the ER, causing ER stress. ER stress beyond the adaptive range can cause cellular dysfunction and death, resulting in neurodegenerative diseases [48].

Finally, synaptogenesis, synaptic transmission and synaptic plasticity are susceptible to the effects of drugs and, during rapid synaptogenesis, neurons are highly sensitive to perturbations in their synaptic environment. The effects of volatile anesthetics, including sevoflurane, on precise neuronal cytoarchitecture at a developmental stage were evaluated by Adrian et al. [49]. They demonstrated that while sevoflurane did not alter important morphofunctional aspects of the gross dendritic arbor of layer 5 pyramidal neurons, it significantly increased dendritic spine density on apical and basal dendritic shafts of these cells. TEM photomicrographs also showed the ultrastructure of synapses in sevoflurane-exposed groups were negatively affected, including reduced numbers of synapses, widened synaptic clefts, and other pathological features.

The neuroprotective mechanism of sevoflurane is based on its anti-inflammatory, anti-oxidative, and analgesic effects. Both preconditioning and post-conditioning with sevoflurane have been shown to be neuroprotective in the setting of neonatal hypoxia-ischemia in multiple animal studies [39, 40, 50], although conclusive human studies are lacking to date. These mechanisms include activation of ATP-dependent potassium channels, the PI3K/AKT/GSK-3β and PI3K/AKT/CREB pathways, reduction of excitotoxic stressors and the cerebral metabolic rate, augmentation of peri-ischemic cerebral blood flow and up-regulation of nitric oxide synthase [39, 51-53].

Interestingly, sevoflurane can induce both an increase and decrease in the activation of the AKT/GSK-3β pathway in the brain tissues of mice and H4 human neuroglioma cells. A short exposure time to sevoflurane treatment might produce neuroprotection via activation of the AKT/GSK3β signaling pathway, but a long exposure time to sevoflurane anesthesia could induce neurotoxicity via inhibition of the AKT/GSK3β signaling pathway [54]. This finding may partly explain why anesthesia with multiple exposures of the commonly used inhalation anesthetic sevoflurane induces neuroinflammation and cognitive impairment in young mice, but anesthesia with a single exposure to sevoflurane does not.

Clinical studies of neurodevelopmental effects of sevoflurane exposure during early childhood

This alarming preclinical evidence of sevoflurane-induced neurotoxicity from *in vitro* and *in vivo* animal studies raises serious concern that the use of the inhaled anesthetic sevoflurane in children might lead to long-term adverse neurodevelopmental outcomes. Although experimental models in animals may not truly represent the pathophysiological processes in humans, as there is known interspecies variability, the potential neurotoxicity of anesthetic agents is widely considered to have effects on cognitive functions in patient populations with a vulnerable neuronal cache. This includes infants and children, who are undergoing critical stages of neurodevelopment and rapid synaptogenesis. Some links between anesthesia exposure to immature brains and subsequent cognitive deficiencies have been identified based on several retrospective cohort studies. Three of these studies derived their data from the Olmstead CountyBirth Cohort [55-57]. Wilder et al. examined the effects of postnatal anesthesia before age 4 and found that learning disability (math, language, or reading) was higher in those children with multiple anesthesia exposures and surgery before age 4. Using the same cohort, Flick et al. also detected that repeated exposure to anesthesia and surgery before age 2 was a significant independent risk factor for the later development of learning dis-

ability. Sprung et al. studied the Olmstead County Birth Cohort to specifically determine the neurocognitive effects of prenatal/fetal exposure during delivery and found that children exposed to general or regional anesthesia during a cesarean delivery were not more likely to develop learning disabilities compared to children delivered vaginally. This finding may suggest that brief perinatal exposure to anesthetic drugs does not adversely affect long-term neurodevelopmental outcomes. Using a prospective birth cohort from the Western Australian Pregnancy Cohort (Raine) Study, Caleb et al. examined the association between exposure to anesthesia in children younger than 3 years of age and three types of outcomes at 10 years of age [58]. They found that, when assessing cognition in children exposed to anesthesia at an early age, the results may depend on the outcome measures used. Specifically, neuropsychological testing and the International Classification of Diseases, 9th Revision, Clinical Modification-coded clinical outcomes were able to measure deficits at age 10 years in children exposed to anesthesia before age 3 years, whereas no differences could be identified using any academic achievement scores. All these studies mentioned above were of great significance but also had several limitations. The study cohorts did not reflect the patient characteristic and cultural and racial/ethnic diversity of the overall population. For lack of detailed anesthetic information and medical records, we did not know the exact anesthetic agents used in these retrospective cohort studies and the relationship between sevoflurane exposure and neurotoxic changes during early childhood. Fortunately, there are two large-scale studies underway that are attempting to address the issue of anesthetic neurotoxicity in infancy [59]. It is anticipated that the results of two randomized controlled trials; i.e., the General Anesthesia Compared to Spinal Anesthesia (GAS) study and the Pediatric Anesthesia and NeuroDevelopment Assessment (PANDA) study, will give more insight into the problem. The GAS study is an international multi-site assessor-masked randomized controlled trial and sevoflurane was used as the only anesthetic in this trial. Between Feb 9, 2007, and Jan 31, 2013, 722 infants who had inguinal hernias were randomly assigned to receive either awake-regional anesthesia or sevoflurane-based general anesthesia at 28 hospitals in Australia, Italy, the US, the UK, Canada, the Netherlands, and New Zealand. Except for data from 190 children who were excluded for various reasons, the available information was collected from 238 children in the awake-regional group and 294 in the sevoflurane-based general anesthesia group. Davidson et al. reported the secondary outcome of this trial and found no evidence that just less than 1 h of sevoflurane anesthesia in infancy increases the risk of an adverse neurodevelopmental outcome at 2 years of age compared with awake-regional anesthesia [60]. However, it should be noted that this was only an analysis of a secondary outcome with the primary outcome planned at 5 years of age, and in view of the limited sensitivity of developmental assessment at 2 years of age, this trial did not provide the definitive answer. The PANDA study is a sibling-matched observational cohort study that examined whether a single anesthesia exposure in healthy children younger than 3 years is associated with an increased risk of impaired global cognitive function (IQ) as the primary outcome, and abnormal domain-specific neurocognitive functions and behavior as secondary outcomes at ages 8 to 15 years. Exposed children (n=105) had a single episode of general anesthesia before 3 years for elective inguinal hernia surgery and were 36 weeks gestational age or older at birth. The unexposed cohort (n=105) were biologically related siblings closest in age (within 3 years) to the exposed child, also 36 weeks gestational age or older at birth but with no anesthesia exposure before 3 years. The study found that mean IQ scores were not significantly different between the exposed and unexposed siblings, with both groups scoring somewhat higher than average. As longer or multiple exposures were not examined in these trials, further research is needed urgently.

Summary

The safety of anesthesia for pediatric surgery has been an important concern for decades. Here we reviewed the current animal and clinical evidence on the neurofunctional effect of sevoflurane. Although it seems a single exposure to sevoflurane did not cause a deleterious impact on the developing brain in the latest GAS study, its potential hazards should not be ignored. From the aforementioned research

studies, we postulated that high dose, long-term and multiple exposures to sevoflurane can be neurotoxic to immature brains, but under some pathological states, such as HIBD, certain exposures may have beneficial effects. Thus, use of the inhaled anesthetic sevoflurane in children at specific developmental states should be considered with prudence. We recommend that surgical procedures performed under sevoflurane anesthesia should be avoided in children under 3 years of age and use should wait until the children are more mature, unless the situation is urgent or potentially harmful if not attended to (as the U.S. Food and Drug Administration and the International Anesthesia Research Society have suggested) [61]. Finally, further experimental and clinical studies are required to analyze the effects of the inhaled anesthetic sevoflurane on the brain of the neonate.

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Disclosure of conflict of interest

None.

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References

[1] Istaphanous GK and Loepke AW. General anesthetics and the developing brain. Curr Opin Anaesthesiol 2009; 22: 368-373.

[2] Jiang H, Huang Y, Xu H, Sun Y, Han N and Li QF. Hypoxia inducible factor-1alpha is involved in the neurodegeneration induced by isoflurane in the brain of neonatal rats. J Neurochem 2012; 120: 453-460.

[3] Paule MG, Li M, Allen RR, Liu F, Zou X, Hotchkiss C, Hanig JP, Patterson TA, Slikker W Jr and Wang C. Ketamine anesthesia during the first week of life can cause long-lasting cognitive deficits in rhesus monkeys. Neurotoxicol Teratol 2011; 33: 220-230.

[4] Yan J, Huang Y, Lu Y, Chen J and Jiang H. Repeated administration of ketamine can induce hippocampal neurodegeneration and long-term cognitive impairment via the ROS/HIF-1alpha pathway in developing rats. Cell Physiol Biochem 2014; 33: 1715-1732.

[5] Xiong M, Li J, Alhashem HM, Tilak V, Patel A, Pisklakov S, Siegel A, Ye JH and Bekker A. Propofol exposure in pregnant rats induces neurotoxicity and persistent learning deficit in the offspring. Brain Sci 2014; 4: 356-375.

[6] Shen X, Liu Y, Xu S, Zhao Q, Guo X, Shen R and Wang F. Early life exposure to sevoflurane impairs adulthood spatial memory in the rat. Neurotoxicology 2013; 39: 45-56.

[7] Johr M and Berger TM. Paediatric anaesthesia and inhalation agents. Best Pract Res Clin Anaesthesiol 2005; 19: 501-522.

[8] Xiao H, Liu B, Chen Y and Zhang J. Learning, memory and synaptic plasticity in hippocampus in rats exposed to sevoflurane. Int J Dev Neurosci 2016; 48: 38-49.

[9] Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vockler J, Dikranian K, Tenkova TI, Stefovska V, Turski L and Olney JW. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science 1999; 283: 70-74.

[10] Servick K. Biomedical research. Researchers struggle to gauge risks of childhood anesthesia. Science 2014; 346: 1161-1162.

[11] Liu F, Rainosek SW, Frisch-Daiello JL, Patterson TA, Paule MG, Slikker W Jr, Wang C and Han X. Potential adverse effects of prolonged sevoflurane exposure on developing monkey brain: from abnormal lipid metabolism to neuronal damage. Toxicol Sci 2015; 147: 562-572.

[12] De Hert S and Moerman A. Sevoflurane. F1000Res 2015; 4: 626.

[13] Doi M and Ikeda K. Airway irritation produced by volatile anaesthetics during brief inhalation: comparison of halothane, enflurane, isoflurane and sevoflurane. Can J Anaesth 1993; 40: 122-126.

[14] Mapleson WW. Effect of age on MAC in humans: a meta-analysis. Br J Anaesth 1996; 76: 179-185.

[15] Katoh T and Ikeda K. Minimum alveolar concentration of sevoflurane in children. Br J Anaesth 1992; 68: 139-141.

[16] Nakajima R, Nakajima Y and Ikeda K. Minimum alveolar concentration of sevoflurane in elderly patients. Br J Anaesth 1993; 70: 273-275.

[17] Shiraishi Y and Ikeda K. Uptake and biotransformation of sevoflurane in humans: a comparative study of sevoflurane with halothane, enflurane, and isoflurane. J Clin Anesth 1990; 2: 381-386.

[18] Lerman J. Inhalational anesthetics. Paediatr Anaesth 2004; 14: 380-383.
[19] Hu ZY, Jin HY, Xu LL, Zhu ZR, Jiang YL and Seal R. Effects of sevoflurane on the expression of tau protein mRNA and Ser396/404 site in the hippocampus of developing rat brain. Paediatr Anaesth 2013; 23: 1138-1144.
[20] Qiu J, Shi P, Mao W, Zhao Y, Liu W and Wang Y. Effect of apoptosis in neural stem cells treated with sevoflurane. BMC Anesthesiol 2015; 15: 25.
[21] Satomoto M, Satoh Y, Terui K, Miyao H, Takishima K, Ito M and Imaki J. Neonatal exposure to sevoflurane induces abnormal social behaviors and deficits in fear conditioning in mice. Anesthesiology 2009; 110: 628-637.
[22] Fredriksson A, Archer T, Alm H, Gordh T and Eriksson P. Neurofunctional deficits and potentiated apoptosis by neonatal NMDA antagonist administration. Behav Brain Res 2004; 153: 367-376.
[23] Liu F, Paule MG, Ali S and Wang C. Ketamine-induced neurotoxicity and changes in gene expression in the developing rat brain. Curr Neuropharmacol 2011; 9: 256-261.
[24] Shen X, Dong Y, Xu Z, Wang H, Miao C, Soriano SG, Sun D, Baxter MG, Zhang Y and Xie Z. Selective anesthesia-induced neuroinflammation in developing mouse brain and cognitive impairment. Anesthesiology 2013; 118: 502-515.
[25] Amrock LG, Starner ML, Murphy KL and Baxter MG. Long-term effects of single or multiple neonatal sevoflurane exposures on rat hippocampal ultrastructure. Anesthesiology 2015; 122: 87-95.
[26] Platholi J, Herold KF, Hemmings HC Jr and Halpain S. Isoflurane reversibly destabilizes hippocampal dendritic spines by an actin-dependent mechanism. PLoS One 2014; 9: e102978.
[27] Wu B, Yu Z, You S, Zheng Y, Liu J, Gao Y, Lin H and Lian Q. Physiological disturbance may contribute to neurodegeneration induced by isoflurane or sevoflurane in 14 day old rats. PLoS One 2014; 9: e84622.
[28] Raper J, Alvarado MC, Murphy KL and Baxter MG. Multiple anesthetic exposure in infant monkeys alters emotional reactivity to an acute stressor. Anesthesiology 2015; 123: 1084-1092.
[29] Raper J, Bush A, Murphy KL, Baxter MG and Alvarado MC. Multiple sevoflurane exposures in infant monkeys do not impact the mother-infant bond. Neurotoxicol Teratol 2016; 54: 46-51.
[30] Zhou L, Wang Z, Zhou H, Liu T, Lu F, Wang S, Li J, Peng S and Zuo Z. Neonatal exposure to sevoflurane may not cause learning and memory deficits and behavioral abnormality in the childhood of Cynomolgus monkeys. Sci Rep 2015; 5: 11145.
[31] Kinoshita H, Matsuda N, Iranami H, Ogawa K, Hatakeyama N, Azma T, Kawahito S and Yamazaki M. Isoflurane pretreatment preserves adenosine triphosphate-sensitive K(+) channel function in the human artery exposed to oxidative stress caused by high glucose levels. Anesth Analg 2012; 115: 54-61.
[32] Kim M, Kim M, Kim N, D'Agati VD, Emala CW Sr and Lee HT. Isoflurane mediates protection from renal ischemia-reperfusion injury via sphingosine kinase and sphingosine-1-phosphate-dependent pathways. Am J Physiol Renal Physiol 2007; 293: F1827-1835.
[33] Sun XJ, Li XQ, Wang XL, Tan WF and Wang JK. Sevoflurane inhibits nuclear factor-kappaB activation in lipopolysaccharide-induced acute inflammatory lung injury via toll-like receptor 4 signaling. PLoS One 2015; 10: e0122752.
[34] Lemoine S, Tritapepe L, Hanouz JL and Puddu PE. The mechanisms of cardio-protective effects of desflurane and sevoflurane at the time of reperfusion: anaesthetic post-conditioning potentially translatable to humans? Br J Anaesth 2016; 116: 456-475.
[35] Altay O, Suzuki H, Hasegawa Y, Caner B, Krafft PR, Fujii M, Tang J and Zhang JH. Isoflurane attenuates blood-brain barrier disruption in ipsilateral hemisphere after subarachnoid hemorrhage in mice. Stroke 2012; 43: 2513-2516.
[36] Burchell SR, Dixon BJ, Tang J and Zhang JH. Isoflurane provides neuroprotection in neonatal hypoxic ischemic brain injury. J Investig Med 2013; 61: 1078-1083.
[37] Chen Y, Nie H, Tian L, Tong L, Deng J, Zhang Y, Dong H and Xiong L. Sevoflurane preconditioning-induced neuroprotection is associated with Akt activation via carboxy-terminal modulator protein inhibition. Br J Anaesth 2015; 114: 327-335.
[38] Xiao Z, Ren P, Chao Y, Wang Q, Kuai J, Lv M, Chen L, Gao C and Sun X. Protective role of isoflurane pretreatment in rats with focal cerebral ischemia and the underlying molecular mechanism. Mol Med Rep 2015; 12: 675-683.
[39] Lai Z, Zhang L, Su J, Cai D and Xu Q. Sevoflurane postconditioning improves long-term learning and memory of neonatal hypoxia-ischemia brain damage rats via the PI3K/Akt-mPTP pathway. Brain Res 2016; 1630: 25-37.
[40] Ren X, Wang Z, Ma H and Zuo Z. Sevoflurane postconditioning provides neuroprotection against brain hypoxia-ischemia in neonatal rats. Neurol Sci 2014; 35: 1401-1404.
[41] Sanders RD, Ma D, Brooks P and Maze M. Balancing paediatric anaesthesia: preclinical insights into analgesia, hypnosis, neuroprotec-

tion, and neurotoxicity. Br J Anaesth 2008; 101: 597-609.
[42] Anand KJ, Garg S, Rovnaghi CR, Narsinghani U, Bhutta AT and Hall RW. Ketamine reduces the cell death following inflammatory pain in newborn rat brain. Pediatr Res 2007; 62: 283-290.
[43] Ruda MA, Ling QD, Hohmann AG, Peng YB and Tachibana T. Altered nociceptive neuronal circuits after neonatal peripheral inflammation. Science 2000; 289: 628-631.
[44] Kotani N and Akaike N. The effects of volatile anesthetics on synaptic and extrasynaptic GABA-induced neurotransmission. Brain Res Bull 2013; 93: 69-79.
[45] Aamodt SM and Constantine-Paton M. The role of neural activity in synaptic development and its implications for adult brain function. Adv Neurol 1999; 79: 133-144.
[46] Song Q, Ma YL, Song JQ, Chen Q, Xia GS, Ma JY, Feng F, Fei XJ and Wang QM. Sevoflurane induces neurotoxicity in young mice through FAS/FASL signaling. Genet Mol Res 2015; 14: 18059-18068.
[47] Orrenius S, Zhivotovsky B and Nicotera P. Regulation of cell death: the calcium-apoptosis link. Nat Rev Mol Cell Biol 2003; 4: 552-565.
[48] Komita M, Jin H and Aoe T. The effect of endoplasmic reticulum stress on neurotoxicity caused by inhaled anesthetics. Anesth Analg 2013; 117: 1197-1204.
[49] Briner A, De Roo M, Dayer A, Muller D, Habre W and Vutskits L. Volatile anesthetics rapidly increase dendritic spine density in the rat medial prefrontal cortex during synaptogenesis. Anesthesiology 2010; 112: 546-556.
[50] Luo Y, Ma D, Ieong E, Sanders RD, Yu B, Hossain M and Maze M. Xenon and sevoflurane protect against brain injury in a neonatal asphyxia model. Anesthesiology 2008; 109: 782-789.
[51] Lee YM, Song BC and Yeum KJ. Impact of volatile anesthetics on oxidative stress and inflammation. Biomed Res Int 2015; 2015: 242709.
[52] Deng J, Lei C, Chen Y, Fang Z, Yang Q, Zhang H, Cai M, Shi L, Dong H and Xiong L. Neuroprotective gases--fantasy or reality for clinical use? Prog Neurobiol 2014; 115: 210-245.
[53] Matchett GA, Allard MW, Martin RD and Zhang JH. Neuroprotective effect of volatile anesthetic agents: molecular mechanisms. Neurol Res 2009; 31: 128-134.
[54] Zhang L, Zhang J, Dong Y, Swain CA, Zhang Y and Xie Z. The potential dual effects of sevoflurane on AKT/GSK3beta signaling pathway. Med Gas Res 2014; 4: 5.
[55] Wilder RT, Flick RP, Sprung J, Katusic SK, Barbaresi WJ, Mickelson C, Gleich SJ, Schroeder DR, Weaver AL and Warner DO. Early exposure to anesthesia and learning disabilities in a population-based birth cohort. Anesthesiology 2009; 110: 796-804.
[56] Flick RP, Katusic SK, Colligan RC, Wilder RT, Voigt RG, Olson MD, Sprung J, Weaver AL, Schroeder DR and Warner DO. Cognitive and behavioral outcomes after early exposure to anesthesia and surgery. Pediatrics 2011; 128: e1053-1061.
[57] Sprung J, Flick RP, Wilder RT, Katusic SK, Pike TL, Dingli M, Gleich SJ, Schroeder DR, Barbaresi WJ, Hanson AC and Warner DO. Anesthesia for cesarean delivery and learning disabilities in a population-based birth cohort. Anesthesiology 2009; 111: 302-310.
[58] Ing CH, DiMaggio CJ, Malacova E, Whitehouse AJ, Hegarty MK, Feng T, Brady JE, von Ungern-Sternberg BS, Davidson AJ, Wall MM, Wood AJ, Li G and Sun LS. Comparative analysis of outcome measures used in examining neurodevelopmental effects of early childhood anesthesia exposure. Anesthesiology 2014; 120: 1319-1332.
[59] Sun L. Early childhood general anaesthesia exposure and neurocognitive development. Br J Anaesth 2010; 105 Suppl 1: i61-68.
[60] Davidson AJ, Disma N, de Graaff JC, Withington DE, Dorris L, Bell G, Stargatt R, Bellinger DC, Schuster T, Arnup SJ, Hardy P, Hunt RW, Takagi MJ, Giribaldi G, Hartmann PL, Salvo I, Morton NS, von Ungern Sternberg BS, Locatelli BG, Wilton N, Lynn A, Thomas JJ, Polaner D, Bagshaw O, Szmuk P, Absalom AR, Frawley G, Berde C, Ormond GD, Marmor J, McCann ME; GAS Consortium. Neurodevelopmental outcome at 2 years of age after general anaesthesia and awake-regional anaesthesia in infancy (GAS): an international multicentre, randomised controlled trial. Lancet 2016; 387: 239-250.
[61] Davidson AJ. Anesthesia and neurotoxicity to the developing brain: the clinical relevance. Paediatr Anaesth 2011; 21: 716-721.