Published in final edited form as:

Anesthesiol Clin. 2016 September ; 34(3): 439–451. doi:10.1016/j.anclin.2016.04.001.

General Anesthetics and Neurotoxicity: How much do we know?

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Synopses

Over a decade ago we have come to alarming findings that an exposure of the very young and very old animals to clinically-used general anesthetics could be detrimental to their brain. The evidence presented in respectable studies suggested that the exposure to commonly-used gaseous and intravenous general anesthetics induces the biochemical and morphological changes in the immature and aging neurons ultimately resulting in their demise. More alarming was the demonstration of significant cognitive and behavioral impairments noted long after the initial anesthesia exposure. Although the causality between behavioral sequelae and neuro-morphological damage cannot be established with certainty, the concerns were raised regarding the safety of general anesthetics, a class of agents that were once considered to be innocuous for a young and aging brain. This article provides a brief overview of anesthesia-induced developmental neurotoxicity and short commentary on the effects of general anesthesia on the aging brain.

Keywords

learning and memory; immature brain; aging brain; synaptogenesis; GABA; NMDA; synaptic transmission

I. Introduction

Over the past several decades the field of clinical anesthesiology has enjoyed an enormous growth with seemingly limitless ability to take care of patients in all age groups regardless of their health status. Over 300 million complex and very painful procedures are being performed annually. To achieve the level of unconsciousness and insensitivity to pain during various interventions, human brains are being subjected to a variety of general anesthetics, alone or in combinations. The long-term outcomes of this practice are being actively investigated and scrutinized in a variety of preclinical and clinical studies.

II. The physiology of neurotransmitters and their receptors during brain development

All key elements of neuronal development depend on fine balance between various neurotransmitters. In particular, it has been known that two major neurotransmitters, glutamate and GABA, control all aspects of neuronal migration, differentiation, maturation and synaptogenesis, the key components of mammalian brain development (1).

Synaptogenesis involves massive dendritic branching and formation of trillion synaptic contacts between neurons thus enabling the formation of meaningful neuronal circuitries and orderly neuronal maps. The processes by which axonal and dendritic projections find the right 'target' and the most appropriate pathways for growth are very complex and not within the scope of this article. However, it is worth mentioning that the synaptogenesis and the development of neuronal processes is based on activity-dependent remodeling suggesting that neuronal firing and interneuronal communications are crucial for timely and proper synaptogenesis (2,3). All aspects of developmental synaptogenesis are tightly controlled by glia, which actively participate in neuron-glia signaling while providing an appropriate milieu for neuron-neuron interaction (4). The electrical activity and synaptic signaling are strategically important during synaptogenesis, so much so that the major inhibitory neurotransmitter, GABA serves as an excitatory neurotransmitter during early stages of synaptogenesis (5). Although synapses are very pliable and undergo constant remodeling where new synapses are being formed and others are being pruned away throughout one's life, the bulk of fundamental neuronal networks and synaptic contacts are formed during developmental synaptogenesis. In humans that time period occurs during the last trimester of *in utero* life and the first few years of post-natal life while being most intense during the first several months of post-natal life (3).

III. Unphysiologic modulation of the neurotransmitters and their receptor systems during brain development may result in neurotoxic damage to the immature neurons

The fact that neuronal activity and communication are crucial for proper formation of synaptic contacts and for the establishment of stable receptor structures which form the foundation for cognitive and behavioral development brings into focus general anesthetics, a class of drugs commonly used in modern anesthesia. Their main goal is unphysiologically 'switching off' or 'turning down' neuronal communication for the purpose of achieving amnesia, analgesia and hypnosis. Both gaseous (e.g. nitrous oxide, isoflurane, sevoflurane, desflurane, etc.) and intravenous (e.g. benzodiazepines, barbiturates, propofol, etomidate, etc.) anesthetics are frequently used for the purpose of assuring the patients' comfort during painful intervention.

General anesthetics are very potent and effective in transiently inhibiting neuronal communication (6). However, despite their widespread use, the mechanisms of their anesthetic action are not fully understood. Based on the studies published over the last few decades, it appears that there are specific cellular targets through which general anesthetics act (7). In general, enhancement of inhibitory synaptic transmission and/or inhibition of excitatory synaptic transmission have been reported. In particular, many intravenous anesthetics, among them barbiturates, benzodiazepines, propofol, and etomidate (7,8), as well as inhalational volatile anesthetics such as isoflurane, sevoflurane, desflurane, and halothane (9,10), promote inhibitory neurotransmission by enhancing GABA_A-induced currents in neuronal tissue. For this reason, they are often referred to as GABAergic agents. On the other hand, a small number of intravenous anesthetics [e.g. phencyclidine (PCP) and its derivative, ketamine] (11) and inhalational anesthetics, nitrous oxide and xenon (12,13)

inhibit excitatory neurotransmission by blocking N-methyl-D-aspartate (NMDA) receptors, a subtype of glutamate receptors.

Although general anesthetics are powerful modulators of GABA and glutamate and thus cause significant imbalance in their functioning, only recently has it been recognized that general anesthetics, given in clinically relevant concentrations and combinations, are potentially damaging to neuronal cells in adult and immature brains thus suggesting that these agents are potentially deleterious. This newly developing knowledge suggests the importance of maintaining a fine balance in neurotransmitters release and their receptor activation during critical stages of synaptogenesis.

IV. The neurotoxic potential of general anesthetics in the developing mammalian brain

More than 3 million general anesthetics are administered to pediatric patients in the USA alone (14). In the majority of cases, the administration of general anesthesia is the result of an exponential increase in the frequency of operating suite visits for relatively minor surgical interventions. However, heroic attempts to save very ill infants and young children have led to multiple surgeries and prolonged, deep sedations in intensive care units, adding to the number of anesthesia interventions during a delicate period of human development. Also, since premature births account for more than 12% of the overall live birth rate (www.marchofdimess.com), and since an increased proportion of these premature babies, some of them as young as 20 weeks post-conception, survive in neonatal intensive care units, more of them undergo anesthesia on a daily basis (e.g. surgical interventions, prolonged ICU sedation, etc.).

It would be overly simplistic to consider children as small adults when it comes to anesthesia management. Of particular interest for this article is the fact that the human central nervous system (CNS) is not completely developed at birth. The brain weighs approximately 335 g at birth, doubles in size by 6 months of age, and almost triples by 12 months (2). This is known as the brain growth spurt period (3), or the period of synaptogenesis. During this time trillions of synaptic connections are being formed, while each neuron is vastly expanding its dendritic surfaces to accommodate incoming axonal contacts. Because of this postnatal maturation of the CNS, the effects of general anesthetics on the immature brain deserve careful consideration.

Many years ago, it was noted that children who, during first two years of life, had undergone surgery and general anesthesia were experiencing a higher incidence of postoperative psychological disturbances than did older children (15,16). It was assumed that these disturbances were a consequence of the emotional and physical trauma of surgery rather than an effect of anesthesia techniques or agents. The possibility that anesthetic agents *per se* might have damaging effects on the developing brain was not rigorously addressed or systematically investigated. Now, however, rapidly emerging animal and human data suggest that common general anesthetics could be detrimental to the developing brain.

During early stages of brain development neurons have to establish their final destination and to form meaningful connections which constitute the morphological and physiological bases for the formation of functional neuronal circuitries. Neurons that are not successful in making meaningful connections are considered redundant and are destined to die by programmed cell death, i.e. apoptosis, which occurs naturally during normal development of the mammalian CNS. Although this is a physiological process, apoptosis during normal development is tightly controlled, resulting in the removal of only a small percentage of neurons (17). A disturbance of the fine balance between glutamatergic and GABAergic neurotransmission by excessive depression of neuronal activity and unphysiological changes in the synaptic environment during a crucial stage of brain development may constitute a generic signal for developing neurons to “commit suicide.” Since the goal of surgical anesthesia is to render the patient unconscious and insentient to pain, the ultimate question becomes whether general anesthetics at doses that ensure substantial receptor occupancy and profound depression of neuronal activity could be promoting excessive activation of neuroapoptosis and the death of large populations of developing neurons.

It appears (17–20) that general anesthetics do indeed cause significant and widespread neuroapoptotic degeneration of developing neurons in various mammalian species. The peak of vulnerability to anesthesia-induced neuroapoptosis in each species coincides with its peak of synaptogenesis, with much less vulnerability observed during late stages of synaptogenesis (20,21). Aside from prominent caspase-3 staining, a hallmark of apoptotic death which could be detected at the light microscopic level, detailed examination at the ultrastructural level suggests that the initial insult, visible mainly in the nucleus, is marked by the clamping of chromatin, followed by disruption of the nuclear membrane, intermixing of the cytoplasm and nucleoplasm, and the formation of apoptotic bodies (17) (Fig. 1). In recent years, we have put forward a considerable effort into elucidating the mechanisms of anesthesia-induced developmental neuroapoptosis and found it to involve several cascades of cellular events that ultimately lead to neuronal deletion and impairment of proper synapse formation.

V. The importance of anesthesia-induced modulation of neurotransmitters in the formation of neuronal networks

The morphological changes described thus far represent substantial changes in neuronal structure that can be detected easily using histological assessments. An important issue that has been brought to light on several occasions is that seemingly subtle changes that cannot be detected morphologically remain in surviving ‘normal’ neurons after the grossly damaged neurons have been removed. Based on presently available evidence, these neurons may not be truly functional; i.e., their communications may be faulty. We first noted that an early exposure to general anesthesia causes long-term impairment in synaptic transmission in the hippocampus of adolescent rats (postnatal day 27–33) exposed to anesthesia at the peak of their synaptogenesis (postnatal day 7) (17). In particular, long-term potentiation was impaired significantly despite the presence of robust short-term potentiation. This observation suggested a long-lasting disturbance in neuronal circuitries in the young hippocampus, a brain region that is crucial for proper learning and memory development. A

deficit in long-term potentiation was confirmed when synaptic transmission was examined using patch clamp recordings of evoked inhibitory post synaptic current (eIPSC) and evoked excitatory post synaptic current (eEPSC) by recording from the pyramidal layer of control and anesthesia-treated rat subiculum, an important component of the hippocampal complex. Again it was noted that anesthesia-treated animals suffered from impaired synaptic transmission with inhibitory transmission affected significantly (22).

Although the precise mechanisms responsible for the long-lasting changes in synaptic communication post-anesthesia remain to be deciphered, some recent findings suggest that anesthetics impair axon targeting and inhibit axonal growth cone collapse, resulting in lack of proper response to guidance cues, thus causing errors in axon targeting (23) (Fig. 2).

VI. Excessive modulation of neurotransmitters during critical stages of brain development and long-term effects on animal behavior

The above pathomorphological findings make it clear that anesthesia exposure results in neuronal deletion (20,24) and long-lasting impairment of synapses in vulnerable brain regions (25–28). The ultimately question is whether and how these observations translate to lasting effects on behavior and the short answer appears to be that they do. Development of cognitive abilities of animals exposed to general anesthetics at the peak of synaptogenesis, lagged behind those of controls, with the gap widening into adulthood (Fig. 3). Even intravenous general anesthetics like propofol or thiopental in combination with ketamine, (but not singly) at post-natal day 10, alters mouse behavior later in young adulthood (29). Similar adult behavioral deficits were noted when mice were exposed at post-natal day 10 to a cocktail containing ketamine and diazepam (30).

Although anesthesia cocktails seem to be most detrimental, ketamine given alone during early stages of brain development in rats also caused later deficits in habituation, and in learning and memory (30). When anesthetic agents with GABAergic and N-methyl-D-aspartate (NMDA) antagonist properties are combined, which is done frequently in the clinical setting (for example, nitrous oxide and volatile anesthetics or propofol and ketamine), cognitive deficits are more profound (17,29,30). Although causality is difficult to establish, it is reasonable to propose that anesthesia-induced neuroapoptosis, is at least in part, responsible for the observed cognitive deficits. Some recently published studies suggest that multiple, shorter-lasting exposures to anesthesia (e.g. sevoflurane, ketamine) during vulnerable periods cause significant impairments in morphological and neurocognitive development (31,32).

General anesthesia is rarely administered in the absence of surgery, and its associated pain and tissue injury. Thus, the reported neurotoxic potential of general anesthesia during brain development needs to be confirmed in the setting of surgical stimulation. Using clinically relevant concentrations of nitrous oxide and isoflurane Shu and his colleagues (33) found that nociception enhanced neuroapoptosis and worsened long-term cognitive impairments when compared to anesthesia alone. These results would seem to undermine the hope that surgical stimulation may somehow be ‘protective’ against anesthesia-induced neurotoxicity.

Results from rodent studies are known to translate poorly to humans, but newly emerging behavioral studies with non-human primates are beginning to suggest that translation is likely. Dr. Paule and his colleagues (34) have examined the effects of continuous neonatal (age 5 or 6 days) ketamine infusion (24 h) sufficient to maintain a light surgical plane of anesthesia on behavioral development in primates. They observed that ketamine-treated primates exhibit long-term disturbances in all important aspects of cognitive development, such as learning, psychomotor speed, concept formation and motivation. These effects occurred despite an absence of physiological or metabolic trespass. Although 24 hours of anesthetic exposure would be considered unusual, it certainly occurs, especially in critically ill patients of all ages. A very recent monkey study shows that multiple exposures to sevoflurane during the first month of postnatal life resulted in higher frequency of anxiety-related behaviors later in life (at 6 months of age) suggesting the impairment of emotional behavior (35).

VII. General anesthesia and cognitive and behavioral development in humans after childhood surgery

Backman and Kopf (36) were the first to suggest a relationship between anesthesia and long-term cognitive delay. In this report children were anesthetized with ketamine and halothane for removal of congenital nevocytic nevi, a fairly minor procedure. Nevertheless, the authors reported an increased incidence of cognitive impairment, described as regressive behavioral changes, lasting up to 18 months after the procedure. Children younger than 3 years were the most sensitive. These authors voiced concern that general anesthetics may cause long-term cognitive effects.

Clinical investigations are still at an early stage, but the evidence that has emerged over the last few years suggests potentially detrimental effects of anesthetic exposure in young children on subsequent behavioral and cognitive development.

In a population-based retrospective birth-cohort study of 5,357 children, Wilder *et al.* (37) found that children who received two or more general anesthetics before the age of 4 years were at increased risk of learning disability as adolescents (Figure 4). Moreover, the risk increased with longer cumulative exposure duration (more than 2 hours). Of particular concern is their finding that the cohort exposed to anesthesia before the age of 4 years had cognitive scores that were two standard deviations below predicted. This implies that early exposure to general anesthesia may have prevented achievement of full cognitive potential. In an even larger population study, Sun *et al.* (14) assessed learning disabilities in 228,961 individuals. Children who had procedures requiring anesthesia before the age of 3 years required more Medicaid services for learning disability than did children not having these procedures. Procedures in premature infants have also been associated with an excess of behavioral disabilities later in life. For example, surgically treated premature infants with patent ductus arteriosus (38) or necrotizing enterocolitis (39,40) had worse neurological outcomes than did premature infants who were treated medically although that maybe contributed to the severity of their disease necessitating surgical intervention.

Some newer retrospective evidence has revealed that the longer the duration of anesthesia, the lower the scores on academic achievement tests (41). Among otherwise healthy Iowa children, the groups exposed to anesthesia at a very young age had a significantly higher percentage (about 12–14%) performing below the 5th percentile on academic achievement tests as compared with the Iowa population as a whole (about 5%). Although the authors caution that there may be explanations other than anesthesia exposure, this result is of concern. Sprung *et al.* (42) reported that after adjustment for co-morbidities, children who underwent two or more exposures to anesthesia before the age of 2 years had a significantly higher risk of developing hyperactivity problems.

Collectively, this emerging clinical evidence suggests that children exposed to general anesthesia before the age of 3 years are particularly vulnerable to developing a variety of behavioral impairments. Furthermore, there appears to be a direct correlation between the duration of exposure to general anesthesia and the risk for developing cognitive impairments, i.e. longer the exposure, more likely it is that the child will exhibit some form of learning disability later in life.

This rapidly emerging human evidence has to be assessed in view of the complexity and interplay between different facets of child development. Not only could the age of exposure be an important determinant of the susceptibility to anesthesia-induced developmental impairments, but the subtlety of the neurocognitive assessment also could be a key factor in our ability to capture potential deficits at any given age (43,44). For example, when children exposed to anesthesia before the age of 3 were assessed at the age of 10, the outcomes depended significantly on the type of assessment tools that were utilized. Although the anesthesia-exposed children did not show a significant difference in academic achievement scores as compared with unexposed children, they showed subtle but significant impairment in language abilities (in particular in receptive and expressive language) and increased risk of disability in cognition that were detected even after a single exposure to anesthesia (44). These results suggest that the choice of assessment tools and outcome measures are crucially important in the ability to capture anesthesia-induced developmental neurotoxicity.

Although very early stages of brain development (before the age of 3 years) suggest higher vulnerability, the latest epidemiologic report suggested that children exposed to a general anesthetic between the ages of 3 and 10 years also may be vulnerable as assessed by neurodevelopment outcomes at the age of 10, although the vulnerability may be of a different nature (45). Using a wide range of neuropsychological tests, the authors uncovered a higher propensity in anesthetic-exposed children to develop motor deficits, even when co-morbidities or earlier injuries were taken into consideration. However, the authors concluded that there were no significant effects on language or cognitive abilities. Thus, developmental milestones may be affected in complex ways by exposure to anesthesia at different developmental stages.

Collectively, the few clinical studies to date suggest that exposure of infants and neonates to surgery and general anesthetics may cause significant neurocognitive impairment and a variety of behavioral sequelae. However, all of the studies were done retrospectively and, thus, could not control for the many variables that come into play during the perioperative

period. The design of randomized, double-blinded prospective clinical studies of very young patients is fraught with complex issues, including ethical considerations, the lack of apoptosis biomarkers that can be used safely in a living organism, the complexity and meaningfulness of various clinical outcomes - especially neurocognitive ones, and the lack of appropriate controls.

VIII. General anesthesia and the aging brain

Although the majority of this article is devoted to anesthesia-induced developmental neurotoxicity, it is important to note that the concerns regarding the effects of general anesthesia on the aging brain are being actively examined. Unlike the findings in children where some correlation between an early exposure to general anesthesia and lasting neurocognitive impairments have been suggested, clinical studies of post-operative cognitive decline (POCD) in elderly human population have been less conclusive. In fact, except for the initial ISPOCD study which has suggested that anesthetic duration could be a significant risk factor, no evidence of long-lasting cognitive influences regardless of the anesthetic agent or the anesthetic approach, has been put forward. Having said that, the possibility that the patient population with ongoing but clinically undiagnosed neuropathology may prove to be the one most vulnerable to anesthesia-induced POCD should be seriously considered.

When it comes to preclinical data, the findings are interesting. Using animal model of POCD in elderly rodents Dr. Culley and her collaborators have shown that isoflurane and nitrous oxide exposure caused cognitive deficits that lasted at least a few weeks (46) where isoflurane was shown in the later study to be more of a culprit than nitrous oxide (47). Although these findings were confirmed in other animal models where general anesthetics were examined in the absence of surgery (48) and were shown to be somewhat anesthetic dependent with isoflurane being most detrimental of all inhaled anesthetics and intravenous anesthetics being less detrimental than any of the inhaled anesthetics, POCD was concluded to be fairly transient in nature.

It is noteworthy that two hallmark lesions of Alzheimer disease, excessive plaque formation and tauopathy, have been shown to be influenced by general anesthetics (49,50) thus suggesting possible interaction between anesthesia and Alzheimer pathogenesis which may underlie post-operative cognitive decline. The evidence regarding the role of anesthesia-induced calcium imbalance and modulation of inflammatory pathways in the development of POCD is actively being investigated.

IX. Conclusion remarks

Modern neuroscience reminds us of the importance of proper functioning and fine modulation of neurotransmitters not only in aging brain but importantly during various stages of brain development. Any degree of 'tempering' with timely neurotransmitter synthesis, release, receptor activation or inhibition could have substantial consequences on synaptogenesis, neuronal network formation and on proper cognitive and behavioral functioning.

References

1. Komuro H, Rakic P. Modulation of neuronal migration by NMDA receptors. *Science*. 1993; 260:95–97. [PubMed: 8096653]
2. Brown, JK., Omar, T., O'Regan, M. Brain development and the development of tone and muscle. In: KJC, HF., editor. *Neurophysiology and the Neuropsychology of Motor Development*. Mackeith Press; 1997. p. 1-41.
3. Dobbing J, Sands J. The brain growth spurt in various mammalian species. *Early Hum Dev*. 1979; 3:79–84. [PubMed: 118862]
4. Allen NJ, Barres BA. Signaling between glia and neurons: focus on synaptic plasticity. *Curr Opin Neurobiol*. 2005; 15:542–8. [PubMed: 16144764]
5. Ben-Ari Y. Excitatory actions of GABA during development: the nature of the nurture. *Nature Reviews Neuroscience*. 2002; 3:728–739. [PubMed: 12209121]
6. Hudetz AG. General anesthesia and human brain connectivity. *Brain Connect*. 2012; 2:291–302. [PubMed: 23153273]
7. Franks NP. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. *Nat Rev Neurosci*. 2008; 9:370–86. [PubMed: 18425091]
8. Hirota K, Roth SH, Fujimura J, et al. GABAergic mechanisms in the action of general anesthetics. *Toxicol Lett*. 1998; 100–101:203–7.
9. Pearce, RA. Effects of volatile anesthetics on GABAA receptors: Electrophysiological studies. In: Moody, EJ., Skolnick, P., editors. *Molecular basis of anesthesia*. Boca Raton, FL: CRC Press; 2000.
10. Nishikawa K, Harrison NL. The actions of sevoflurane and desflurane on the gamma-aminobutyric acid receptor type A: effects of TM2 mutations in the alpha and beta subunits. *Anesthesiology*. 2003; 99:678–84. [PubMed: 12960553]
11. Lodge D, Anis NA. Effects of phencyclidine on excitatory amino acid activation of spinal interneurons in the cat. *Eur J Pharmacol*. 1982; 77:203–204. [PubMed: 7037432]
12. Jevtovic-Todorovic V, Todorovic SM, Mennerick S, Mennerick S, et al. Nitrous oxide (laughing gas) is an NMDA antagonist, neuroprotectant and neurotoxin. *Nature Medicine*. 1998; 4:460–463.
13. Franks NP, Dickinson R, deSousa SLM, et al. How does xenon produce anesthesia? *Nature*. 1998; 396:324. [PubMed: 9845069]
14. Sun LS, Li G, Dimaggio C, et al. Anesthesia and neurodevelopment in children: time for an answer? *Anesthesiology*. 2008; 109:757–761. [PubMed: 18946281]
15. Levy DM. Psychic trauma of operations in children and a note on combat neurosis. *American Journal of Disease of Children*. 1945; 69:7–25.
16. Jackson K. Psychological preparation as a method of reducing emotional trauma of anesthesia in children. *Anesthesiology*. 1951; 12:293–300. [PubMed: 14838345]
17. Jevtovic-Todorovic V, Hartman RE, Izumi Y, et al. Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. *J Neurosci*. 2003; 23:876–882. [PubMed: 12574416]
18. Loepeke AW, Istaphanous GK, McAuliffe JJ 3rd, et al. The effects of neonatal isoflurane exposure in mice on brain cell viability, adult behavior, learning, and memory. *Anesth Analg*. 2009; 108:90–104. [PubMed: 19095836]
19. Young C, Jevtovic-Todorovic V, Qin YQ, et al. Potential of ketamine and midazolam, individually or in combination, to induce apoptotic neurodegeneration in the infant mouse brain. *Br J Pharmacol*. 2005; 146:189–97. [PubMed: 15997239]
20. Rizzi S, Carter LB, Ori C, Jevtovic-Todorovic V. Clinical anesthesia causes permanent damage to the fetal guinea pig brain. *Brain Pathol*. 2008; 18:198–210. [PubMed: 18241241]
21. Yon J-H, Daniel-Johnson J, Carter LB, Jevtovic-Todorovic V, Jevtovic-Todorovic V. Anesthesia induces neuronal cell death in the developing rat brain via the intrinsic and extrinsic apoptotic pathways. *Neuroscience*. 2005; 35:815–827.
22. Sanchez V, Feinstein SD, Lunardi N, et al. General Anesthesia Causes Long-term Impairment of Mitochondrial Morphogenesis and Synaptic Transmission in Developing Rat Brain. *Anesthesiology*. 115:992–1002.

23. Mintz CD, Barrett KM, Smith SC, et al. Anesthetics interfere with axon guidance in developing mouse neocortical neurons in vitro via a γ -aminobutyric acid type A receptor mechanism. *Anesthesiology*. 2013; 118:825–33. [PubMed: 23364597]
24. Nikizad H, Yon JH, Carter LB, Jevtovic-Todorovic V. Early exposure to general anesthesia causes significant neuronal deletion in the developing rat brain. *Ann NY Acad Sci*. 2007; 1122:69–82. [PubMed: 18077565]
25. Lunardi N, Ori C, Erisir A, Jevtovic-Todorovic V. General anesthesia causes long-lasting disturbances in the ultrastructural properties of developing synapses in young rats. *Neurotox Res*. 2010; 17:179–188. [PubMed: 19626389]
26. Head BP, Patel HH, Niesman IR, et al. Inhibition of p75 neurotrophin receptor attenuates isoflurane-mediated neuronal apoptosis in the neonatal central nervous system. *Anesthesiology*. 2009; 110:813–825. [PubMed: 19293698]
27. Briner A, Nikonenko I, Roo M De, et al. Developmental Stage-dependent persistent impact of propofol anesthesia on dendritic spines in the rat medial prefrontal cortex. *Anesthesiology*. 2011; 115:282–293. [PubMed: 21701379]
28. Briner A, De Roo M, Dayer A, Dayer D, et al. Volatile anesthetics rapidly increase dendritic spine density in the rat medial prefrontal cortex during synaptogenesis. *Anesthesiology*. 2010; 112:546–556. [PubMed: 20124985]
29. Fredriksson A, Pontén E, Gordh T, Eriksson P. Neonatal exposure to a combination of N-methyl-D-aspartate and gamma-aminobutyric acid type A receptor anesthetic agents potentiates apoptotic neurodegeneration and persistent behavioral deficits. *Anesthesiology*. 2007; 107:427–36. [PubMed: 17721245]
30. Fredriksson A, Archer T, Alm H, Alm H, et al. Neurofunctional deficits and potentiated apoptosis by neonatal NMDA antagonist administration. *Behav Brain Res*. 2004; 153:367–76. [PubMed: 15265631]
31. Han T, Hu Z, Tang YY, et al. Inhibiting Rho kinase 2 reduces memory dysfunction in adult rats exposed to sevoflurane at postnatal days 7–9. *Biomed Rep*. 2015; 3(3):361–364. 2015. [PubMed: 26137236]
32. Zou X, Patterson TA, Sadovova N, et al. Potential neurotoxicity of ketamine in the developing rat brain. *Toxicol Sci*. 2009; 108:149–58. [PubMed: 19126600]
33. Shu Y, Zhou Z, Wan Y, et al. Nociceptive stimuli enhance anesthetic-induced neuroapoptosis in the rat developing brain. *Neurobiol Dis*. 2012; 45:743–50. [PubMed: 22075165]
34. Paule MG, Li M, Allen RR, et al. Ketamine anesthesia during the first week of life can cause long-lasting cognitive deficits in rhesus monkeys. *Neurotoxicol Teratol*. 2011; 33:220–30. [PubMed: 21241795]
35. Raper J, Alvarado MC, Murphy KL, Baxter MG. Multiple Anesthetic Exposure in Infant Monkeys Alters Emotional Reactivity to an Acute Stressor. *Anesthesiology*. 2015 Sep 2. Epub ahead of print.
36. Backman ME, Kopf AW. Iatrogenic effects of general anesthesia in children: considerations in treating large congenital nevocytic nevi. *J Dermatol Surg Oncol*. 1986; 12:363–367. [PubMed: 3514716]
37. Wilder RT, Flick RP, Sprung J, et al. Early exposure to anesthesia and learning disabilities in a population-based birth cohort. *Anesthesiology*. 2009; 110:796–804. [PubMed: 19293700]
38. Chorne N, Leonard C, Piecuch R, et al. Patent ductus arteriosus and its treatment as risk factors for neonatal and neurodevelopmental morbidity. *Pediatrics*. 2007; 119:1165–1174. [PubMed: 17545385]
39. Rees C, Pierro MA, Eaton S. Neurodevelopmental outcomes of neonates with medically and surgically treated necrotizing enterocolitis. *Arch Dis Child Fetal Neonatal Ed*. 2007; 92:F193–8. [PubMed: 16984980]
40. Hintz SR, Kendrick DE, Stoll BJ, et al. Neurodevelopmental and growth outcomes of extremely low birth weight infants after necrotizing enterocolitis. *Pediatrics*. 2005; 115:696–703. [PubMed: 15741374] Hirota K, Roth SH, Fujimura J, et al. GABAergic mechanisms in the action of general anesthetics. *Toxicol Lett*. 1998; 100–101:203–7.

41. Block RI, Thomas JJ, Bayman EO, et al. Are anesthesia and surgery during infancy associated with altered academic performance during childhood? *Anesthesiology*. 2012; 117:494–503. [PubMed: 22801049]
42. Sprung J, Flick RP, Katusic SK, et al. Attention-deficit/hyperactivity disorder after early exposure to procedures requiring general anesthesia. *Mayo Clin Proc*. 2012; 87:120–129. [PubMed: 22305025]
43. Ing C, DiMaggio C, Whitehouse A, et al. Long-term differences in language and cognitive function after childhood exposure to anesthesia. *Pediatrics*. 2012; 130:e476–85. [PubMed: 22908104]
44. Ing CH, DiMaggio CJ, Malacova E, et al. Comparative analysis of outcome measures used in examining neurodevelopmental effects of early childhood anesthesia exposure. *Anesthesiology*. 2014a; 120:1319–32. [PubMed: 24694922]
45. Ing CH, DiMaggio CJ, Whitehouse AJ, et al. Neurodevelopmental Outcomes After Initial Childhood Anesthetic Exposure Between Ages 3 and 10 Years. *J Neurosurg Anesthesiol*. 2014b Epub ahead of print.
46. 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. [PubMed: 12651650]
47. Culley DJ, Baxter MG, Crosby CA, et al. Impaired acquisition of spatial memory 2 weeks after isoflurane and isoflurane-nitrous oxide anesthesia in aged rats. *Anesth Analg*. 2004; 99:1393–7. [PubMed: 15502036]
48. Bianchi SL, Tran T, Liu C, et al. Brain and behavior changes in 12-month-old Tg2576 and nontransgenic mice exposed to anesthetics. *Neurobiol Aging*. 2008; 29:1002–10. [PubMed: 17346857]
49. Tang JX, Eckenhoff MF. Anesthetic effects in Alzheimer transgenic mouse models. *Prog Neuropsychopharmacol Biol Psychiatry*. 2013; 47:167–71. [PubMed: 22705294]
50. Quiroga C, Chaparro RE, Karlinski R, et al. Effects of repetitive exposure to anesthetics and analgesics in the Tg2576 mouse Alzheimer's model. *Neurotox Res*. 2014; 26:414–21. [PubMed: 24927827]

Key points

1. The developing and aging brain could be vulnerable to anesthesia-induced neurotoxicity;
2. An important mechanism for anesthesia-induced developmental neurotoxicity is widespread neuroapoptosis;
3. An early exposure to anesthesia causes long-lasting impairments in neuronal communication and faulty formation of neuronal circuitries;
4. Exposure to anesthesia during both extremes of brain age could result in long-lasting impairments in cognitive and behavioral performance in animals and potentially in humans.

Figure 1. Triple anesthetic cocktail induces apoptotic neurodegeneration
 (a–l) Light micrographic views of various brain regions of either a control rat (a, f, h, j) or a rat exposed to the triple anesthetic cocktail (0.75-vol% isoflurane with midazolam at 9 mg/kg, s.c., and nitrous oxide at 75-vol% for 6 hrs) (b–e, g, i, k, l). Some sections were stained by the DeOlmos silver method (a, b, d, f, g, k); the others were immunocytochemically stained to reveal caspase-3 activation (c, e, h–j, l). The regions illustrated are the posterior cingulate/retrosplenial cortex (a–c), subiculum (d, e), anterior thalamus (f, g), rostral CA1 hippocampus (h, i), and parietal cortex (j–l). The individual

nuclei shown in the anterior thalamus (*f, g*) are laterodorsal (*LD*), anterodorsal (*AD*), anteroventral (*AV*), anteromedial (*AM*), and nucleus reuniens (*NR*). *m* and *n* are electron micrographic scenes depicting the ultrastructural appearance of neurons undergoing apoptosis. The cell in *m* displays an early stage of apoptosis in which dense spherical chromatin balls are forming in the nucleus while the nuclear membrane remains intact; few changes are evident in the cytoplasm. The cell in *n* exhibits a much later stage of apoptosis in which the entire cell is condensed, the nuclear membrane is absent, and there is intermixing of nuclear and cytoplasmic constituents. [Reproduced with permission from *Journal of Neuroscience*, 2003; 23(3):876–882].

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Figure 2. Isoflurane causes immediate effects on axonal growth cone sensitivity to Semaphorin3A but requires a longer exposure for effects on axon guidance

A time-response curve is shown depicting the percent axonal growth cone collapse to Semaphorin3A at increasing time of exposure to isoflurane, which shows a loss of collapse in as little as 15 min (A). Trajectory diagrams from a slice overlay assay conducted at 5h, the minimum time for appropriate targeting of controls (B) shows that a disruption of axon guidance is seen with 1.8% isoflurane at this time point. Axes in (B) and (C) in μm .

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MORRIS WATER MAZE

MORRIS MAZE

RADIAL ARM MAZE

Figure 3. Effects of neonatal triple anesthetic cocktail treatment on spatial learning

(a) Rats were tested at P32 for their ability to learn the location of a submerged (not visible) platform. (b) Rats were retested as adults (P131) for their ability to learn a different location of the submerged platform. The graph on the left shows the path-length data from the first five place trials when all rats were tested. The graph on the right shows the data from rats given 5 additional training days as adults. During these trials, rats in the control group improved their performance and appeared to reach asymptotic levels, whereas the rats given the anesthetic cocktail showed no improvement. (c) Probe trial performance of rats given the

anesthetic cocktail and control rats during adult testing. The dotted line represents the amount of time that animals would be expected to spend in the target quadrant based on chance alone. (d, e) Data are shown from the radial arm maze test done on P53 to evaluate spatial working memory capabilities. (d) This histogram shows that rats given the anesthetic cocktail rats required significantly more days to reach a criterion demonstrating learning (8 correct responses out of the first 9 responses for 4 consecutive days) compared with controls. (e) This plot shows the days to criterion data as the cumulative percentage of rats reaching criterion in each group as a function of blocks of training days. Numbers in parentheses in each graph indicate sample sizes. * $p < 0.05$; Bonferroni corrected level: $^{\dagger}p < 0.005$ in *a*; $^{\dagger}p < 0.01$ in *b*. [Reproduced with permission from *Journal of Neuroscience*, 2003; 23(3): 876–882].

Figure 4.

Cumulative percentage of learning disabilities diagnosis by the age at exposure shown separately for those that have zero, one, or multiple anesthetic exposures before age 4 yr.

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