HHS Public Access

Author manuscript

Anesthesiology. Author manuscript; available in PMC 2019 April 01.

Published in final edited form as:

Anesthesiology. 2018 April ; 128(4): 832–839. doi:10.1097/ALN.0000000000002047.

Exposure of developing brain to general anesthesia: what is the animal evidence?

Vesna Jevtovic-Todorovic, MD, PhD, MBA

Department of Anesthesiology, University of Colorado School of Medicine, Aurora, CO, USA.

I. Introduction

Over the past 15 years we have been witness to numerous research reports bringing an uncomfortable concern that very young mammalian brain could be susceptible to disturbances in homeostasis during critical stages of neuronal networks formation. Despite scrutiny of initial animal reports, more recent reports suggest that there is a real possibility that clinically-used general anesthetics and sedatives could cause powerful disruptions of brain development. This is likely due to perturbations of normal neuronal and glial activity, which has to occur in order for an individual to be rendered unconscious and insensitive to pain. Since undisturbed neuronal and glial activity and communication are crucially important for neuronal circuits formation[1,2], these pharmacological agents have been thought to be detrimental to normal neuronal and glial development, resulting in alterations in cognitive and socio-emotional behavioral development.

II. What is the importance of anesthesia-induced activation of developmental apoptosis?

Initial studies were focused on rodent models of anesthesia-induced developmental neurotoxicity because of their relative simplicity and high degree of reproducibility. Because rodent brains undergo brain maturation fairly quickly (i.e. over the course of the first three weeks of postnatal life)[3] and since the time frame of their synaptogenesis (i.e. a period when synapses are massively developing and basic neuronal networks are being formed) is fairly well defined, a number of mechanistic and behavioral studies were conducted using very young rodents.

For quite a long time our work and the work of others centered around the finding that an early exposure to anesthesia resulted in highly reproducible neuronal apoptosis. Although apoptosis is often referred to as ‘physiological cell death’ it became clear early on that there was nothing ‘physiological’ about the intensity of the neuronal apoptosis observed after an early exposure to clinically-used general anesthetics and sedatives[4–6]. Numerous reports have suggested substantial upregulation in ‘naturally-occurring’ neuronal death (as much as

Corresponding Author: Vesna Jevtovic-Todorovic, MD, PhD, MBA, Department of Anesthesiology, University of Colorado School of Medicine, 12401 East 17th Avenue, Aurora, CO 80045, Phone:720-848-6723; Fax:720-848-7375, vesna.jevtovic-todorovic@ucdenver.edu.

Conflict of interest: None.

70-fold) when compared to age-matched controls[6–11]. The reason for it being referred to as 'natural' is that detailed ultrastructural analyses of the immature neurons undergoing anesthesia-induce neuroapoptosis suggested that this dying process was not of unique nature but rather that it followed a strictly controlled step-wise process as described with physiological cell death[4,6]. It is important to note that developing neurons have an active apoptotic machinery designed to eliminate neurons that become redundant by the virtue of not being successful in migrating to their final destination, not maturing in timely fashion and /or not properly connecting with other neurons. The issue, though, is that unlike neurogenesis, only a very small percentage of neurons are expected to be removed during intense synaptogenesis (less than 2% with some variations from one brain region to another) *via* this unique form of programmed cell death[4–6]. The scientific community realized that general anesthetics, by perturbing homeostatic milieu, 'force' many previously healthy neurons into the redundant category destined to die.

At that time, apoptosis was thought to be the leading mechanism of anesthesia-induced developmental neurotoxicity. A substantial amount of energy was focused on understanding all aspects of apoptotic activation so that the protective strategies could be developed[11–16]. The apoptotic pathways initiated by damage to mitochondria and/or endoplasmic reticuli became the primary targets of investigation since general anesthetics were reported to be particularly damaging to these intracellular organelles. In addition to activating the mitochondria-dependent apoptotic pathway which involves the down-regulation of anti-apoptotic proteins from the bcl-2 family (e.g., bcl-x_L), an increase in mitochondrial membrane permeability, followed by an increase in cytochrome c and the activation of a series of caspases[10,11], general anesthetics cause a significant and long lasting disturbance in mitochondrial morphogenesis. This is marked by disturbances in mitochondrial fission and fusion[17], two dynamic processes that assure proper regeneration of mitochondria[18]; deranged fusion leads to mitochondrial fragmentation while deranged fission leads to mitochondrial enlargement. General anesthetics may disturb mitochondrial dynamics favoring excessive mitochondrial fusion and impaired fission[17] which in turn may explain excessive free oxygen radical formation and disturbances in metabolic support for newly developing synapses. This may lead to impaired plasticity of dendritic spines and the formation, stability and function of developing synapses[14,19–21]. Since mitochondrial ATP production at the vicinity of an active synapse regulates all the elements of neurotransmitter synthesis, release (*via* exocytosis) and uptake, it is clear that mitochondrial dysfunction during critical stages of synaptogenesis may lead to the elimination of developing neurons.

As a regulator and primary source of releasable Ca^{2+} in neurons the endoplasmic reticulum (ER) plays an important role in neuronal function and survival. Since intracellular Ca^{2+} regulates many aspects of neuronal development, including synapse development and functioning, membrane excitability, protein synthesis, neuronal apoptosis and autophagy[22–24] the ER is considered an important initial target of anesthesia-induced developmental neurotoxicity and an instigator of a series of events resulting in mitochondrial dysfunction. Indeed, Zhao and colleagues[25] have shown that the inhalational anesthetic, isoflurane activates inositol 1,4,5-trisphosphate receptors to induce significant Ca^{2+} release from the ER, resulting in modulation of mitochondrial bcl-xL protein, which then promotes apoptotic neuronal death in the immature rat brain. Similar modulation of inositol 1,4,5-

trisphosphate receptors was reported with the general anesthetics propofol, desflurane and sevoflurane, with resultant cytosolic Ca^{2+} overload and an increase in mitochondrial permeability transition pore activity resulting in mitochondrial swelling and uncontrolled release of pro-apoptotic factors[26].

III. Anesthesia-induced developmental neurotoxicity involves complex functional changes in neuronal networks that go beyond apoptosis

The morphological changes described thus far represent modifications in neuronal structure that can easily be detected using histological assessments. Importantly though, it has been brought to light on several occasions that seemingly subtle changes which cannot be detected morphologically, remain in surviving 'normal' neurons after 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)[6]. In particular, long-term potentiation was impaired 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 had impaired synaptic transmission with inhibitory transmission affected significantly[17].

Although the precise mechanisms responsible for the long-lasting changes in synaptic communication post-anesthesia still need 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[27].

IV. The challenges of rodent model of anesthesia-induced developmental neurotoxicity: how relevant are rodent studies to higher mammalian species?

Initial studies were scrutinized due to the fact that rodent brain development is substantially shorter that human brain development (weeks as opposed to years)[3] and that many rodent models used exposures that were considered to be lengthy – 4 to 6 hours- thus drawing perhaps a simplified conclusion that hours of exposure to anesthesia in rodents would equate to weeks and months of exposure to humans. Another important concern was an obvious difference in the complexity of neuronal networks in rodents compared to humans. To begin to address and challenge this trepidation, the scientific community looked at shorter exposure in rodents and discovered that brief exposure to inhaled anesthetic, sevoflurane (30 minutes) may not cause obvious long-term effects on behavioral development but may

induce subtle modifications in spine density and synaptic plasticity[28]. When guinea pigs, a rodent species with more complex brain networks and about three-fold longer duration of synaptogenesis compared to rats and mice[9] were studied, it was discovered that a 4-hour exposure to general anesthesia causes substantial activation of neuroapoptosis comparable in intensity, timing, and distribution to the ones commonly described in rats and mice after a 6-hour exposure[9]. This confirmed that a relationship between duration of synaptogenesis and the length of general anesthesia exposure is very complex and as such cannot be explained by simple mathematical modeling. The findings with guinea pigs and rats suggest that the timing, rather than a duration, could be a much more compelling consideration, since the same anesthetic regimen causes substantial damage at the peak of synaptogenesis but substantially lesser one during other time points of synaptogenesis[9,10].

Despite a number of highly reproducible rodent findings, it is becoming clear that we cannot rely exclusively on rodent data if we are to make inroads into understanding the potential relevance of animal data to humans. Accordingly, the scientific community has begun to rely on a growing body of work being done with non-human primates. Hence, a review of presently available evidence in non-human primates deserves serious attention.

Initial studies with neonatal monkeys have shown that exposure of a 6-day-old rhesus macaques to surgical plane of isoflurane anesthesia (from 0.7 to 1.5 end-tidal Vol%) for 5h resulted in significant histopathological changes despite vigilant physiological monitoring comparable to human setting[29]. The authors reported substantial neuroapoptotic activation in the cerebral cortex of isoflurane-treated monkeys represented as a 13-fold increase in acute apoptotic neurodegeneration when compared to age-matched controls. In a study that soon followed, this group confirmed that a 5-hour exposure to isoflurane of 6-day-old rhesus macaques significantly upregulates neuroapoptosis not only in cerebral cortex, but that it causes significant apoptosis in gray matter throughout the brain[30]. Interestingly, the authors also report a substantial and wide spread activation of apoptosis in oligodendrocytes in the white matter, suggesting that a larger proportion of apoptotic cells are glia, i.e. about 52% of dying cells were glia whereas about 48% were neurons. Considering the importance of oligodendroglia in timely myelination of neuronal axons, the concern was raised that early exposure to general anesthesia may impair proper myelogenesis. To further examine this notion, the authors focused on a very early stage of myelogenesis which in rhesus macaques occurs during *in utero* life. Hence, they exposed fetal monkeys at gestational age of 120 days for 5 hours to isoflurane anesthesia. When they systematically examined all brain regions looking for the evidence of neuronal and glial apoptosis they found not only a widespread neuronal apoptosis affecting several cerebrocortical regions as well as putamen, caudate, amygdala and cerebellum but diffusely dispersed apoptotic oligodendrocytes in many white matter regions[31]. Again, they noted that their glia was more vulnerable than neurons based on the higher prevalence of apoptotic oligodendrocytes[31]. Similar observations were made when the monkeys were exposed to commonly used intravenous anesthetic, propofol[32]. Namely, when fetal (120-days of *in utero* life) or neonatal (6 days postnatal life) were exposed to 5 h of propofol anesthesia sufficient to achieve a surgical plane of anesthesia they reported a significant increase in apoptotic degeneration in both neurons and oligodendrocytes although the pattern of apoptotic damage was somewhat different when fetal and neonatal brains were compared. The authors noted that the severity of apoptotic

damage was less that reported with isoflurane which would suggest some difference in the neurotoxic potential among clinically used anesthetics. Very recent report suggests that even shorter exposure of infant monkeys to general anesthesia (i.e. isoflurane for 3 hours) resulted in a four-fold increase in neuronal and oligodendroglia apoptosis compared to controls[33].

As stated previously, the timing of anesthesia exposure based on rodent studies was suggested to be more important than the duration or the type of anesthesia for determining the susceptibility and neurotoxic potential. This notion was examined in non-human primates as well and it was concluded that neuronal vulnerability indeed diminishes with age but the glial vulnerability does not. When 20- and 40-day old rhesus macaques were exposed to 5 hours of isoflurane anesthesia the authors discovered that the neuroapoptosis was somewhat lower, reporting that 'the window of vulnerability for neurons is beginning to close while the vulnerability of oligodendrocytes remained high[34].

Aside from neuronal and glial apoptotic damage, a recent study with prolonged exposure to sevoflurane (at 2.5% for 9 hours) demonstrated that the non-human primate brain is susceptible to substantial modulation of gene expression, changes in cytokine levels, and impairment of lipid metabolism[35]. The fact that not only lipid content but also lipid composition is affected suggests that changes in brain biochemistry could result from exposure to anesthetics. The authors suggest that changed lipid composition could be used as a potential biomarker of anesthesia-induced developmental neurotoxicity.

The scientific community initially grappled with concerns centered on the ability to monitor and maintain proper physiologic homeostasis as assessed by vigilant monitoring of vital signs during the general anesthesia state as is commonly done in a clinical setting. Since rat and mice pups are small in size, the assessment of physiologic parameters was challenging. Although the initial studies did offer some assessment of the arterial blood gas composition[6,12], the method of obtaining the arterial blood sample was considered invasive and certainly not clinically relevant. The studies with guinea pigs helped to alleviate some concerns since the continuous monitoring was performed with the placement of an arterial line whereby the blood pressure recordings and arterial blood gas analyses were performed on repeated basis[9]. Despite the maintenance of proper homeostasis, it was confirmed that general anesthesia exposure at the peak of brain development caused neuroapoptotic damage to very young neurons thus confirming that close monitoring and maintenance of physiologic homeostasis has no bearings on the severity of anesthesia-induced neuronal damage. This line of rigorous assessment of the role of proper homeostasis during moderate plane of anesthesia has been carried forward to include non-human primates. Dr. Martin and colleagues[36] examined the effects of three commonly used anesthetics (isoflurane, ketamine, and propofol) on physiologic parameters commonly monitored in clinical practice such as heart rate, blood pressure, respiratory rate, end-tidal carbon dioxide levels, oxygen saturation, and body temperature. The measurements were done every 15 min and venous blood was collected to determine blood gases and metabolic status at baseline and at regular intervals during a 5 h-anesthesia as well as 3 h-post-anesthesia. The authors concluded that the maintenance of all physiologic parameters was overall adequate and that among all three anesthetics, isoflurane caused more hypotensive episodes than propofol or ketamine thus necessitating an increased volume of intravenous fluids.

V. Presently available evidence for behavioral and cognitive outcomes of an early exposure to general anesthesia: are rodent findings relevant to higher mammalian species?

Although above reviewed patho-morphological and functional changes in neuronal development are of scientific interest, a true test of practical relevance is in assessing long-term behavioral sequelae. Researchers have devoted substantial attention to examining the development of cognitive abilities of animals exposed to general anesthetics at the peak of synaptogenesis and concluded that they lagged behind those of controls, with the gap widening into adulthood. Not only can a single long exposure to general anesthetics lead to cognitive deficits[6,37,38] but the data suggest that multiple, shorter-lasting exposures to anesthesia during vulnerable periods cause significant impairments in neurocognitive development[39,40].

An early studied performed at NCTR/FDA showed for the first time that ketamine, an intravenous anesthetic widely used in pediatric anesthesia when administered to rhesus monkeys during three stages of development - 122 days of gestation and 5 and 35 postnatal days - intravenously for 24 h to maintain a surgical plane of anesthesia, produced a significant increase in the number of apoptotic and necrotic neurons in the cortex of gestational and 5- but not 35-day old monkeys[41]. The authors concluded that earlier developmental stages (122 days of gestation and 5 postnatal day-old) appear more sensitive to ketamine-induced neuronal death than later ones (at 35 post-natal day) thus confirming previously reported observation with rodents that the timing, rather than a duration is the most important contributing factor[9,10]. The authors went on to examine the effects of this anesthesia regimen on behavioral development in primates. They reported that primates exposed to continuous infusion of ketamine (24h) at age 5 or 6 days exhibit long-term disturbances in cognitive development, including learning, psychomotor speed, concept formation, and motivation when examined over next few years[42]. These effects occurred despite an absence of physiological or metabolic derangements during anesthesia. Although 24 hours of anesthetic exposure could be considered unusual, it certainly does occur, especially in critically ill children who are sedated in the intensive care unit. Interestingly, subsequent studies with ketamine showed that even substantially shorter exposures, i.e. for 5h at either 120 days of gestation or at post-natal day 6 result in significant neuroapoptosis[43] when compared to age matched controls. Interestingly, the authors report that the fetal brain is more susceptible as shown by a 2.2-fold greater neuroapoptosis in fetal monkey brain compared to an infant brain.

In clinical practice, children may be exposed to multiple anesthetics during critical stages of brain development thus raising the concern that this practice could be detrimental. A monkey study suggests that this concern may not be unfounded. When Dr. Baxter and his colleagues[44] exposed rhesus monkeys of both sexes to three sevoflurane anesthetics during the first month of their postnatal life and compared them to control monkeys six months later using human intruder test, they discovered a higher frequency of anxiety-related behaviors in sevoflurane-exposed monkeys, thus suggesting the impairment of emotional behavior. Interestingly, Dr. Baxter[45] has previously reported that the thalamus, in particular

its mediodorsal nucleus, plays an important role in controlling plasticity and flexibility of prefrontal-dependent cognitive processes. Lesion studies have suggested that an injury to this brain region affects a wide array of subcortical relays thus damaging neuronal networks important for cognitive functioning. This is an interesting observation in view of the fact that commonly used general anesthetics are known to cause substantial neuroapoptotic damage to a variety of gray matter structures not only in rodents[4–11] but importantly in baby monkeys as well[29,31,32,33,43]. Although anesthesia-induced damage is not focal in nature but rather encompasses many brain regions, the fact that the thalamus is a vulnerable gray matter raises some concerns that an early exposure to anesthesia may indeed impair a wide array of subcortical relays crucially important for cognitive development.

As we are learning more about the long-term behavioral and cognitive sequelae in non-human primates, very recent evidence where a single 5-hour exposure to isoflurane was compared to multiple exposures (total of three times) confirms that when compared to controls, multiple- but not single-exposed monkeys- exhibited motor reflex deficits at 1 month of age and responded to their new social environment with increased anxiety and affiliative/appeasement behavior at 12 months of age. The authors concluded that an early exposure to isoflurane results in long-lasting and detrimental effects on socioemotional development[46].

It is noteworthy that although new pathomorphological data suggest that single shorter exposure to general anesthesia can result in apoptotic neurodegeneration[33], there are no published reports to date examining the possibility that short exposure may result in cognitive and/or behavioral development.

VI. Scientific limitations

It is important to note that currently used animal models suffer from some important limitations. For one, they all expose healthy animals to general anesthesia whereas in clinical setting we often take care of ill children. Hence, comorbidities should be considered as a potential confounder, especially since those children get exposed to prolonged surgeries and often have complicated post-operative course marked by pain, anxiety, fluid imbalance and surgery-induced trauma.

This brings up another important consideration with currently used animal models - a lack of surgical and/or painful stimulations. At present, there is very limited number of animal studies and they seem to be conflicting on this issue. For example, a study by Dr. Shu and colleagues published in 2012[47] suggests that rat pups who received general anesthesia for 6 hours and were subjected to surgical and chemical nociception with hind paw incision or formalin injection respectively, exhibited higher degree of neuroapoptosis in brain cortex and spinal cord and showed enhanced expression of the pro-inflammatory cytokine, IL-1β (in cortex). Additionally, when examined on learning paradigm in young adulthood, these subjects demonstrated worsened long-term cognitive impairments compared to age-matched animals exposed to anesthesia alone without nociceptive stimulus. The authors conclude that nociceptive stimulations and prolonged anesthesia are more detrimental than prolonged anesthesia exposure alone when it occurs during the peak of brain development.

Interestingly, a study by Liu et al. published the same year[48] suggests that a 6-hour exposure of rat pups to general anesthesia with chemical nociception induced by complete Freund's adjuvant resulted in attenuated anesthesia-induced neuroapoptotic response although cognitive behavior later in life was not assessed. While it is difficult to discern the reason for these seemingly opposite observations, one possible explanation could be the difference in the choice of general anesthesia- the first study used a combined nitrous oxide and isoflurane anesthesia whereas the other was ketamine-based. Regardless of the point of view we may want to adopt, the fact is that the nociceptive and/or surgical stimulations are a complicated factor that may or may not potentiate anesthesia-induced developmental neurotoxicity. Importantly, it reminds us that the choice of anesthesia may play a critical role in morphological and behavioral outcomes.

As more animal evidence emerges attempting to improve clinical relevance to multiple exposures in humans, we should be cautious not to assume that multiple exposures using consecutive daily patterns in animals are as relevant to clinical setting where young children are less likely to be exposed on daily basis (unless exposed to prolonged sedation in the intensive care unit).

The preponderance of animal and *in vitro* findings show overwhelming evidence that young neurons and glia are vulnerable to anesthesia-induced morphological and functional impairments. Not only that significant apoptotic damage could be detected, but importantly, there is an evidence of synaptic dysfunction[6, 17] and attrition[19–21] as well as impaired connectivity and faulty formation of neuronal circuits[27]. Although there is a multitude of behavioral evidence suggesting the impairment of cognitive and emotional development, the scientific community has not been able to prove the relevance and causality between observed morphological and functional impairments at the cellular level and the impairments in behavioral development.

The translational value of many preclinical designs and models has lately been under scrutiny and the field of anesthesia-induced developmental neurotoxicity is no exception. In addition to important caveats discussed earlier, there are the concerns well-described in a recent review manuscript by Dr. Disma and his colleagues.[49] The authors point at the fact that published animal studies are heterogeneous in terms of the type of general anesthetics examined, the duration and the timing of exposures, age of the animals and importantly, the level of physiological monitoring. This would suggest that establishing a clear relevance of the animal studies to clinical practice would be challenging. However, although these concerns are well-taken, the fact is that our clinical practice is very heterogeneous as well and the choice of anesthesia and the approach vary based on the child’s age and medical/surgical history, type of surgical procedure and the duration, timing, and the nature of anesthesia exposure. In addition, although physiological monitoring is certainly more standardized in the clinical setting, it is not uniform and is guided by clinical judgement in any given situation. Importantly for us as practicing anesthesiologists, the use of anesthetics in various combinations is common so one can argue that the plethora of different experimental conditions and designs in preclinical studies in many ways mimics heterogeneity inherent to our clinical practice.

VII. Conclusion Remarks

In clinical practice, the decision to anesthetize young children with general anesthetics or prolonged sedation is often necessary for life-saving interventions. Nevertheless, the latest FDA warning tells us that, ‘we should discuss with parents, caregivers, and pregnant women the benefits, risks, and appropriate timing of surgery or procedures requiring anesthetic and sedation drugs.’ The majority of animal studies on the topic would suggest that this is important, although the relevance of animal findings remains to be confirmed in humans. As physicians, it is our responsibility to relentlessly evaluate ways to make general anesthetics and sedatives safe for those children needing interventions that require administration of anesthetics and sedatives.

Acknowledgments

This study was supported by the grants R0144517 (NIH/NICHD), R0144517-S (NIH/NICHD), R01 GM118197 (NIH/NIGMS), R21 HD080281 (NIH/NICHD), John E. Fogarty Award 007423-128322 (NIH) and March of Dimes National Award, USA (to Vesna Jevtovic-Todorovic). Vesna Jevtovic-Todorovic was an Established Investigator of the American Heart Association.

References

1. Allen NJ, Barres BA. Signaling between glia and neurons: focus on synaptic plasticity. Curr. Opin. Neurobiol. 2005; 15:542–8. [PubMed: 16144764]
2. Hudetz AG. General anesthesia and human brain connectivity. Brain Connect. 2012; 2:291–302. [PubMed: 23153273]
3. Dobbing J, Sands J. The brain growth spurt in various mammalian species. Early Hum. Dev. 1979; 3:79–84. [PubMed: 118862]
4. Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vöckler J, Dikranian K, Tenkova TI, Stefovska V, Turski L, Olney JW. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science. 1999; 283:70–4. [PubMed: 9872743]
5. Ikonomidou C, Bittigau P, Ishimaru MJ, Wozniak DF, Koch C, Genz K, Price MT, Stefovska V, Hörster F, Tenkova T, Dikranian K, Olney JW. Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. Science. 2000; 287:1056–60. [PubMed: 10669420]
6. Jevtovic-Todorovic V, Hartman REY, Izumi Y, Benshoff ND, Dikranian K, Zorumski CF, Olney JW, Wozniak DF. Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. J. Neurosci. 2003; 23:876–882. [PubMed: 12574416]
7. Loepke AW, Istaphanous GK, McAuliffe JJ 3rd, Miles L, Hughes EA, McCann JC, Harlow KE, Kurth CD, Williams MT, Vorhees CV, Danzer SC. 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]
8. Young C, Jevtovic-Todorovic V, Qin YQ, Tenkova T, Wang H, Labruyere J, Olney JW. 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]
9. 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]
10. Yon J-H, Daniel-Johnson J, Carter LB, 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.
11. Yon J-H, Carter LB, Reiter RJ, Jevtovic-Todorovic V. Melatonin reduces the severity of anesthesia-induced apoptotic neurodegeneration in the developing rat brain. Neurobiol Dis. 2006; 21:522–530. [PubMed: 16289675]

12. Lu LX, Yon JH, Carter LB, Jevtovic-Todorovic V. General anesthesia activates BDNF-dependent neuroapoptosis in the developing rat brain. Apoptosis. 2006; 11:1603–1615. [PubMed: 16738805]
13. Boscolo A, Starr JA, Sanchez V, Lunardi N, DiGruccio MR, Ori C, Erisir A, Trimmer P, Bennett J, Jevtovic-Todorovic V. The abolishment of anesthesia-induced cognitive impairment by timely protection of mitochondria in the developing rat brain: the importance of free oxygen radicals and mitochondrial integrity. Neurobiol Dis. 2012; 45:1031–41. [PubMed: 22198380]
14. 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; 110:813–825. [PubMed: 19293698]
15. Noguchi KK, Johnson SA, Kristich LE, Martin LD, Dissen GA, Olsen EA, Olney JW, Brambrink AM. Lithium protects against anaesthesia neurotoxicity in the infant primate brain. Sci Rep. 2016; 6:22427. [PubMed: 26951756]
16. Straiko MM, Young C, Cattano D, Creeley CE, Wang H, Smith DJ, Johnson SA, Li ES, Olney JW. Lithium protects against anesthesia-induced developmental neuroapoptosis. Anesthesiology. 2009; 110:862–8. [PubMed: 19293695]
17. Sanchez V, Feinstein SD, Lunardi N, Joksovic PM, Boscolo A, Todorovic SM, Jevtovic-Todorovic V. General anesthesia causes long-term impairment of mitochondrial morphogenesis and synaptic transmission in developing rat brain. Anesthesiology. 2011; 115:992–1002. [PubMed: 21909020]
18. Chan DC. Mitochondrial fusion and fission in mammals. Annu Rev Cell Dev Biol. 2006; 22:79–99. [PubMed: 16704336]
19. 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]
20. Briner A, De Roo M, Dayer A, Muller D, Habre W, Vutskits L. Volatile anesthetics rapidly increase dendritic spine density in the rat medial prefrontal cortex during synaptogenesis. Anesthesiology. 2010; 112:546–556. [PubMed: 20124985]
21. Briner A, Nikonenko I, De Roo M, Dayer A, Muller D, Vutskits L. Developmental Stage-dependent persistent impact of propofol anesthesia on dendritic spines in the rat medial prefrontal cortex. Anesthesiology. 2011; 115:282–293. [PubMed: 21701379]
22. Berridge MJ. Inositol trisphosphate and calcium signaling mechanisms. Biochim Biophys Acta. 2009; 1793:933–940. [PubMed: 19010359]
23. Decuypere JP, Monaco G, Bultynck G, Missiaen L, De Smedt H, Parys JB. The IP(3) receptor-mitochondria connection in apoptosis and autophagy. Biochim Biophys Acta. 2011; 1813:1003–1013. [PubMed: 21146562]
24. Hanson CJ, Bootman MD, Roderick HL. Cell signalling: IP3 receptors channel calcium into cell death. Curr Biol. 2004; 14:R933–935. [PubMed: 15530388]
25. Zhao Y, Liang G, Chen Q, Joseph DJ, Meng Q, Eckenhoff RG, Eckenhoff MF, Wei H. Anesthetic-induced neurodegeneration mediated via inositol 1,4,5-trisphosphate receptors. J Pharmacol Exp Ther. 2010; 333:14–22. [PubMed: 20086058]
26. Inan S, Wei H. The cytoprotective effects of dantrolene: a ryanodine receptor antagonist. Anesth Analg. 2010; 111:1400–1410. [PubMed: 20861418]
27. Mintz CD, Barrett KM, Smith SC, Benson DL, Harrison NL. 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]
28. Qiu L, Zhu C, Bodogan T, Gómez-Galán M, Zhang Y, Zhou K, Li T, Xu G, Blomgren K, Eriksson LI, Vutskits L, Terrando N. Acute and Long-Term Effects of Brief Sevoflurane Anesthesia During the Early Postnatal Period in Rats. Toxicol Sci. 2016; 149:121–33. [PubMed: 26424773]
29. Brambrink AM, Evers AS, Avidan MS, Farber NB, Smith DJ, Zhang X, Dissen GA, Creeley CE, Olney JW. Isoflurane-induced neuroapoptosis in the neonatal rhesus macaque brain. Anesthesiology. 2010; 112:834–41. [PubMed: 20234312]
30. Brambrink AM, Back SA, Riddle A, Gong X, Moravec MD, Dissen GA, Creeley CE, Dikranian KT, Olney JW. Isoflurane-induced apoptosis of oligodendrocytes in the neonatal primate brain. Ann Neurol. 2012a; 72:525–35. [PubMed: 23109147]

31. Creeley CE, Dikranian KT, Dissen GA, Back SA, Olney JW, Brambrink AM. Isoflurane-induced apoptosis of neurons and oligodendrocytes in the fetal rhesus macaque brain. Anesthesiology. 2014; 120:626–38. [PubMed: 24158051]
32. Creeley C, Dikranian K, Dissen G, Martin L, Olney J, Brambrink A. Propofol-induced apoptosis of neurones and oligodendrocytes in fetal and neonatal rhesus macaque brain. Br J Anaesth. 2013; 110(Suppl 1):i29–38. [PubMed: 23722059]
33. Noguchi KK, Johnson SA, Dissen GA, Martin LD, Manzella FM, Schenning KJ, Olney JW, Brambrink AM. Isoflurane exposure for three hours triggers apoptotic cell death in neonatal macaque brain. Br J Anaesth. 2017; 119:524–531. [PubMed: 28969320]
34. Schenning KJ, Noguchi KK, Martin LD, Manzella FM, Cabrera OH, Dissen GA, Brambrink AM. Isoflurane exposure leads to apoptosis of neurons and oligodendrocytes in 20- and 40-day old rhesus macaques. Neurotoxicol Teratol. 2016:30141–6. pii: S0892-0362.
35. Liu F, Rainosek SW, Frisch-Daiello JL, Patterson TA, Paule MG, Slikker W Jr, Wang C, Han X. Potential Adverse Effects of Prolonged Sevoflurane Exposure on Developing Monkey Brain: From Abnormal Lipid Metabolism to Neuronal Damage. Toxicol Sci. 2015; 147:562–72. [PubMed: 26206149]
36. Martin LD, Dissen GA, McPike MJ, Brambrink AM. Effects of anesthesia with isoflurane, ketamine, or propofol on physiologic parameters in neonatal rhesus macaques (Macaca mulatta). J Am Assoc Lab Anim Sci. 2014; 53:290–300. [PubMed: 24827572]
37. 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]
38. Fredriksson A, Archer T, Alm H, Gordh T, Eriksson P. Neurofunctional deficits and potentiated apoptosis by neonatal NMDA antagonist administration. Behav. Brain Res. 2004; 153:367–76. [PubMed: 15265631]
39. Han T, Hu Z, Tang YY, Shrestha A, Ouyang W, Liao Q. Inhibiting Rho kinase 2 reduces memory dysfunction in adult rats exposed to sevoflurane at postnatal days 7–9. Biomed Rep. 2015; 3:361–364. [PubMed: 26137236]
40. Zou X, Patterson TA, Sadovova N, Twaddle NC, Doerge DR, Zhang X, Fu X, Hanig JP, Paule MG, Slikker W, Wang C. Potential neurotoxicity of ketamine in the developing rat brain. Toxicol Sci. 2009; 108:149–58. [PubMed: 19126600]
41. Slikker W Jr, Zou X, Hotchkiss CE, Divine RL, Sadovova N, Twaddle NC, Doerge DR, Scallet AC, Patterson TA, Hanig JP, Paule MG, Wang C. Ketamine-induced neuronal cell death in the perinatal rhesus monkey. Toxicol Sci. 2007; 98:145–58. [PubMed: 17426105]
42. Paule MG, Li M, Allen RR, Liu F, Zou X, Hotchkiss C, Hanig JP, Patterson TA, Slikker W Jr, Wang C. 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]
43. Brambrink AM, Evers AS, Avidan MS, Farber NB, Smith DJ, Martin LD, Dissen GA, Creeley CE, Olney JW. Ketamine-induced neuroapoptosis in the fetal and neonatal rhesus macaque brain. Anesthesiology. 2012b; 116:72–84.
44. Raper J, Alvarado MC, Murphy KL, Baxter MG. Multiple Anesthetic Exposure in Infant Monkeys Alters Emotional Reactivity to an Acute Stressor. Anesthesiology. 2015; 123:1084–92. [PubMed: 26313293]
45. Baxter MG. Mediodorsal thalamus and cognition in non-human primates. Front Syst Neurosci. 2013; 7:38. [PubMed: 23964206]
46. Coleman K, Robertson ND, Dissen GA, Neuringer MD, Martin LD, Cuzon Carlson VC, Kroenke C, Fair D, Brambrink AM. Isoflurane Anesthesia Has Long-term Consequences on Motor and Behavioral Development in Infant Rhesus Macaques. Anesthesiology. 2017; 126:74–84. [PubMed: 27749311]
47. Shu Y, Zhou Z, Wan Y, Sanders RD, Li M, Pac-Soo CK, Maze M, Ma D. Nociceptive stimuli enhance anesthetic-induced neuroapoptosis in the rat developing brain. Neurobiol Dis. 2012; 45:743–50. [PubMed: 22075165]

48. Liu JR, Liu Q, Li J, Baek C, Han XH, Athiraman U, Soriano SG. Noxious stimulation attenuates ketamine-induced neuroapoptosis in the developing brain. Anesthesiology. 2012; 117:64–71. [PubMed: 22617253]

49. Disma N, Mondardini MC, Terrando N, Absalom AR, Bilotta F. A systemic review of methodology applied during preclinical anesthetic neurotoxicity studies: important issues and lessons relevant to the design of future clinical research. Paediatr Anaesth. 2016; 26:6–36. [PubMed: 26530523]

Summary of key points

Recently, the FDA issued an official warning to all practicing physicians regarding potentially detrimental behavioral and cognitive sequelae of an early exposure to general anesthesia during *in utero* and an early postnatal life. The FDA concern is focused on children younger than three years of age who are exposed to clinically used general anesthetics and sedatives for three hours or longer. Although human evidence is limited and controversial, a large body of scientific evidence gathered from several mammalian species demonstrates that there is a potential foundation for concern. Considering this new development in public awareness, this review focuses on non-human primates because their brain development is the closest to humans in terms of not only timing and duration, but in terms of complexity as well. The review compares those primate findings to previously published work done with rodents.

Summary Statement

Newest studies with monkeys confirm the large body of evidence presented by rodent studies and brings us a step closer to understanding the implications of early exposure to general anesthesia.

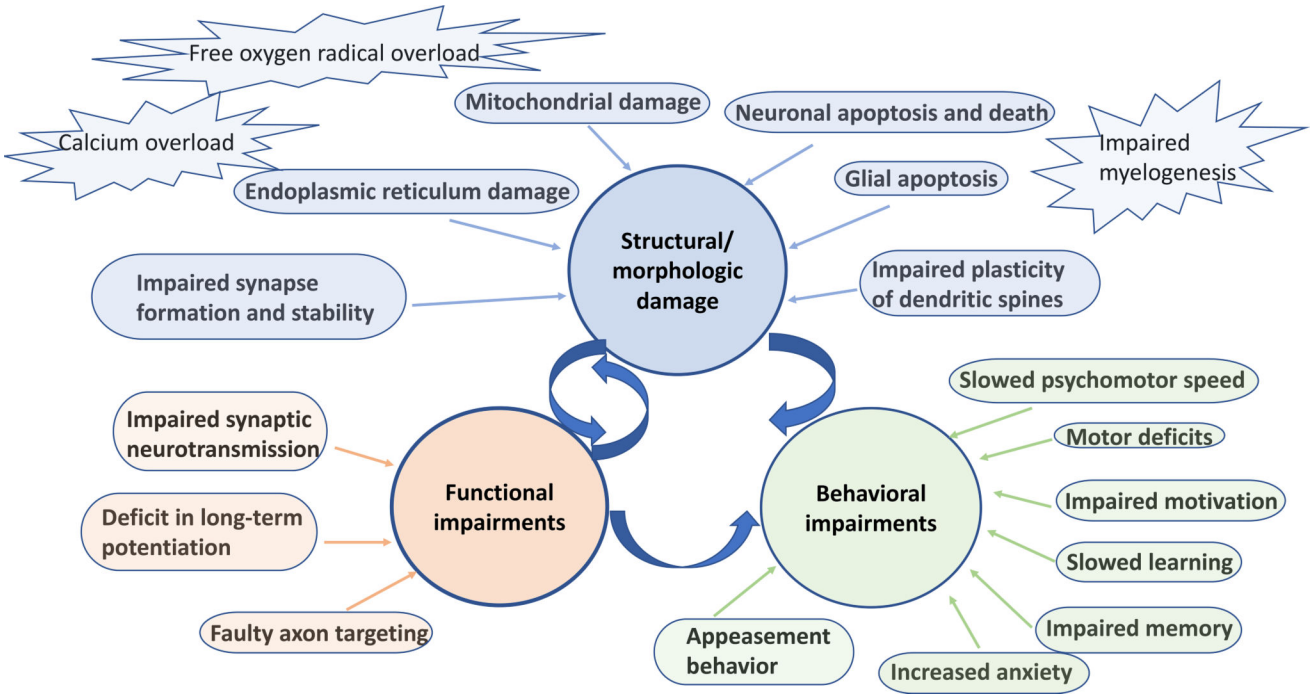

Figure 1.