

# Safety of anesthetic exposure on the developing brain – Do we have the answer yet?

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## Abstract

During the past two decades, a vast number of studies done on rodents and nonhuman primates have implicated general anesthetic exposure of developing brains in producing neurotoxicity leading to various structural and functional neurological abnormalities with cognitive and behavioral deficits later in life. However, it is still unclear whether these findings translate to children and whether single exposure to anesthesia in childhood can have long-term neuro-developmental risks. Considering the fact that a large number of healthy young children are undergoing elective surgery under general anesthesia globally, any such potential neurocognitive risk of pediatric anesthesia is a serious public health issue and is therefore important to understand. This review aims to assess the current preclinical and clinical evidence related to anesthetic neurotoxicity.

**Keywords:** Anesthesia, child, developing brain, neurotoxicity

## Introduction

Neurotoxicity refers to a type of toxicity in which a chemical, physical, or biological substance produces harmful structural or functional effect on the neuroaxis at the peripheral or central level.<sup>[1]</sup> Neurotoxin is an agent exposure which can cause alteration in the normal activity of nervous system, causing permanent or reversible damage to nervous tissue. Toxicity of any agent is directly related to its dose and is less dependent on its chemical properties. Neurocognitive functions are closely related to functions of specific neural pathways, cerebral regions, or cortical links in the substrate layers of brain or neural matrix at the molecular level in cell. A neurocognitive deficit can be defined as a lessening or damage of cognitive functions in these regions.<sup>[2]</sup>

During the past two decades, a vast number of animal studies done on rodents and non-human primates have implicated

general anesthetics in causing various structural and functional neurological abnormalities in developing animal brain with cognitive and behavioral deficits later in life, thereby engendering a big debate among the clinicians regarding their safety for young children. The first evidence on this aspect was from a rodent study that was published in 1999.<sup>[3]</sup> The study concluded that blocking N-methylD-aspartate (NMDA) glutamate receptors even for a short span of few hours during later part of pregnancy or early newborn period could trigger widespread apoptosis and neurodegeneration in the immature rat brain, suggesting that the neurotransmitter glutamate which is excitatory in nature, acting through the NMDA receptors, may have a role in controlling neuronal survival. They suggested in the light of these findings that exposure to NMDA receptor blockers (many anesthetics) may have implication in various neurodevelopment diseases.

With the widespread use of general anesthetic agents in the vulnerable neonates and young children, both during

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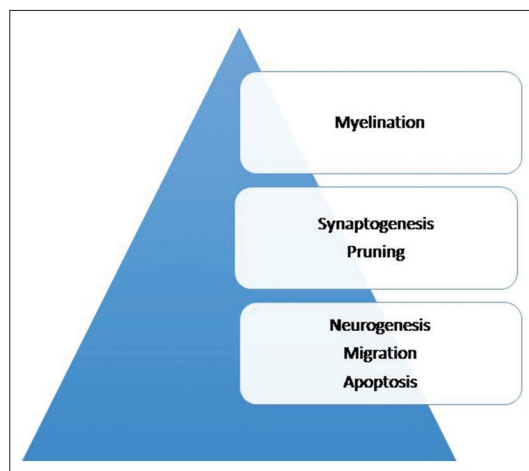
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surgery and nonsurgical interventions, their safety is a burning public health problem and has drawn interest of the general public, regulatory bodies, and the anesthesiologists globally. To address these issues in the United States, a partnership between International Anaesthesia Research Society and Food and Drug Administration (FDA), known as Smart Tots (Strategies for Mitigating Anesthesia-Related neurotoxicity in Tots), was formed. It works toward coordination and funding research toward safe delivery of anesthetic/sedatives to children enduring surgery. In a recent update regarding the use of anesthetic and sedation agents in pregnant woman and young kids, issued in April 2017, FDA has approved two label changes with regard to their use in the aforementioned population. The first change includes a notice mentioning that long duration or multiple exposures to these agents may potentially affect brain development in children <3 years. The second warning is added information to pregnancy and pediatric use section labels describing research pertaining to pregnant and young animals which have demonstrated that exposure to these anesthetic drugs for a duration of >3 h may lead to widespread neural loss in the developing brain with behavioral and learning deficits later in animal's life.<sup>[4]</sup> This review aims to understand the impact of the issue concerning neurotoxicity of anesthetics in neonate and young children in the present day context, based on the clinical and nonclinical studies conducted so far.

## Development of Normal Human Brain

Brain development follows a predetermined organized pattern [Figure 1] that correlates with functions that a newborn performs at various stages of development. The process of neurogenesis begins in the early weeks of gestation after conception. The majority of the cortical neurons develop and proliferate in germinal matrix and subventricular zone. Migration to their final destination in cortex ensues between



**Figure 1:** Major neurodevelopmental processes in human brain

weeks 12 and 20 of gestation.<sup>[5]</sup> Rapid automated death of cells (apoptosis) happens from 24 weeks of gestation to 4 weeks after birth causing 50% reduction in the neurons. Although cortical neurons develop before birth, they are poorly linked. Synaptogenesis (proliferation and organization of neuronal synapses) starts at about 20<sup>th</sup> week of gestation and the number of synapses reaches a peak at about 1–2 years of age. This period of massive burst of synapse formation is known as the “exuberant period.”<sup>[6-9]</sup> Regionally, specific loss of synaptic connections in the process of pruning occurs following this. The major indicators of developmental stage are sulcation and myelination. Myelination begins by the end of second trimester and continues at a much slower rate throughout life.

Neuroplasticity is the ability of the brain to change throughout life. Various stimuli travelling through sensory afferents to the brain before and after birth cause change in the neuronal function and their pathways. This process of neuroplasticity is double-edged sword as it increases the capability of the human brain, on one hand, but, on the other hand, makes it vulnerable to changes in environment.<sup>[10]</sup>

## Mechanisms of anesthetic neurotoxicity

A report based on the Salzburg Seminar discussed in detail the various mechanisms leading to anesthesia-induced neurotoxicity based on current evidence from human and animal studies.<sup>[11]</sup> Several mechanisms have been proposed to be causal in anesthesia-induced neurotoxicity.

The developmental stage and the degree of anesthetic exposure (both the frequency of exposure and cumulative anesthetic dose) are the two significant factors that determine anesthetic neurotoxicity.

It is clear from animal models that anesthesia-induced neuroapoptosis or defective synaptic development occurs in phase of synaptogenesis.<sup>[12]</sup> As a result, the local differences in neurotoxicity could be related to dissimilarity in synaptogenesis in different brain regions during its development.

Certain brain regions such as the hippocampal dentate gyrus and the subventricular zone undergo neurogenesis all throughout life. In the hippocampus, this process of neurogenesis is thought to be important for learning and memory. In an animal study, repeated exposure of isoflurane for 35 min every day for 4 days to young rodents resulted in impaired memories which turned out to be more pronounced with the growth of the animals. However, adult rodents did not show such impairment with similar exposures. Why this isoflurane-induced loss of stem cells is seen in the young but not seen in the adult brains is still not clear.<sup>[13]</sup>

A primary cultured model of hippocampal neurons, used to study neurotoxicity, exposed to 1.4% isoflurane or 2 $\mu$ M propofol for 4 h was shown to cause significant reduction in synaptic density. At the cellular level, there is activation of Ras homolog gene family (RhoA) and the growth factor receptor p75. Administration of Pep5 (an inhibitor of p75 receptor) along with isoflurane reduces its effect on synaptogenesis.<sup>[14]</sup>

Mitochondrial dysfunction and oxidative stress also promote anesthesia-induced neurotoxicity. Anesthetics and sedatives acting through GABA<sub>A</sub> receptor activation increase intracellular calcium, disturb the mitochondrial membrane potential, and ultimately lead to cell death.<sup>[15,16]</sup> Anesthetic agents such as propofol, isoflurane (along with midazolam and nitrous oxide), and sevoflurane are shown to increase reactive oxygen species (ROS).<sup>[17]</sup> Hence, one should avoid redundant hyperoxic ventilation or may use antioxidants under anesthesia to protect against anesthesia-induced neurotoxicity.<sup>[18,19]</sup> Anesthetic preconditioning has been postulated to reduce oxidative stress and mitochondrial dysfunction in brain and heart due to production of subtoxic ROS and subsequent antioxidant gene expression.<sup>[20,23]</sup>

Nitrous oxide neurotoxicity is mediated by blocking of the NMDA receptors and increasing the plasma homocysteine levels. In a study, an 8-h exposure to nitrous oxide was shown to be associated with an eight-fold increase in blood homocysteine level.<sup>[24,25]</sup> However, future research is needed to assess whether this effect is important for short- or long-term neurocognitive outcomes.

Glial cells, an important anesthetic target, play an important role during early phase of brain growth.<sup>[26,27]</sup> Isoflurane impairs glial cytoarchitecture in immature astrocytes, which could impair morphological growth and proliferation.

Surgery by itself has an additive effect on anesthesia-induced neurotoxicity.<sup>[28]</sup> Interleukin-1 beta (a key proinflammatory factor elevated during surgery) increases the surface expression of GABA<sub>A</sub> receptors on neurons and may increase neurotoxicity.<sup>[29]</sup>

Isoflurane activates the complement cascade and inflammatory pathways in the absence of apoptosis or overt changes in the number or morphology of microglia. Hence, the anesthetic effect is much more complex than activation of apoptosis during synaptogenesis.<sup>[30,31]</sup>

### Effect of *in utero* and maternal exposure to anesthetics

The recent past has seen a growing number of fetal intervention programs and cases. Some of these would

require a prolonged duration of general anesthesia involving the use of significant concentrations of volatile anesthetics to maintain uterine quiescence and to anesthetize both the mother and the fetus.<sup>[32]</sup> Another important concern is that such fetus is likely to undergo multiple surgical procedures after birth also and thereby are prone to repeated anesthetic exposures later in life. Exposure of fetal brain to significant levels of GABAergic agents as a result of the anesthetic exposure can potentially increase the risk of neurodegenerative changes. In cases of general anesthesia to the mother, the present data on fetal well-being focus on the teratogenic effects during early gestation and APGAR scores along with acid-base status at or near term. There are very limited data on neurodevelopmental consequences on the fetus of the *in utero* anesthetic exposure.<sup>[33,34]</sup> There are several mechanisms that collectively increase the susceptibility of the fetal brain to anesthetic neurotoxicity, that is, ease of transfer of most anesthetic agents across placenta due to their lipophilicity,<sup>[35]</sup> relatively longer duration of general anesthesia required for such procedures,<sup>[36,37]</sup> higher concentrations of volatile agents that may be needed, and finally the high sensitivity of the neurodevelopment process of neurogenesis and neuronal migration to environmental influences.

### Difficulty in assessing the toxic effects of anesthetic agents on human brain

In the past two decades, a large number of nonclinical and clinical trials have studied the neurotoxic effects of the anesthetics on the developing brain. There still exists a clinical equipoise with some studies showing a possible negative effect and others showing no effect. The dominant question regarding the translatability of the results of animal studies to human population remains unanswered. However, due to obvious ethical concerns, exposures cannot be performed prospectively, nor can the anesthetic exposure be given to children when not indicated, limiting our ability to get precise answers.<sup>[38]</sup>

### What is the evidence of harm related to anesthetic agent exposure till now?

Recently, a number of studies on the same subject have been published in humans [Table 1].<sup>[39-49]</sup> Most of these are retrospective cohort studies showing inconsistent results. Till date, only one large multicentric randomized controlled trial, the GAS Trial, has been done.<sup>[47]</sup> In this trial, infants undergoing hernia repair prospectively received either sevoflurane anesthesia or awake regional anesthesia, and the risk for cognitive decline in sevoflurane-exposed children was assessed using a cognitive test battery at 2 years. The trial failed to identify any increased risk for negative cognitive outcomes in the exposed group. The 5-year follow-up data on GAS study are awaited. With the available evidence, a

**Table 1: Clinical studies on the effects of anesthetic agents in childhood on neurological outcomes**

| Authors   | Study design                    | Study/<br>controls                                     | Duration of<br>exposure<br>(min) | Exposure<br>age<br>(years) | Assessment<br>age (years)                      | Outcome<br>measure                                 | Effects   |
|---|---------------------------------|--|----------------------------------|----------------------------|--|--|---|
| Hansen <i>et al.</i> 2013 <sup>[39]</sup>       | Nationwide birth CS (1986-90)   | 2689/14,575  | NS                               | 0-1                        | 15-16  | APin 9 <sup>th</sup> grade                         | ND  |
| Bartels <i>et al.</i> <sup>[40]</sup>           | Twin study                      | 384/759  | NS                               | 0-3                        | 7-10   | AP   | ND  |
| Fan <i>et al.</i> 2013 <sup>[41]</sup>          | Prospective CS                  | 72   | 67.3 (9.8)                       | 4-7                        | Day prior, day 1 of surgery and 6 months later | WPPSI (3 <sup>rd</sup> ed..n)                      | ND  |
| Stratmann <i>et al.</i> , 2014 <sup>[42]</sup>  | Matched CS                      | 28/28  | NS                               | <1                         | 6-11   | Recognition memory Tasks, WASI, CBCL               | Recollection memory significantly lower in both color and spatial tasks, ND in familiarity, IQ or behavioral indices                |
| Taghon <i>et al.</i> , 2015 <sup>[43]</sup>     | Gender-andage-matched CS        | Exposed - 15<br>Control -yes                           | 65-317                           | <2                         | 10-17  | MRI imaging ("go/no-go" attention task)            | ND in performance accuracy and response time; activation differences detected in cerebellum, cingulate gyrus and paracentral lobule |
| Backeljauw <i>et al.</i> , 2015 <sup>[44]</sup> | Retrospective-matched CS        | Exposed - 53<br>Control - yes                          | 5-170                            | <4                         | 5-18   | WISC/WAIS, WLS, MRI                                | Lower scores and performance IQ, associated with low gray matter density in occipital cortex and cerebellum                         |
| Sun <i>et al.</i> , 2016 <sup>[45]</sup>        | Sibling-matched CS              | Exposed - 116<br>Control -yes                          | 20-240                           | <3                         | 8-15   | IQ tests for neurocognitive functions and behavior | ND  |
| Clausen <i>et al.</i> , 2016 <sup>[46]</sup>    | Nationwide birth CS (1986-1990) |  | NS                               | 2.8 months (median)        | 15-16  | 9 <sup>th</sup> grade exams                        | Lower scores in children with cleft palate repair   |
| Davidson <i>et al.</i> , 2016 <sup>[47]</sup>   | RCT                             | 358 (GA); 361 (RA)                                     | 54                               | NS                         | 2  | BSID-III, Mc Arthur Bates                          | ND  |
| Hu <i>et al.</i> , 2017 <sup>[48]</sup>         | retrospective CS (1996-2000)    | 116 >1 surgery, 457-1 surgery, 463- unexposed          |                                  | <3 years                   | 14-18  | AP   | Multiple exposures are more likely to develop adverse outcomes related to learning and attention                                    |
| Glatz <i>et al.</i> , 2017 <sup>[49]</sup>      | Nationwide CS (1972-1993)       | 33,512 (single exposure)<br>37,231 (multiple exposure) |                                  | <4                         | 16-18  | Mean school grades and IQ test score               | Small association   |

NS=Not specified; AP=Academic performance; WPPSI=Wechsler Preschool and Primary Scale of Intelligence; RCT=Randomized control trial; CS=Cohort study; ND=No difference; GA=General anesthesia; RA=Regional anesthesia

meaningful answer cannot be drawn regarding the impact of age on neurotoxicity of anesthetic agents. The most vulnerable period or the age beyond which anesthetic exposure can be safe remains unknown. This is important to know so that elective surgeries can be postponed till the time beyond which anesthetic exposure is considered to be safe.

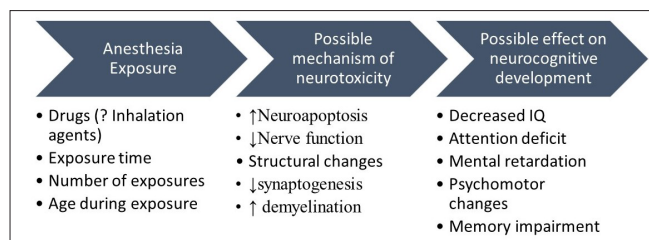
### Framework for studying anesthetic neurotoxicity

*In vitro* and *in vivo* research conducted in animals have suggested that exposure to anesthetics leads to structural impairments in brain structure by the process of increased apoptosis, decreased neurogenesis, synaptogenesis, and alterations in dendritic spine architecture and other undefined biological mechanisms. These collectively lead to neurotoxicity which manifests as adverse health effects in the form of

neurocognitive dysfunctions and functional impairment.<sup>[38,50,51]</sup> A conceptual framework [Figure 2] for studying neurotoxicity caused due to anesthetics was suggested by Guohua Li.<sup>[6]</sup> To extrapolate these results from preclinical studies and analyze data from clinical studies, a wide range of end points including abnormalities in behavior and learning, performance in school, direct neuropsychological testing, and motor performance have been used.<sup>[50]</sup>

### Limitation of using preclinical evidence for studying anesthetic neurotoxicity

Animal studies by design are uniform unlike human studies where there is variability in exposure times and ages during exposure. Development of neurological abilities such as language, skills, intelligence, and academic performance takes



**Figure 2:** Framework to study anesthetic-agent-induced neurotoxicity

a long period in humans, and so it is difficult to model these in animals. Language development may be especially susceptible to anesthetic exposure during infancy and toddler years.<sup>[44,52]</sup> This aspect cannot be reasonably tested in preclinical studies. In addition, the developmental stages of rodents and even primates do not exactly parallel the corresponding human phases and there can be considerable lag in the various milestones, making a sound comparison difficult.

### Anesthetic neuropharmacology, neurotoxicity, and neuroplasticity

Alcohol fetal syndrome shares many features in common with the apoptosis induced by anesthetics and sedatives. The possibility of long-term effect of anesthetic exposures on developing brain had been explored as an extension of animal research into occupational exposures and fetal alcohol syndromes.<sup>[53,54]</sup> Various important aspects of anesthetic-induced neurotoxicity include the following given below.

#### Effect of exposure time

In both human and animal studies with exposure time of less than 1 h, the ratio of positive-to-negative studies ranged between 40% and 50%.<sup>[38]</sup> Positive studies were the ones where at least one abnormality, even transiently, was reported and negative studies were those which did not identify any abnormal structural and/or functional outcome. For exposure times of more than 3 h, the ratio exceeded 80%.<sup>[38]</sup> In line with these findings, many recent studies including only randomized controlled trial have not demonstrated any significant difference between children with a single brief exposure in early childhood or no exposure.<sup>[47]</sup> Similarly, the PANDA study did not show any differences in IQ between siblings with and without anesthesia exposure of 20–240 min (median duration of 80 min).<sup>[45]</sup> In a recent cohort study from Western Australia in 2608 children around 10 years of age, extensive neurobehavioral testing was done.<sup>[55]</sup> It was seen that 321 children who were anesthetized and underwent surgery before 3 years of age had more probability of having defective language and abstract reasoning compared with unexposed children. However, it was noteworthy that more than one-third of them underwent surgery which lasted less than 15 min. Furthermore, this subset of population is known to suffer from later language and learning problems

due to the primary disease, thereby raising doubts on the reliability of the results.

#### Effect of number of anesthetic exposures

There is wide variability in the results of studies done on this topic with some studies reporting functional and structural brain alterations following single anesthesia exposure,<sup>[44,55]</sup> while others reporting increased risk only with multiple exposure<sup>[49,56]</sup> and some reporting no negative results.<sup>[45]</sup> However, multiple exposures of anesthesia have been consistently found to be linked with later learning disabilities. This may be related to the cumulated dose of exposure or the actual number of times the child got anesthetized. In a study by Wilder *et al.*, single exposure did not increase the risk of cognitive impairment in 449 children studied, while more than one incidence of anesthesia exposure directly correlated with later learning disabilities among 144 children.<sup>[56]</sup> Another study by Glatz *et al.* also observed proportionately worse school performance with higher episodes of anesthesia exposures.<sup>[49]</sup> Limitations of these studies are that confounding variables are likely to increase with increasing number of surgical procedures. In addition, the duration of procedures was not analyzed.

#### Age during exposure and outcome

In most of the animal studies, immature stages of human brain development in antenatal period showed intense vulnerability to anesthetics. Interestingly, studies on humans and animals beyond the prenatal period did not show decline in positive studies with increasing age, during exposure as would be expected, based on the above findings. We cannot conclusively refer to a safe age after which anesthetic can be safely administered.

Two clinical studies on the preterm infants in neonatal intensive care unit examined the effects of exposure to long-standing sedatives which could not demonstrate any impaired cognition at 2–5 years following exposure to sedatives.<sup>[57,58]</sup> The PANDA study was a large-scale multisite, ambi-directional sibling matched cohort study that included siblings within 36 months of age and assessed neurocognitive and behavioral outcomes prospectively in children at 8–15 years of age to ensure neurocognitive impairment if any.<sup>[45]</sup> The data on anesthesia exposure were assessed retrospectively. They found no statistically significant difference in full-scale IQ score (mean difference of 0.2 IQ points) between siblings with and without single anesthesia exposure before 3 years of age. The secondary outcomes (mean scores of memory, language, attention, executive function, visuospatial function, motor and processing speed, or behavior) were also comparable.

#### Future direction for research

The impact of different anesthetic agents and the dosages on the neurodevelopment varies and safe thresholds need to

be determined. The exposure time threshold also needs to be ascertained to know the “safe” time limits within which procedures can be performed under anesthesia. This will help in planning whether the surgery can be done in a single sitting or multiple. In addition, if multiple exposures are found to have increased risk, then procedures could be performed jointly at the cost of increasing exposure duration. The impact of surgery and pain vis-à-vis the neurotoxicity on the developing human brain needs to be assessed.

## Conclusion

The current animal and human literature regarding anesthetic neurotoxicity is largely inconclusive and insufficient to make unambiguous recommendation regarding the safety of their use for neonates or older children. Further research is required to clearly identify the type of anesthetic agent and techniques, the threshold for age, and duration of exposure which can be clearly defined as safe for this vulnerable patient population. Till that time, there is no indication for pediatric anesthesiologists to modify their routine practice other than adopting various mitigating strategies.

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## Conflicts of interest

There are no conflicts of interest.

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