

# Anesthesia Induced Developmental Neurotoxicity (AIDN)

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**Abstract:** "How do we determine what are physical, chemical and psychological hazards of occupation and in particular those that are rare and not easily recognized?" and "... the available human studies ... cannot exclude the possibility that the anesthesia-induced neurotoxicity observed in many animal studies may also occur in children" Sir Austin Bradford Hill, Royal Society of Medicine, 1965

**Keywords:** Neonate, Infant, Child, Anesthesia, Toxicity

## 1. Introduction

Pediatric patients comprise a significant proportion of the total number of patients treated with general anesthesia, a trend that will continue well into the future. Pediatric patients differ significantly from adults with respect to anatomical, physiological, and pharmacological characteristics.

In December 2016, the US Food and Drug Administration (FDA) issued a drug safety announcement warning that repeated or lengthy (>3 hours) use of general anesthetic and sedating drugs during surgeries or procedures in children <3 years of age or in pregnant women during their third trimester may affect the development of children's brains. The FDA now also requires drug manufacturers to add warning labels regarding children <3 years of age being exposed to general anesthetics and sedative medications: desflurane, etomidate, halothane, isoflurane, ketamine, lorazepam injection, methohexital, midazolam injection and syrup, pentobarbital, propofol, and sevoflurane. (1, 2, 3, 4, 5)

Patients admitted to the pediatric intensive care unit (PICU) from the emergency department (ED) or operating room routinely require continuous and prolonged infusions of sedatives and opioids for management of pain and anxiety, tolerance of mechanical ventilation, control of intracranial hypertension, and/or provision of immobility. These infusions may continue for days, weeks, or even months. In addition, patients admitted to the PICU may require imaging studies or other procedures outside the operating room for which sedation may be provided by a team using anesthetics such as ketamine or propofol.

Use of these sedative and anesthetic agents can result in cognitive decline, leading to lower IQs, deteriorating school performance, and shortfalls in attention and memory. The risk of learning difficulties has been seen to progressively increase with repeated exposure to anesthesia. Behavioral disorders were 60% more frequent among those who received anesthesia in comparison to those who did not. The estimated hazard ratio for developmental and behavioral disorders is 1:1 for those who received anesthesia once before the age of 3, 2:9 for those exposed twice, and 4 for

those who have been exposed to anesthesia three or more times. (6,7,8,9,10)

Most of the data related to anesthetic neurotoxicity originate from studies related to fetal alcohol syndrome. Ethanol is a known *N*-methyl-D-aspartate (NMDA) receptor antagonist and  $\gamma$ -aminobutyric acid (GABA) receptor agonist. During periods of synaptogenesis ethanol was seen to cause widespread apoptotic neurodegeneration.

When the effects of anesthesia used during cesarean procedures were examined in children, infants born under regional anesthesia exhibited fewer learning difficulties in the later stages of their life when compared with those delivered under general anesthesia.

The Mayo Anesthesia Safety in Kids (MASK), General Anesthesia Compared to Spinal Anesthesia (GAS) and the Pediatric Anesthesia Neurodevelopmental Assessment (PANDA) studies have all advised caution in subjecting children <3 years of age to general anesthetics.

## 2. Pediatric Neurology

Neonates are born with approximately 100 billion neurons, and the number of neurons does not increase over time. The neonatal brains weigh approximately 300–400 g. Increased myelination, synapse formation, neuronal maturation, and proliferation of glial cells increase the weight of the brain to 1100 g at 3 years of age and 1300–1400 g at adulthood.

A newborn infant has approximately 50 trillion synapses, increasing to 1000 trillion within the first year of life and decreasing to 500 trillion in adulthood. Synaptogenesis has been defined as the most important period of brain development, also described as the "fragile period" or "critical period." The brain's sensitivity to environmental stimuli is at maximum during the neonatal and infancy period when synaptogenesis is also maximized. Synaptogenesis consists of five phases. The greatest leap in synapse formation occurs in the neonatal phase 3, which is sometimes referred to as the "big bang." Following phase 3, synaptogenesis continues with the same speed during phase 4. This phase is referred to as the plateau phase, corresponding to infancy and adolescence. During phase 5, which occurs during adulthood, synaptogenesis continues,

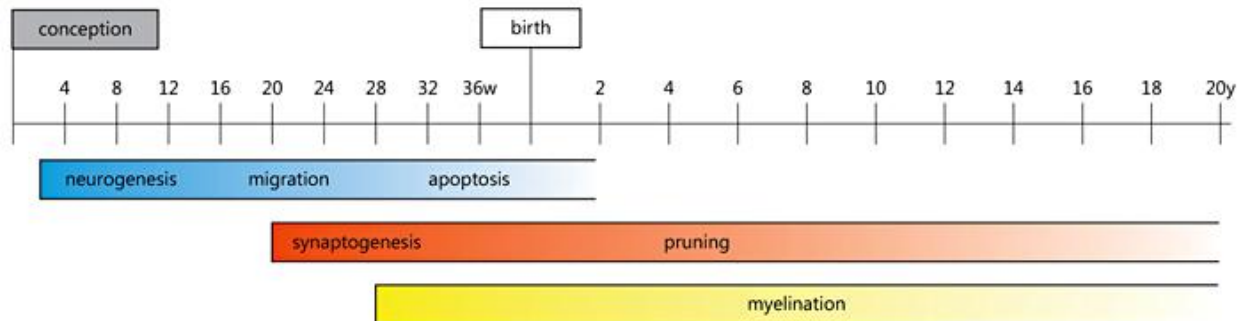
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but it is limited and localized. The initiation, duration, and end of these critical periods (phase 3 and phase 4) are controlled by multiple genetic and epigenetic mechanisms. The most prominent manifestations of anesthesia-induced developmental neurotoxicity (AIDN) are often observed at post-natal day 7, which is the peak period for synaptogenesis

Critical periods for brain development are the intrauterine period, the first 3 years of life and puberty. Brain maturation is not complete at birth, and there is a heterogeneous maturation process in the brain following birth, and this is particularly slow in the cortex and the limbic system. Alteration of neurotransmission in the immature brain due to anesthesia exposure may lead to impairments in the future. (Fig 1)



**Figure 1:** Timing and intensity of key neurodevelopmental processes in the human brain

Neuroplasticities or increase in intercellular connections are neurophysical and neurochemical ability to improve compliance against environmental changes and damage. Agents that enhance neuroplasticity have raised new hope for the treatment of neurodegenerative diseases.

During neonatal brain development, GABA facilitates cell proliferation, neuroblast migration, and dendritic maturation, and unlike in adults, it acts as an excitatory neurotransmitter during infancy rather than an inhibitory neurotransmitter. This is because GABA increases the permeability of the cell membrane to chloride ions through intrinsic chloride-conducting ion pores. Thereafter, KCC2 K<sup>+</sup>/Cl<sup>-</sup> co transporter aids in influx of chloride ion when the neuron is hyperpolarized and its activity is suppressed. However, because KCC2 expression is low during the early period of development, the chloride action potential is reversed by GABA<sub>A</sub> and glycine receptor activity, leading to neuronal depolarization and increased permeability to chloride. (11)

The major excitatory neurotransmitters glutamate and aspartate are present in the brain at very high concentrations (glutamate 10 mmol/L and aspartate 4 mmol/L). Both direct synaptic signaling at nerve terminals and control ion intake to neurons and influence synaptogenesis, neuronal plasticity, learning, and memory. The excitatory neurotransmitters are normally responsible for nerve conduction, but they are also potential sources of neurotoxicity. An abnormal decrease in glutamate may disturb normal excitation, and abnormal increases may cause excitotoxicity and cell death by disturbing calcium homeostasis. Glutamate and similar amino acids have been shown to cause acute swelling in the neuron body, dendrites, and glia and also promote prolonged neuronal degeneration. Under normal conditions regulation of glutamate levels in the synaptic gap involves reuptake of excess glutamate from the synaptic gap through receptors present in presynaptic end of nerve terminal and glial cells. Despite being a strong and rapid-acting toxin under physiological conditions, this mechanism ensures that even direct application of glutamate to the brain does not cause damage. Nevertheless, pathological conditions that affect

this system or cause release of large amounts of glutamate has the potential to cause neuronal loss. (12)

#### **Non Anesthetic Neuronal Damage**

Hemodynamic disturbances due to hypotension, hypertension, tachycardia, bradycardia, asystole, or other arrhythmias, and respiratory system issues such as apnea, hypoxia, hypocapnia, or hypercapnia can lead to disturbances in microcirculation to the central nervous system (CNS) and cause CNS damage. Patient-related factors, such as genetic anomalies, prematurity, sepsis, infection, and vascular diseases, hormonal, metabolic, inflammatory, or cardiovascular changes caused by trauma or surgery, hypo-/hyperglycemia, electrolyte imbalances, and temperature variations that occur due to anesthesia can also contribute to the development of perioperative neurotoxicity.

Although the rate of complications has been reduced through improved understanding of the anatomical, physiological, and pharmacological characteristics of pediatric patients, advances in monitoring methods and practitioner specialization, the risks are never completely eliminated.

#### **Anesthesia and Neuronal Damage**

General anesthetics are highly lipid soluble and can dissolve in every membrane, penetrate into organelles and interact with numerous cellular constituents. Their actions have long been considered rapid and fully reversible, with the pharmacodynamic time course of anesthesia dictated solely by the pharmacokinetic profiles of anesthetic uptake and elimination. In the last decade, it has become apparent that anesthetics can affect gene expression, protein synthesis and processing, as well as cellular function. Neurotoxicity of anesthetic substances on the developing brain is determined by a reduction in neural density and apoptosis that can lead to disturbances in memory, attention, learning, and motor activity.

Anesthetics elicit their effects by enhancing the activity of major inhibitory neurotransmitters gamma-aminobutyric acid (GABA) and glycine or antagonizing the N-methyl-D-

aspartate (NMDA) receptors of the major excitatory neurotransmitter glutamate. (Table 1)

**Table 1:** Actions of anesthetics at receptor level

Agent	GABA	NMDA	$\mu$ -Opioid	$\alpha_2$ -Adrenergic
Halogenated anesthetics (sevoflurane, isoflurane, desflurane)	+			
Nitrous oxide		-		
Benzodiazepines	+			
Propofol	+			
Barbiturates	+			
Etomidate	+			
Chloral hydrate	+			
Ketamine		-		
Opioids			+	
Dexmedetomidine				+

GABA,  $\gamma$ -aminobutyric acid; NMDA, N-methyl-D-aspartate; +, agonist; -, antagonist.

Sevoflurane, isoflurane, and propofol cause excitability in electroencephalogram in neonates. For these reasons, anesthesia applications are believed to disrupt the balance between excitatory and inhibitory neurotransmission and thus cause neuronal injury and reduction in neuron density. Anesthesia has also been incriminated in causing neurotoxicity through neuronal apoptosis, impaired neurogenesis, neuroinflammation, modulation of neuronal plasticity, disruption of glial cytoarchitecture in immature astrocytes, and disturbances in the ultrastructural properties of developing synapses, morphological changes in pyramidal cells and suppressed release of excitatory neurotransmitters.

Memory defects have been observed in patients administered repeated low doses or a single high-dose anesthetic.

A 4-hour sevoflurane exposure (2.5%) has been seen to reduce hippocampal postsynaptic density protein-95 expression without causing any neuronal loss, causing learning and memory disturbances. 0.5% minimum alveolar concentration (MAC) sevoflurane applied for 6 hours has no significant effect on apoptosis and S100 $\beta$  levels. Sevoflurane inhalation lasting less than one hour duration is safe for infants. Sevoflurane, isoflurane, and desflurane increase caspase-3 levels. (13)

With Isoflurane, modalities of neurotoxicity observed are:-

- Dying cells are cleared by microglia before markers of cell death can be detected.
- Progenitor cells differentiate into glial cells instead of neurones.
- The normal, age-related disappearance of hippocampal neural stem cells, the appearance of new astrocytes, and the decline in the production of new neurones may be accelerated.
- Loss of stem cells and reduction of neurogenesis.
- Activation of the complement cascade and inflammatory pathways *via* modulation of C1q+ and C3 and by induction of a variety of cytokines and chemokines. Complement cascade activation can occur

in the absence of apoptosis or overt changes in microglial number or morphology; effects on C1q appear after relatively short exposure to isoflurane (as short as 2 h). (14,15)

Halothane administered during the prenatal period is associated with neurodegeneration and behavioral changes. Neurotoxicity induced by nitrous oxide-induced block of N-methyl-d-aspartate (NMDA) receptors is manifest by massive swelling of neuronal organelles including mitochondria and endoplasmic reticulum. Nitrous oxide also increases plasma homocysteine caused by oxidation of methionine synthase. As levels of homocysteine can be easily measured in blood, they can be used as biomarkers of nitrous oxide-induced modulation of methionine synthase activity. After an 8 h exposure to nitrous oxide, an eight-fold increase in blood homocysteine levels could be detected. Nitrous oxide application for 6 hours does not cause neuroapoptosis; however, apoptosis is increased when nitrous oxide is administered with isoflurane.

Nitrous oxide, sevoflurane, and isoflurane given to children at <12 months of age impairs recollection when these children are 6–11 yr old. Recollection is an important component of recognition memory and is supported by anatomic brain structures that are affected by anesthesia-induced cell death. When the outcomes of spatial tasks were explored, young boys were more affected than young girls, although difficulties with the colour recognition task were detected equally in boys and girls. The performance was worse when children were exposed for a longer time (several hours).(16)

Xenon, the currently preferred anesthetic, does not cause neuroapoptosis when used alone; on the contrary, xenon reduced the effects of other inhalation anesthetics when administered first.

Three hours exposure to ketamine does not produce any significant histochemical change, whereas profound brain cell death is observed in the frontal cortex among subjects that are under the effect of ketamine for 9 or 24 hours. In

cell culture ketamine decreases neuronal viability time and dose dependently, leads to neuronal ultrastructural abnormalities, causes depolarization of mitochondrial membrane potential, induces apoptotic pathway, causes cytochrome c release from mitochondria into cytosol, and induces free oxygen radical production.

The manufacturer of propofol does not recommend the use of propofol as a general anesthetic agent for children under the age of 3.(12)

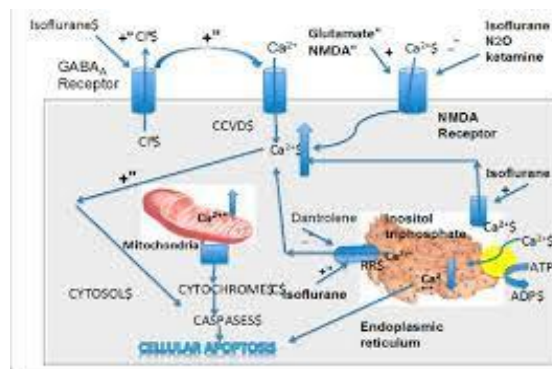
Because dexmedetomidine has no interaction with either GABA or NMDA receptors, it is an attractive agent for study in anesthetic neurotoxicity. At clinical concentrations, there is no increase from baseline neuroapoptosis, but with very high doses, it does result in neuroapoptosis in the sensory cortex and the thalamus. Dexmedetomidine is neuroprotective and this effect is neutralized by the  $\alpha_2$ -antagonist atipamezole. Dexmedetomidine alone does not affect cognitive performance, and it reverses the deficit in cognitive performance produced by isoflurane. (17)

General Anesthesia Compared to Spinal Anesthesia (GAS), the Pediatric Anesthesia Neurodevelopmental Assessment (PANDA) and MASK study observe no statistically significant difference in the full-scale IQ score between siblings at 8–15 years, with and without a single anesthesia exposure before the age of 3 years.(18)

**Neuronal Damage markers**

Apoptosis is a programmed cell death that can occur in both physiological and pathological conditions. Apoptosis is physiologically present in the developing brain, occurring at a rate of approximately 1%. However, apoptosis that occurs following pathological processes like hypoxia and ischemia is increased following anesthesia exposure. It is difficult to estimate the rate of apoptosis following anesthesia exposure in humans and quantify to what extent this apoptosis affects maturation of the developing brain.

Experimental studies have shown that anesthesia induces apoptosis via intrinsic and extrinsic pathways. Anesthesia application causes leakage of cytochrome c and translocation of Bax protein to the mitochondria, leading to activation of the apaf-1 and caspase pathways, respectively. This in turn results in lipid peroxidation via release of free oxygen radicals. Apoptosis occurs not only in intrinsic pathway but also in extrinsic pathway which activates Fas protein. (Fig 2, 3)



**Figure 2 & 3:** Proposed mechanism of anesthetic-induced neurodegeneration

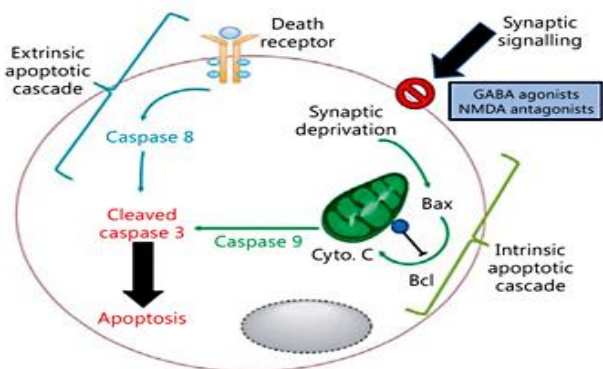
Toxic effects are mediated by the accumulation and aggregation of amyloid-peptides into a variety of soluble oligomers. Amyloid  $\beta$  peptides of various lengths are released after proteolytic cleavage of the amyloid precursor protein (APP). Depending on various conditions, these peptides can self-assemble into toxic oligomers which correlate with the severity of cognitive impairment. Insoluble deposits of  $A\beta$  that appear amorphous under light microscopy are found in abnormally high amounts in the brains of attention deficit (AD) patients and to a lesser extent in normal age-matched humans. While it is not entirely clear how these products of oligomerization cause neurodegeneration, considerable evidence supports the ‘ $A\beta$  hypothesis of AD’. Specifically, mutations of APP that increase cleavage of APP to the toxic form of  $A\beta$  ( $A\beta_{42}$ ) are associated with AD. Synthetic  $A\beta$  peptides are toxic to hippocampal and cortical neurons *in vitro*, and injection of  $A\beta$  into the cerebral ventricles reversibly prevents induction of long-term potentiation, a cellular form of learning and memory. Within the framework of the  $A\beta$  hypothesis, the neurotoxic element is the intermediate-sized  $A\beta$  oligomer rather than the mature fibril. The possibility that volatile anesthetics might favor oligomerization of  $A\beta_{42}$  under physiologically relevant conditions is considered as a possible reason for neurotoxicity. (19, 20)

Propofol downregulates microRNA-21, ketamine upregulates microRNA-34a, microRNA-34c, and microRNA-124 and downregulates microRNA-137.

In cell culture models, it has been demonstrated that neuron development is highly dependent on the actin cytoskeleton, and anesthetics affect actin regulation adversely.

Tau protein hyperphosphorylation at serine 404 demonstrates neurodegeneration and is induced by ketamine. This disrupts and damages microtubules.(21)

Brain-derived neurotrophic factor (BDNF) is formed by destruction of proBDNF in the synaptic gap by the action of plasmin. Mature BDNF binds to the TrkB receptors present on the postsynaptic membrane and enhances viability of the target cell. However, in conditions where plasmin release is reduced or blocked, such as when anesthesia is applied, proBDNF cannot be converted to the mature form, and it stimulates p75NTR instead of the TrkB receptor. Activation of p75NTR receptor, also called the “death receptor,” leads



to actin depolymerization and apoptosis. Isoflurane causes cerebral neuronal apoptosis through this pathway.(22)

Translocator protein (TSPO, 18 kDa) is a biomarker that could be used for evaluation of reactive gliosis and microglia activity. It has the potential for use in noninvasive imaging using positron emission tomography and single photon emission computed tomography. The relationship between anesthesia-associated neurotoxicity and DNA methylation and gene expression is under investigation.

A 2-hour isoflurane anesthetic exposure activates biomarkers compatible with a transient neurotoxic effect: an increase in caspase 3 (a marker of apoptosis) 6 h after anesthesia is followed by elevated A $\beta$  24 h after exposure. (23,24)

### Treatment of Neuronal Injury

Treatment strategies to reduce neurodegeneration induced by anesthetics have been widely investigated. Lithium, melatonin, estradiol, pilocarpine, dexmedetomidine, xenon, erythropoietin, L-carnitine, hydrogen gas, and pramipexole are among the leading candidates for this emerging therapy. Co-administration of apocynin, an NADPH oxidase inhibitor, prevents long-term memory loss following exposure to sevoflurane by preserving glutamatergic neurons in the basolateral amygdala.

Continuous infusion of vitamin B12, which is an enzyme co-factor of methionine synthase prevents nitrous oxide induced neurotoxicity.

Although opioid-based anesthesia and opioids co administered with inhalation anesthetics have been shown to reduce apoptosis, their safety has not been confirmed. Fetal and neonatal chronic exposure to opioids has been associated with neuronal changes.

Dexmedetomidine, the current intravenous anesthetic, reduces prenatal toxicity caused by propofol and isoflurane.

### 3. Conclusion

The potential for anesthetic neurotoxicity in the neonate, young infant, and fetus is the most pressing question facing the field of pediatric and fetal anesthesia. The very rapid development of the fetal brain potentially makes this the patient group most vulnerable to neurotoxicity from anesthesia. The increasing number of fetal interventions requiring anesthesia make this a very important field of research inquiry.

Single brief anesthetic exposures do not appear to produce a measurable effect whereas repeated exposures consistently demonstrate associations between exposure and subsequent deficits in learning and behavior.

Pain itself has a neurotoxic effect, anesthesia-analgesia application in painful conditions may have a net neuroprotective effect. In cases of hypoxia-ischemia or trauma, the administration of anesthetics reduces the infarct volume by reducing the metabolic rate, decreasing

intracranial pressure, eliminating free oxygen radicals, and reducing secondary injury.

The strength of evidence for neuroimaging varies by diagnosis and a substantial number of children with new-onset seizure and status epilepticus will have urgent or emergent intracranial pathology identified on neuroimaging. But a vast majority of children with mild traumatic brain injury do not require neuroimaging especially with known neurodevelopmental vulnerability, e.g. congenital heart disease, other complex neonatal surgeries, and the fetus. Anesthetic exposure in this subset can be avoided. (25)

The focus on development of biomarkers for risk prediction and alternative agents to prevent damage to the developing brain is warranted.

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