REVIEW ARTICLE

Maternal anesthesia and fetal neurodevelopment

A. Palanisamy
Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, USA

ABSTRACT
It is clear from animal studies that commonly used anesthetic agents affect early brain development both histologically and functionally. With human epidemiologic evidence suggesting an association between anesthesia and surgery early in life and late-onset learning disabilities, investigators have focused their attention on the subtle long-term effects of anesthesia exposure. Most obstetric anesthesia studies, however, have focused on either the teratogenic effects of anesthetic agents in the first trimester or on the neonatal status immediately after delivery. Not much attention has been paid to the human second trimester, a period of active fetal brain development typified by neurogenesis and neuronal migration. Of concern though, is that these events are easily perturbed by environmental and pharmacological influences. New research studies have raised significant questions about the fetal impact of maternal anesthesia for non-obstetric and fetal surgery. This review summarizes the major findings in the field of developmental neurotoxicity of anesthetic agents, discusses the susceptibility of the fetal brain to anesthetic effects in a trimester-specific style, and outlines the pitfalls in extrapolating animal research to humans.

Introduction
Emerging lines of animal evidence suggest that anesthetic agents, when administered during the critical ‘growth spurt’ phase of early brain development, cause neurodegenerative changes and learning abnormalities in the offspring.1–3 This phase of rapid brain growth, also called synaptogenesis, is a postnatal phenomenon in the rodent, unlike humans where it begins as early as the third trimester and extends to the first few years of life. Most studies on fetal wellbeing during maternal general anesthesia are typically limited to teratogenic effects earlier in gestation, and Apgar scores and acid-base status at or near term.4,5 Until now, not much has been known about the subtle long-term effects of fetal anesthetic exposure. With the rising incidence of non-obstetric surgery and fetal interventions, the relevant questions then become: are these findings of anesthetic neurotoxicity in postnatal rodents directly applicable to the fetus in utero? Are there any anatomical or physiological characteristics that distinguish the fetal brain from a postnatal brain? If so, do these characteristics alter the vulnerability of the fetal brain to maternal anesthesia in any way? The purpose of this review, therefore, is to elaborate upon the common features underlying anesthetic-related neurotoxicity in the developing brain, to highlight the differences between the fetal and the postnatal brain, to help delineate why second trimester could be a vulnerable period, and to understand how these mechanisms of anesthetic neurotoxicity are applicable to obstetric anesthetic practice. Because of the distinct and unique nature of fetal neurodevelopment, this review will be oriented with the fetus in mind, and, for brevity, focus only on anesthetic and not analgesic agents.

Features of anesthetic-related developmental neurotoxicity
Much of our understanding of anesthetic-related developmental neurotoxicity comes from animal studies performed at a critical time point during early brain development, i.e., the phase of synapse formation, which is a postnatal event at least in rodents. Organization of early neural circuitry, including formation of synapses, depends on ongoing electrochemical activity within the body of immature neurons; firing neurons tend to seek out and establish synaptic connections with other predestined cells, a process called activity-dependent network formation that involves activation of calcium...
channels.6–9 In immature neurons, GABA and NMDA-
subtype of glutamate receptors indirectly regulate these
calcium channels and it is easy to see why interference
with these channels, as would happen with inhalational
and intravenous anesthetic administration, could derail
early neurodevelopment. Apoptotic neuronal death, a
common feature of these developmental neurotoxicity
studies, was certainly not without functional conse-
quences. It was accompanied by electrophysiological
changes in the hippocampus, persistent deficits in learn-
ing and memory, and abnormal social behavior reminis-
cent of autism spectrum disorders.1–3,10 These
pioneering studies established the fundamental princi-
pies in the field and remain the foundation of current re-
search; they indicate that a combination of anesthetic
agents acting through multiple receptor mechanisms is
more neurotoxic than a single agent administration, and
that this neurotoxic effect is developmental stage-
dependent.1,2,10 Although the exact mechanisms remain
unclear, it appears that this cell death is not due to direct
cytotoxicity of anesthetic agents.11 Of more concern is
that similar findings are reported in primates, a species
that is phylogenetically proximate to humans.12–15

Although neuronal death was purported to occur due
to a lack of synapse formation, there was no evidence to
suggest that anesthetics directly affected the structure of
the synapse. Recently though, elegant electron micro-
scopy studies have shed light on this hitherto unstudied
aspect. A combination of anesthetic agents that included
nitrous oxide, isoflurane, and midazolam, caused mor-
phologically abnormal synapses when administered dur-
ing synaptogenesis. Specifically, there was a significant
decrease in volumetric densities of synapses in the subic-
ulum, a region of the hippocampal formation,16 along
with structural changes indicative of mitochondrial
degeneration.17 Moreover, both inhalational and intrave-
nous anesthetic agents altered dendritic spine formation,
a prerequisite to synaptogenesis, in a developmental stage-dependent manner.18,31 The effects of propofol,
in this regard, were intriguing in that it either permanently
decreased or increased dendritic spine formation in the
rat medial prefrontal cortex depending upon the time of
exposure.19 Collectively, these rodent studies have con-
firmed that commonly used anesthetic agents cause
during adverse effects on synaptic morphology and
neural circuit assembly.

Table 1 Salient features of developmental anesthetic
neurotoxicity

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
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<tbody>
<tr>
<td>Apoptotic neuronal death</td>
<td>During synaptogenesis</td>
</tr>
<tr>
<td>Suppression of neurogenesis</td>
<td>During synaptogenesis</td>
</tr>
<tr>
<td>Morphologically abnormal synapse formation</td>
<td>During synaptogenesis</td>
</tr>
<tr>
<td>Altered dendritic spine formation</td>
<td>During synaptogenesis</td>
</tr>
<tr>
<td>Impairment of hippocampal long-term potentiation</td>
<td>During synaptogenesis</td>
</tr>
<tr>
<td>Suppression of neurogenesis</td>
<td>During synaptogenesis</td>
</tr>
<tr>
<td>Aberrant cell cycle re-entry during neuronal mitosis</td>
<td>During synaptogenesis</td>
</tr>
<tr>
<td>Neuronal mitochondrial dysfunction</td>
<td>During synaptogenesis</td>
</tr>
<tr>
<td>Abnormal intra-neuronal calcium homeostasis</td>
<td>During synaptogenesis</td>
</tr>
</tbody>
</table>

...8%) decrease rodent neural progenitor cell prolifera-
tion by 20–30%.22 Given the anti-mitotic effects of inhala-
tional anesthetic agents on multiple cell lines,23–25 it is
perhaps not so surprising that isoflurane affects neuronal
proliferation. In humans, neurogenesis begins and peaks
in utero, and therefore, these findings of a reduced neural
stem cell pool following isoflurane administration may
have a direct bearing on maternal anesthesia during
mid-gestation.

Despite such convincing evidence, mechanistic as-
pects still remain unresolved due to logistic concerns
with current experimental paradigms. For example, it
is not possible to administer a GABA-antagonist, or
an NMDA-receptor agonist to prevent such adverse ef-
fects of inhalational anesthetics in vivo, without disrupt-
ing the anesthetic effect. Adding to this concern is the
pleiotropic nature of anesthetic drugs; anesthetic agents,
for instance, cause apoptosis by interacting with inositol
1,4,5-triphosphate receptors to cause calcium dysregula-
tion,26,27 depolymerize actin, a cytoskeleton protein,
through activation of p75 neurotrophin receptor,28 im-
pair mitochondrial function directly,17 affect the cytos-
ketalon of immature astroglial cells,29 upregulate
NMDA receptors increasing the susceptibility to gluta-
matergic signaling,30 and enhance aberrant cell cycle
reentry (Table 1).31 In aggregate, it is clear that admin-
istration of anesthetic agents during critical neurodevel-
opmental time points does much more than transient
suppression of synaptic function, a hitherto widely held
dogma of anesthetic action.

The uniqueness of fetal brain development

Before extrapolating research studies, it is essential to
acknowledge important temporal differences in brain
development both between experimental animals and
humans (Fig. 1) and qualitative differences between pre-
and postnatal neurodevelopment. The formation of the
human nervous system consists of a sequential ser-
ies of well-orchestrated steps – neurogenesis, differentia-
tion, and neuronal migration – that precisely positions
the appropriate cell type at a preordained location fol-
lowed by establishment of synaptic connections, i.e.,
synaptogenesis. In rodents, at least, the majority of synaptogenesis occurs postnatally and the preceding processes, such as neurogenesis and neuronal migration, begin and peak in utero. From a developmental perspective, therefore, the fetal rodent brain is qualitatively unlike the postnatal rodent brain, where synaptogenesis is a major developmental event and the focus of a majority of anesthesia-induced developmental neurotoxicity studies. Because this vulnerable phase extends from the third trimester to the first few years of life in humans, a question arises whether the fetal brain is affected as well. This marginal developmental overlap notwithstanding, the fetal brain appears to be developmentally distinct from the postnatal brain in a myriad of other ways, and it is unclear, at present, if the fetal brain is differentially vulnerable to anesthetic neurotoxicity.

Neurogenesis refers to the generation and survival of functional post-mitotic neurons from neural stem/progenitor cells derived primarily from the subventricular zone (SVZ) and the subgranular zone (SGZ). Less well-studied are the interneuron progenitor cells that arise from ganglionic eminences. Regardless of their origins, immature neurons generated from these neurogenic niches migrate to their final destinations, a complex phenomenon that requires structural and humoral assistance. An apt analogy would be trekking an equivalent of thousands of miles using just anatomical landscape and paracrine signaling cues. What is commonly understood is that there are at least two known subtypes of this bewildering process: radial and tangential migration. Briefly, pyramidal neurons that make up the majority of the cortical layers migrate radially from the SVZ, whereas interneurons, which constitute 10–15% of the neurons in the brain but play an indispensable role in local neural circuitry, migrate tangentially from the ganglionic eminences. Despite these processes occurring at varying rates in different brain regions, both neurogenesis and neuronal migration accelerate and reach a peak during and after the second trimester.

Although neurogenesis and neuronal migration are genetically programmed, environmental cues play a pivotal role in shaping the final three-dimensional architecture of the brain. One of the major nurturing factors during early neurodevelopment is the immediate availability of glutamate and GABA in the vicinity of these neurogenic niches. Unlike the adult brain where glutamate and GABA act primarily as neurotransmitters engaged in synaptic communication, they serve a trophic role during early brain development. For example, both glutamate and GABA influence neuronal proliferation, cell fate determination, and neuronal differentiation, and GABA, per se, serves as a ‘stop’ signal for neuronal migration. Pharmacological interventions that act directly or indirectly on these powerful neuromodulator systems during pregnancy, such as ethanol and anti-epileptic drugs, induce a long-lasting impairment of brain development mainly due to impaired neurogenesis and/or altered neuronal migration. Given that anesthetic agents act precisely through the same receptor mechanisms, it is conceivable that maternal...
administration of anesthetics could potentially alter one or more of these processes in the fetal brain.

Another distinguishing feature of fetal brain development is its symbiotic relationship with maternal hormones, a phenomenon that ceases after severance of the placenta and is notably absent in the postnatal brain. Pregnancy is characterized by a multifold increase in the plasma levels of serum progesterone, estradiol, and their derivatives compared to the non-pregnant state. These hormones freely cross the placenta, suffice the fetal brain, and profoundly influence neurodevelopment. For example, estradiol and progesterone influence neural stem cell proliferation, modulate apoptosis and synaptogenesis in a region-specific manner, alter subcellular signaling mechanisms, and promote dendritic growth and spinogenesis through specific receptor mechanisms. In addition, maternal plasma oxytocin level gradually increases during pregnancy and reaches a peak during the second stage of labor. Researchers have now established that oxytocin has significant effects on GABAergic signaling in the fetal neuron, transiently switching the action of GABA on immature rodent fetal neurons from ‘depolarizing’ to ‘hyperpolarizing’ at term gestation. Complicating this picture further are the neuroprotective effects of oxytocin, and the intricate interactions between oxytocin, GABA, and glutamatergic signaling. Currently, knowledge of these complex interactions during the third trimester is rudimentary at best, and therefore, it is possible that a combination of these factors, i.e., a different stage of neurodevelopment along with the presence of various hormonal effects, may differentially alter the vulnerability of the fetal brain to anesthetic agents in a trimester-specific manner.

Is the fetal brain vulnerable? And if so, when?

There are several reasons to believe that the developing fetal brain may be at risk from maternally-administered anesthetic agents. First, most general anesthetic agents are lipophilic and cross the placenta easily. Specifically, at least in a rodent model, this transplacental transfer was associated with a directly measurable concentration of isoflurane in the fetal brain. Second, non-obstetric surgeries including fetal intervention procedures are, compared to cesarean deliveries, relatively long and general anesthesia is often necessary. Third, high concentrations of anesthetic (~1–1.5 MAC) are usually used to facilitate uterine quiescence and minimize the risk of preterm labor. Most importantly, the neurodevelopmental processes occurring during that time – neurogenesis and neuronal migration – are exquisitely sensitive to environmental and pharmacological influences. For example, in rodents, even a seemingly minor intervention such as abdominal ultrasound can prevent migrating neurons from reaching their proper position in the cortex, and maternal consumption of ethanol or intake of GABAergic agents, during early to mid gestation disrupts neuronal

<table>
<thead>
<tr>
<th>Trimester</th>
<th>Agent</th>
<th>Model</th>
<th>Type of exposure</th>
<th>Dose</th>
<th>Neurodevelopmental effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>First trimester</td>
<td>Halothane</td>
<td>Mouse</td>
<td>Multiple</td>
<td>1–2%</td>
<td>Impaired learning&lt;sup&gt;84&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Isoflurane</td>
<td>Rat</td>
<td>Multiple</td>
<td>1.05%, 6 h/day</td>
<td>No obvious effects&lt;sup&gt;68&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Nitrous oxide</td>
<td>Mouse</td>
<td>Multiple</td>
<td>5%, 15%, or 35%</td>
<td>Impaired startle reflex reactivity&lt;sup&gt;100&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Nitrous oxide</td>
<td>Rat</td>
<td>Single</td>
<td>70–75%, 24 h</td>
<td>Encephalocele and hydrocephalus&lt;sup&gt;77&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Nitrous oxide</td>
<td>Rat</td>
<td>Single</td>
<td>0.55%, 4 h</td>
<td>Neuronal apoptosis at multiple brain regions, worse with combination of anesthetics&lt;sup&gt;89&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Nitrous oxide</td>
<td>Mouse</td>
<td>Single</td>
<td>75%, 8 h</td>
<td>Delayed acquisition of spatial memory, decreased anxiety&lt;sup&gt;96&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Ketamine</td>
<td>Primate</td>
<td>Single</td>
<td>75%, 6 h</td>
<td>Impaired spatial memory, changes in synaptic ultrastructure&lt;sup&gt;88&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Isoflurane</td>
<td>Rat</td>
<td>Single</td>
<td>1.3%, 4 h</td>
<td>Impaired spatial memory, decreased synaptic number&lt;sup&gt;77&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Isoflurane</td>
<td>Rat</td>
<td>Single</td>
<td>1.3%, 2 h</td>
<td>Hyperactivity&lt;sup&gt;99&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Isoflurane</td>
<td>Rat</td>
<td>Single</td>
<td>1.3%, 4 h</td>
<td>Developmental delay and hypoactivity&lt;sup&gt;125&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Isoflurane</td>
<td>Rat</td>
<td>Single</td>
<td>1.3%, 6 h</td>
<td>No change in cell proliferation&lt;sup&gt;126&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Isoflurane</td>
<td>Rat</td>
<td>Single</td>
<td>1.3%, 6 h</td>
<td>Neuronal cell death&lt;sup&gt;15&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Isoflurane</td>
<td>Rat</td>
<td>Single</td>
<td>3%, 1 h</td>
<td>No effect on learning and memory&lt;sup&gt;59&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Isoflurane</td>
<td>Rat</td>
<td>Single</td>
<td>3%, 1 h</td>
<td>Hippocampal neurodegeneration&lt;sup&gt;80&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Multiple exposures indicate that pregnant animals received the indicated anesthetic agent multiple times during the respective trimester. Only neuroteratogenic effects have been included for clarity. There are no data for the effects of propofol on fetal neurodevelopment in pregnant animal models.
proliferation, migration, and corticogenesis in the developing fetal brain and produces behavioral disturbances.\textsuperscript{45,46,63–67} Thus, it is plausible that clinical necessity and practice may inadvertently place the fetal brain at risk. Historically, the teratogenic effects of anesthetic agents were studied in the context of occupational exposure, which required chronic exposure to subanesthetic or trace concentrations of anesthetic agents. Because technological advances have helped monitor and minimize occupational exposure, what is more important now, though, is to determine if a single exposure to a clinically relevant concentration of anesthetic agent causes adverse effects during fetal neurodevelopment. To facilitate easy understanding, the effects of maternal anesthesia on fetal neurodevelopment are described in Table 2 in a trimester-specific style.

**First trimester: evidence for the lack of teratogenic effects of anesthetic agents**

Traditionally, most elective surgical procedures during the first trimester (<12 gestational weeks) are delayed to a later gestational age to negate the potential fetal risks of perioperative stress, surgery, and the teratogenic effects of anesthetic drugs. Although there is considerable disquiet, no data exist to support the teratogenicity of anesthetic agents in the setting of a clinically relevant anesthesia exposure during first trimester. Most research in this area originates from multiple exposure paradigms in pregnant rodents, and occupational exposure to trace anesthetic concentrations in humans.

Even if one discounts the obvious lack of clinical relevance of such studies, the teratogenic data are still very weak and unconvincing. For example, a majority of rodent studies suggest that halogenated volatile agents are devoid of teratogenic effects in rats,\textsuperscript{68,69} a finding that was confirmed in a detailed review of anesthesia exposure during first trimester.\textsuperscript{70} The reported incidence of major birth defects after first trimester surgery and anesthesia was approximately 2–3.9%,\textsuperscript{71,72} a rate that was not higher than the baseline rate for birth abnormalities.\textsuperscript{73} However, when only neural tube defects were analyzed, the picture gets obfuscated with studies suggesting a link between surgery and anesthesia and subsequent central nervous system defects such as hydrocephalus.\textsuperscript{74,75} A practical difficulty with these studies is teasing out the cause-effect relationship; a majority of women with acute surgical illnesses during pregnancy are febrile and require intra-abdominal surgeries that necessitate general anesthesia. Since maternal fever per se is an independent risk factor for neural tube defects,\textsuperscript{76} it is impossible to clearly delineate the risks due to anesthetic agents. Another drawback in extrapolating these studies is that the data set is from a bygone era of anesthesia, i.e., 1970–1985, when an earlier generation of inhalational agents such as halothane and methoxyflurane were commonly administered. It is unclear if any of the currently used anesthetic agents have similar effects. Conversely, teratogenic data are arguably convincing for one inhalational agent that has withstood the test of time, i.e., nitrous oxide. Nitrous oxide exposure during early pregnancy appeared to be associated with increased fetal resorptions, and skeletal and ocular abnormalities in rodents,\textsuperscript{77} and an increased incidence of spontaneous abortions and preterm births in human occupational studies.\textsuperscript{31,78–80} Nevertheless, numerous studies undoubtedly confirm the relative innocuousness of a single, clinical exposure to anesthesia and surgery during the first trimester.\textsuperscript{71,72,81,82}

**Second trimester: a period of vulnerability**

Historically, the second trimester was considered a safe period for maternal anesthesia primarily because embryogenesis was complete and, therefore, scant attention was given to the effects of maternal anesthesia on the offspring. Worryingly enough, human neural ontogeny suggests that the second trimester is a period of bustling fetal brain development. For example, neuroblast proliferation peaks between the 5th and 25th postmenstrual week and neuronal migration starts around the 12th postmenstrual week.\textsuperscript{36} Because GABA and glutamate play a crucial role in these processes,\textsuperscript{39–41,83} it is reasonable to hypothesize that prolonged and unphysiological modulation of fetal GABA and glutamatergic systems, as might occur during second trimester maternal anesthesia, might affect neurogenesis and/or neuronal migration. Given the frequent use of maternal general anesthesia for non-obstetric surgery and fetal interventions in the second trimester, is it possible that we are inadvertently placing the actively developing fetal brain at risk?

Most early studies were geared to investigate anesthetic effects on the fetus in the setting of occupational exposure to anesthesia; these experimental paradigms studied the fetal effects of a chronic, low-dose exposure or multiple exposures to anesthesia during gestation.\textsuperscript{68,84,85} The first study to simulate a clinically-relevant scenario used a second trimester pregnant rat model (embryonic day 14 where total gestational period is ~22.5 days). In this study, a single exposure to 1.4% isoflurane (1 MAC) for 4 h during the second trimester caused long-lasting impairment of spatial working memory in the rodent offspring.\textsuperscript{86} Currently, it is unknown if these behavioral abnormalities are due to impaired neurogenesis, altered neuronal migration, or cell death. Recent evidence, though, points to cell death as a possible mechanism. Investigators in these studies reported that mid-gestational exposure to 1.3% isoflurane for 4 h caused memory impairment in the offspring, and hence, corroborated previous findings.\textsuperscript{87,88} Furthermore, they
were also able to show that maternal isoflurane activated apoptotic mechanisms and decreased synapse numbers in the hippocampal subregions of the offspring.\(^{87,88}\) Notably, cell death was not specifically studied but only inferred in this paradigm. However, a study in pregnant guinea pigs that investigated fetal exposure to an anesthetic combination during all three trimesters, noted significant apoptotic changes and neuronal death in multiple brain regions following anesthesia exposure in the first and second, but not the third, trimesters.\(^{89}\) Of more concern though, is the demonstration of apoptotic neuronal death in the fetal brain following ketamine anesthesia during late second trimester in pregnant macaques, albeit after a 24-h exposure.\(^{15}\) With histological evidence of neuronal death and subsequent behavioral abnormalities in disparate animal models, it is increasingly clear that exposure to second trimester anesthesia may have functional consequences for the offspring.

**Third trimester: a relatively safe period?**

Only a handful of studies have analyzed the fetal impact of maternal anesthesia during the third trimester and the results are mixed. In one rodent study, 1.3% isoflurane anesthesia for 6 h during the third trimester had no effect on the neurodevelopment of offspring,\(^{59}\) while another dose–response study in term rodents by the same group reported that 3% isoflurane for 1 h, but not 1.3%, caused hippocampal neurodegeneration in the fetal rat brain.\(^{90}\) Thus, it seems that at least during third trimester, toxicity might be a function of isoflurane dose, rather than the duration of exposure, because even 1 h of exposure was able to trigger apoptosis.

Given the relative rarity of general anesthesia and the short duration of anesthesia exposure in the context of a cesarean delivery at or near term, these animal studies may have limited clinical relevance. To examine whether such toxicity occurs during general anesthesia for cesarean delivery in humans, investigators mined the Rochester Epidemiology Project database. Using a robust, population-based birth cohort study design (Olmsted County, MN), the authors sought to determine the incidence of learning disability (LD) in children following general or neuraxial anesthesia during cesarean or vaginal delivery.\(^{91}\) The authors concluded that fetuses exposed to general anesthesia during cesarean delivery were not more likely to develop LD compared to those born vaginally. However, a number of issues limit the external validity of the reported findings. Of major concern is that birth data are from 1976 to 1982; the practice of obstetrics and obstetric anesthesia, as well as the criteria for detecting learning and behavioral deficits, has undergone drastic change since then. The incidence of cesarean delivery has increased as dramatically (from the 10% reported in the study) as the decline in general anesthesia. The anesthetic agents in current practice have evolved beyond the halothane and methoxyflurane used predominantly in the study. The impact of these changing clinical trends and the use of newer anesthetic agents remain to be seen. Second, the study did not evaluate common antecedents for LD (socioeconomic status, fetal alcohol or drug exposure, presence of LD or low IQ in parents or family members, etc.), and the exclusively Caucasian population prevents generalization of the results. Lastly, it must be recognized that behavioral abnormalities span a wide spectrum, and LD is only a small, albeit important, component. It is plausible that subtle disruptions of emotional or affective behaviors may have been missed. From a clinical perspective, maternal general anesthesia at term is usually performed in the setting of a distressed fetus and fetal exposure to maternally administered anesthetics is typically brief, i.e., in minutes. Given the overall consensus that general anesthesia has its place in obstetric anesthesia for select cases, concerns about possible neurotoxicity should not necessitate a need to alter anesthetic approach.

But, what about the use of nitrous oxide for labor analgesia at term gestation and an obvious elephant in the room? Following Tunstall’s pioneering article in 1961,\(^{92}\) nitrous oxide was commercially introduced for use in labor analgesia. Marketed as Entonox, a 50:50 mix of nitrous oxide and oxygen, it achieved widespread popularity due to easy availability and the convenience of self-administration. However, clinical studies on the analgesic efficacy of nitrous oxide have remained inconclusive,\(^{93,94}\) in addition, its use is associated with an increased risk of nausea and vomiting, hypoxemia, and a potential for myelotoxic and environmental effects.\(^{95–98}\) Nevertheless, nitrous oxide still continues to enjoy popularity in some countries despite preclinical evidence suggesting that it causes behavioral teratogenicity.\(^{85,99,100}\) The intermittent administration of Entonox, a preparation that readily crosses the placenta, exposes the term fetal brain to subanesthetic concentrations of nitrous oxide throughout labor. The neurobehavioral consequences of such prolonged exposure remain, hertofofe, unknown. Since learning disabilities usually manifest later in life, it is hard to decipher the effects of nitrous oxide, and as such, no conclusive human behavioral data exist to support or discourage its use. With rising concern about the subtle long-term effects of perinatally-administered anesthetic agents, the role of nitrous oxide in labor certainly demands judicious scrutiny and focused animal studies.

**Caveats in interpreting studies of developmental neurotoxicity of anesthetic agents**

Despite robust evidence for anesthetic-related developmental neurotoxicity in rodent, and now primate, models it requires a certain leap of faith to believe such a phenomenon
occurs in humans. The reasons to not believe are fairly strong as there are numerous pitfalls in extrapolating rodent studies to humans;\(^\text{101}\) these shortcomings are often a necessary deterrent to limit anthropomorphization of animal studies. Even if one disregards the obvious differences in the complexity of the rodent and human brains, there are numerous other factors that potentially interfere with the validity of animal studies.\(^\text{102}\)

The first major concern is that the anesthesia exposure time (4–6 h) is considerably longer relative to the duration of rodent gestation (19–22 days). Simplicistic mathematics would translate this to approximately 48 h of anesthesia in a pregnant woman making it a clinically irrelevant model except, possibly, in the setting of intensive care unit sedation with intravenous anesthetic drugs. However, the human brain is vastly more complex both in terms of neuronal connectivity and function, making it potentially more vulnerable to the detrimental effects of anesthesia.\(^\text{103}\) More concerning is that this phenomenon has now been demonstrated in primates, a species closer to humans on the phylogenetic tree, even with a relatively short duration of anesthesia exposure.\(^\text{12}\) Second, is the lack of hemodynamic and metabolic stability during animal anesthesia, in sharp contrast to clinical anesthesia where every effort is expended to ensure physiological homeostasis. Recent studies, however, have emphatically addressed this concern by invasively monitoring blood pressure, acid–base balance, oxygenation and ventilation, temperature, and blood glucose yet reporting similar results.\(^\text{89}\) This reaffirms that the observed results were directly related to anesthesia exposure and not due to metabolic or acid–base derangement.

Third, considerable inter-species variability in the sequence and timing of neurodevelopmental processes exists, mandating the need for further gestational comparisons between rodents, primates, and humans.\(^\text{101}\) Researchers have simplified this conundrum by collating neurodevelopmental data from various models to generate a user-friendly online tool (http://www.translating-time.net) for translating time across mammalian species.\(^\text{104,105}\) Although useful, it is too simplistic to untangle a complicated knot that is early human neurodevelopment. Fourth, potentially fallacious conclusions can be derived from a misconceived study paradigm for animal behavior. At present, our understanding of animal ethology is, at best, rudimentary with the possible exception of hippocampally-mediated spatial navigation. Moreover, our knowledge of the neurobiological pathways mediating animal behavior is not comprehensive. Finally, just as how animal studies on brain protection failed to bear out in humans, it is possible that developmental neurotoxicity of anesthetic agents seen in animal models may fail to bear out in prospective, randomized human studies. Until then, a prudent approach is to be open to new research findings with a healthy degree of skepticism.

**Is there a safe anesthetic agent?**

Currently, there is no scientific justification to prefer the use of one anesthetic agent to the other. Among inhalational agents, the class of agents for whom data are more robust, researchers have compared the relative neurotoxicity profiles and come up with entirely different conclusions depending on the concentration of anesthetic agent used. To illustrate this further, at an anesthetic dose of 0.5 MAC based on MAC determined in adult humans, isoflurane, but not sevoflurane, increased the plasma levels of S100beta, a putative plasma marker for neurodegeneration, and caused more apoptotic degeneration in neonatal mice.\(^\text{106}\) However, other investigators have challenged the use of MAC determined in adult humans for neonatal rodent anesthesia; not surprisingly, the neonatal rodent MAC appears to be much higher in these studies (desflurane MAC = 11.5–12.2%, isoflurane MAC = 2.7%, and sevoflurane MAC = 3.8–5.4%).\(^\text{107,108}\) A trend that is widely observed in clinical pediatric anesthesia. When these anesthetic agents were administered at 0.5–0.6 of predetermined neonatal rodent MAC (greater than typically seen with human anesthesia), their propensity to cause apoptotic neurodegeneration and working memory disturbance were either similar,\(^\text{107}\) or greater for desflurane.\(^\text{108}\) With this confusion surrounding inhalational agents, is there an alternative?

Among available alternatives, propofol holds the most promise. Although unlicensed for obstetric use, the use of propofol for induction of general anesthesia for cesarean delivery is rising steadily, partly because of clinician preference but also because of the commercial unavailability of time-tested thiopental. That propofol crosses the placenta was never in doubt,\(^\text{109,110}\) but data on the clinical safety profile of propofol are mixed.\(^\text{111}\) Propofol studies at term gestation report an entire gamut of findings from low Apgar scores and poor neurobehavioral adaptation,\(^\text{112,113}\) even in the absence of an inhalational agent for maintenance of anesthesia, to no discernible effects on the newborn.\(^\text{114}\) Preclinical data on propofol are worryingly consistent though; propofol is now known to cause apoptotic neurodegeneration in neonatal rodents,\(^\text{2,115,116}\) interfere with neuronal circuit assembly by altering dendritic spines both in vitro and in vivo,\(^\text{15,117}\) and cause growth cone collapse and neurite retractions in vitro.\(^\text{118}\) High doses of propofol (20–60 mg/kg administered either subcutaneously or intraperitoneally) were used in rodent studies, but because plasma levels were not determined it is unclear how such studies can be extrapolated to clinical anesthetic practice. Interestingly, a low dose of propofol (subcutaneous 10 mg/kg) was shown to have no neurotoxic effects suggesting that a dose–response relationship might, in fact, exist.\(^\text{2}\) Preliminary in vitro data from our laboratory suggest that clinically relevant concentrations of propofol
Nevertheless, as obstetric anesthesiologists, there are anesthetic agents remains an imposing ethical challenge. A search on the fetal effects of maternally administered to the unborn fetus particularly during the third trimester. It may generate interesting results that are directly applicable guide us in the next 3–5 years. However, these studies it is unlikely that we will have enough information to tant time points for behavioral assessments in children, Given the prospective nature of these trials and the dis- Neurodevelopmental Outcome and Apnea in Infants). The biggest question right now is: where do we go from here? And, how do we get there? Even before we attempt to answer these questions, it is clear that suggestive ani- mal and epidemiological evidence about potential neuro- toxic effects of anesthetic agents during early neural development needs to be confirmed with well-conducted prospective randomized trials in humans. This research area is of critical interest to the US Food and Drug Administration (FDA) and has been instrumental in setting up at least two large-scale human studies to study the long-term effects of anesthetic exposure during early infancy – the Pediatric Anesthesia Neuro-development Assessment Study (PANDA) and the GAS study (Comparing Regional and General Anesthesia for Effects on Neurodevelopmental Outcome and Apnea in Infants). Given the prospective nature of these trials and the dis- tant time points for behavioral assessments in children, it is unlikely that we will have enough information to guide us in the next 3–5 years. However, these studies may generate interesting results that are directly applicable to the unborn fetus particularly during the third trimester.

For now though, performing prospective clinical re- search on the fetal effects of maternally administered anesthetic agents remains an imposing ethical challenge. Nevertheless, as obstetric anesthesiologists, there are three clinical arenas where these questions can be asked and answered in a retrospective manner. These include long-term neurological and behavioral data of children exposed to anesthesia in utero (non-obstetric surgery, fe- tal interventions), to GABAergic drugs administered for sedation of critically-ill pregnant women in the intensive care unit, and to nitrous oxide during labor and deliv- ery. Given the numerous confounding influences and limited data sets, these studies may, at best, give us the direction to pursue. The best way moving forward is to combine both animal data (for understanding mech- anisms) and human data (for direct clinical relevance) until consilience is achieved.

**The path forward**

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**Are there any neuroprotective strategies?**

Given the lack of viable alternatives, are there any neuroprotective strategies that can mitigate the reported adverse effects of anesthetic agents? So far, only lithium and xenon are known to confer anti-apoptotic protection in the setting of developmental neurotoxicity of anesthetic agents, a finding that has limited practical utility in clinical anesthetic practice. Magnesium, an agent frequently administered for fetal neuroprotection in preterm labor, and an agent familiar to most obstetric anesthesiologists, may be a promising option. However, running counter to prevailing knowl- edge, a recent study reported neuroapoptosis after high dose magnesium administration in neonatal mice, tempering the optimism surrounding potential magne- nium use. Sequentially, until mechanisms of anesthetic neurotoxicity are clearly understood, it may be difficult to generate a ‘magic bullet’ that alleviates the potential adverse effects of anesthetic agents on early neurodevelopment.

**Summary**

In summary, it is remarkably clear from animal studies that anesthetic agents influence early brain development by altering both the anatomical organization of the brain as well as inducing functional consequences in the form of learning and memory deficits. Although data on the fetal brain are limited, most animal studies indicate that the developing fetal brain is at risk from maternal anesthesia especially during the second trimester. Inhalational anesthetics are frequently implicated, but at this point it is not known if propofol has similar widespread effects on fetal neurodevelopment. Until fur- ther studies delineate the effects of propofol on fetal brain development, it is unwise to advocate the use of one agent over the other. These preclinical findings be- hoove the obstetric anesthesiologist to keep a close eye on latest developments in developmental neurotoxicity literature, which might, one day, be applicable to obstet- ric anesthesia.

**Disclosure**

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