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Anesthesia and Developing Brains: Unanswered Questions and Proposed Paths Forward

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ABSTRACT

Anesthetic agents disrupt neurodevelopment in animal models, but evidence in humans is mixed. The morphologic and behavioral changes observed across many species predicted that deficits should be seen in humans, but identifying a phenotype of injury in children has been challenging. It is increasingly clear that in children, a brief or single early anesthetic exposure is not associated with deficits in a range of neurodevelopmental outcomes including broad measures of intelligence. Deficits in other domains including behavior, however, are more consistently reported in humans and also reflect findings from nonhuman primates. The possibility that behavioral deficits are a phenotype, as well as the entire concept of anesthetic neurotoxicity in children, remains a source of intense debate. The purpose of this report is to describe consensus and disagreement among experts, summarize preclinical and clinical evidence, suggest pathways for future clinical research, and compare studies of anesthetic agents to other suspected neurotoxins.

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Questions regarding the safety of general anesthetic drugs in children emerged nearly 20 yr ago with the finding of neuronal apoptosis and functional deficits in rodents after exposure to these medications.[1] Over the course of the subsequent two decades, exposure to anesthetic agents during brain maturation has been found to consistently disrupt neurodevelopment in animal models.[2] In response, in December 2016, the U.S. Food and Drug Administration (Silver Spring, Maryland) issued a Drug Safety Communication regarding all commonly used anesthetics that bind to γ-aminobutyric acid and *N*-methyl-D-aspartate receptors.[3] The Food and Drug Administration warning that "repeated or lengthy use of general anesthetic and sedation drugs during surgeries or procedures in children younger than 3 yr old or in pregnant women during their third trimester may affect the development of children's brains" was largely based on preclinical animal models and the limited clinical data available at the time.[4] While preclinical data are convincing, the interpretation of the human data has been more complex, with some studies reporting an increased incidence of neurodevelopmental deficits in young children exposed to anesthetics and other studies finding no such differences.

Given the mixed results of the studies in children, the fundamental questions of the safety of commonly used anesthetics and whether this line of research inquiry should continue remains a source of intense debate. The purpose of this report is to provide expert consensus opinion regarding the state of the current preclinical and clinical evidence, the remaining questions, suggestions for future research, and comparisons to the evolution of research of other suspected neurotoxins, with the ultimate goal of ensuring that the millions of children who undergo procedures requiring anesthetic agents do so safely.[5–7]

Preclinical Data from Animal Models and Translation to Humans

Animal studies convincingly show that general anesthetic medications induce a variety of morphofunctional alterations during brain development.[2] Although most preclinical studies have used rodent models, potentially more informative data are available from studies of nonhuman primates. The neonatal rhesus macaque is similar to human neonates with regard to physiology, pharmacology, early development, and behavior.[8] In addition, unlike rodents,

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neonatal rhesus macaques can be cared for using anesthetic equipment and techniques that are similar to those used for human neonates, ameliorating concerns that cognitive deficits in animal models are purely the result of physiologic instability rather than the effect of anesthetics.[9]

The Food and Drug Administration Drug Safety Communication was informed by preclinical nonhuman primate data, particularly regarding the duration of anesthesia and the risk of multiple anesthetic exposures. Neuronal apoptosis and behavioral changes were also seen in younger (5 to 6 days old) but not older nonhuman primates.[10] However, given the lack of specific evaluations of nonhuman primates and humans at various ages between infancy and adulthood at the time of the release of the Drug Safety Communication in 2016, the 3-yr human age cutoff was a "best estimate" for a period of risk in humans based on the translational data for rapid synaptogenesis available at that time.[11–13] Data from the nonhuman primate model have confirmed some of the *in vitro* effects of anesthetics on cultured neural tissue and *in vivo* effects observed in rodent models. Overall, these results have supported the concern from the Food and Drug Administration that γ-aminobutyric acid– and *N*-methyl-D-aspartate–binding anesthetics have adverse neurodegenerative effects that could compromise young children, including changes in behavioral, motor, and cognitive parameters.[10,14–18]

Although these early nonhuman primate studies included only limited behavioral assessments, additional data published after the Food and Drug Administration warning was released further confirmed that behavioral changes (*i.e.*, anxiety, fear, and socialization behavior) characterize the nonhuman primate phenotype and also extended the period of vulnerability to at least postnatal day 40.[19–22] Notably, in one study that evaluated both behavioral and cognitive abilities, alterations in spontaneous and provoked behavior were observed with no decrement in cognitive abilities such as working memory, executive function, and cognitive flexibility.[23]

In attempting to translate preclinical data to humans, there are strengths and limitations to each animal model.[14] Rodent models have found similar apoptotic injury patterns as those identified in nonhuman primates and can offer several advantages given the cost and ethical concerns of studies using nonhuman primates.[24] Recommendations on increasing the translational relevance of laboratory models alongside improving laboratory reporting standards have recently been published by the SmartTots Preclinical Working Group.[14] Choosing appropriate outcomes that are relevant and translatable represents an ongoing challenge,[2] particularly in the absence of a clearly identifiable human phenotype. The lack of an obvious phenotype stems from the fact that the potential for pathology was first recognized in animals rather than the more typical sequence of developing an animal model in response to recognized human pathology. Additional caveats of extrapolation between animal models and humans include physiologic monitoring issues, the developmental stage at the time of drug exposure, the length of exposure, and the absence of surgery and resulting inflammation. In the single study that included an assessment that is potentially directly translatable from studies of nonhuman primate to humans (the Operant Test Battery utilized by the Food and Drug Administration investigators), the detrimental effects observed in anesthetic-exposed rhesus macaques were not found in children[25]; however, the duration of exposure to anesthetic drugs was much greater in the macaques.

Current State of the Clinical Evidence

Designing clinical studies based on outcomes assessed in animals has proven to be challenging for a variety of reasons.[26,27] In particular, issues include the difficulty or impossibility of randomizing a child to not receive anesthesia for surgery, the long length of time required before neurodevelopmental deficits can be adequately assessed, and the challenges of identifying a phenotype of injury when any effect of anesthesia is likely to be at worst modest and therefore difficult to recognize in routine clinical assessments. In addition, since most clinical studies of anesthetic neurotoxicity are observational, they potentially have a multitude of potentially confounding factors, and appropriately accounting for these factors is also challenging.

Given the difficulty of performing randomized controlled trials or any prospective studies in this field, most studies are observational in nature and rely on data collected for other purposes. Among the available studies, there is significant heterogeneity in the types of surgical procedures, numbers of exposures, comparators, methods used to adjust for potential confounding (if any), and the outcomes examined. One systematic review published in 2017 found that even at that time, there were 67 studies evaluating neurodevelopmental outcomes after surgery and anesthesia that reported results from 73 different outcome measures.[28] By 2019, there were 90 studies published evaluating neurodevelopmental outcomes after surgery and anesthesia.[29]

In most of these clinical studies, exposure to anesthesia and surgery is not consistently associated with deficits in clinically relevant outcomes including academic achievement,[30–33] general intelligence,[29,30,34–36] or memory and language.[34–37] However, a more consistent association has been reported in subsets of studies that evaluated deficits in behavior,[29] executive function,[29,36] social communication,[37] motor function,[37] and diagnoses of attention deficit hyperactivity disorder.[38] These deficits have been reported even after single exposures to anesthesia, which for most children is less than 1 h.[6,39] That exposure-related differences are found in some outcomes but not others is plausible and may be particularly evident for outcomes that involve longer periods of observation in less structured settings such as parental reports of behavior and executive function.[29] In

the relatively few studies that have evaluated children with multiple exposures, adverse changes in behavior, executive function, motor function,[36] and the increased incidence of attention deficit hyperactivity disorder[40,41] are more pronounced in multiply exposed compared to singly exposed children. However, because children who receive multiple or prolonged exposures also have a higher rate of comorbid conditions including prematurity, low birth weight,[6] and higher American Society of Anesthesiologists status[39] compared with singly exposed children, addressing issues of unmeasured confounding and bias may be more complicated in studies of those children. As discussed in the following section, there are several potential confounding factors that complicate any causal interpretations of the relationships between anesthesia exposure and outcomes, including factors associated with both exposure and outcome. Although most observational studies employ various strategies to account for known differences in potentially relevant factors that differ between exposed and unexposed groups, there are still risks of unmeasured confounding that cannot be completely mitigated and should be considered when interpreting the study findings.

The ideal method to establish a causal relationship between an exposure and an outcome is by performing a randomized controlled trial. Unfortunately, because of the challenges in design and conduct, only one such study has been completed: the General Anesthesia or Awake-regional Anesthesia in Infancy (GAS) study, which randomized infants to receive either a brief sevoflurane anesthetic or an awake regional anesthetic[34,42] and evaluated intelligence and a variety of secondary outcomes. In this equivalence trial, of all outcomes assessed, only the executive function secondary outcome was found to have both bounds of the 95% CI favoring evidence for a worse outcome with general anesthesia exposure.[34] While in the GAS study behavioral differences were found to be equivalent, in a meta-analysis that combined results from the GAS study with other studies using prospective outcome assessment (including the Pediatric Anesthesia NeuroDevelopment Assessment [PANDA] and Mayo Anesthesia Safety in Kids [MASK] studies),[35,36] results from all included studies found general anesthetic–exposed children displaying consistent but small behavioral score differences but no differences in intelligence.[29] Of note, this phenotype consisting of deficits in behavioral function with no difference in measures of cognitive function after anesthetic exposure is similar to that reported in nonhuman primate studies.

Other Perioperative Factors that Could Affect Measured Outcomes

Other than a causal effect of anesthesia exposure, the most frequently proposed mechanisms to explain the observed associations between exposure to anesthesia and surgery and affected outcomes include perioperative physiologic disturbances, inflammation and psychologic stresses associated

with surgery, and the underlying conditions necessitating surgery (*i.e.* confounding by indication). The proposed relevant physiologic disturbances include hypoxia, hypocapnia, hypercapnia, and especially hypotension.[43] Blood pressures in anesthetized children are considerably lower than those found in nonanesthetized children,[44] and mild intraoperative decreases in cerebral oxygenation are common,[45] but a clear definition of intraoperative hypotension in pediatric patients does not exist.[46] The impact of lower blood pressures in children also remains unclear. While intraoperative hypotension has been associated with postoperative delirium in adults,[47,48] a study evaluating multiply exposed children found no association between low intraoperative blood pressure values and neurodevelopmental outcomes.[49] Inflammation has also been hypothesized as a mechanism behind postoperative cognitive dysfunction in adults,[50] although the effects in children have not been examined. Although chronic illness or severe acute illness can cause psychologic distress that could impair development, even children with chronic significant illness can be surprisingly resilient in terms of cognitive development,[51] and studies examining children undergoing common procedures find most children do not exhibit persistent postoperative psychologic distress.[52] Perhaps the most frequently cited factor in the literature is confounding by indication, which refers to the fact that children requiring anesthesia may be more likely to have conditions that themselves are associated with adverse neurodevelopmental outcomes. While this is an important consideration, most children who require anesthesia do not have these conditions,[6] and the results of some observational studies have not been sensitive to the exclusion of children undergoing major procedures such as cardiac surgery.[36] It is also not clear what conditions representing indications for surgery would consistently be associated with increased risk of adverse outcomes.[53] Indeed, surgery can improve conditions that cause adverse developmental outcomes such as attention deficit hyperactivity disorder[54] (*e.g.*, tonsillectomy and adenoidectomy normalizes measures of behavior and cognition in children with obstructive sleep apnea).

Consensus and Differences of Opinion in the Field

Biologically it is illogical to consider that morphologic changes that are consistently seen in the laboratory across so many species would not also occur humans. The challenge is to determine how these changes translate to clinically relevant human functional outcomes. Given the available preclinical and clinical evidence, there is still a broad, albeit not universal, consensus that based on the current evidence, there remains a possibility that anesthetic agents may cause a relevant long-term neurodevelopmental effect in children. As a result, there is also agreement that further research on this topic is justified. Some authors express concern that the challenges inherent in the design and conduct of these studies[26,27] may preclude a definitive study or studies, while

others suggest that the appropriate studies are indeed possible using novel approaches. All authors of this report, however, agree that further research would provide improved evidence that would aid in clinical decision making (discussed below). Several potential research approaches have been suggested by the authors that may help generate the necessary evidence to answer the central question of whether anesthesia causes long-term neurodevelopmental problems in children (table 1).

What Evidence Would Be Required to Show that Exposure to General Anesthesia Causes Long-term Neurodevelopmental Deficits in Children?

Further Randomized Clinical Trials

Randomized controlled trials are often considered the gold standard for proving the causal effect of an exposure. Although these trials are very challenging to perform, particularly because children cannot be randomized to a placebo anesthetic for surgery, they may still be feasible and provide useful evidence. The Neurodevelopmental Outcome After Standard Dose Sevoflurane Versus Low-dose Sevoflurane/Dexmedetomidine/Remifentanil Anaesthesia in Young Children (T REX) trial, which evaluates children undergoing longer durations of anesthetic exposure, is currently underway.[55] The purpose of this trial is to evaluate a method to mitigate possible injury caused by anesthetic exposure and compares children exposed to low-dose sevoflurane (0.3 to 0.6%) in conjunction with remifentanil and dexmedetomidine to a traditional higher dose sevoflurane anesthetic. The hypothesis is that remifentanil and dexmedetomidine in conjunction with lower dose sevoflurane is either neuroprotective or less neurotoxic than higher dose sevoflurane alone. The primary outcome of interest in this study is intelligence as measured by full-scale intelligence quotient and is also examining other domains of neurodevelopment as secondary outcomes. If low-dose sevoflurane with remifentanil and dexmedetomidine is found to be beneficial, that may imply a dose response in sevoflurane neurotoxicity and either the safety of a lower dose or a mitigating effect of dexmedetomidine and remifentanil. A finding such as this would be important and could have immediate clinical applicability. However, if low- and high-dose sevoflurane are found to be equivalent, the interpretation would be more complicated because this could mean that these two dosing regimens could be either equally safe or equally toxic. Because there are limited data in animal models suggesting that a dexmedetomidine and remifentanil anesthetic differs from sevoflurane on histologic and functional outcomes, the preclinical data may not help with the interpretation of such a result.

The T REX trial evaluates an important question using an international consortium and is currently the largest clinical trial evaluating anesthetic neurotoxicity in children. The limitations in such a study attest to the difficulty of performing ethical and logistically feasible trials to evaluate the basic question of whether anesthetics cause measurable brain injury in children.[26] While further clinical trials would undoubtedly be helpful, at this time the optimal feasible study design is not obvious.

Observational Studies and Causal Theory

Uncommon events in large populations are difficult to study using traditional methods such as randomized controlled trials and prospective cohort studies. These situations are common in areas of public health such as environmental toxicology. In these settings, the application of causal theory can help establish the plausibility of a causal link between an exposure and an outcome. For example, a randomized controlled trial has not evaluated whether exposure to lead in children results in neurodevelopmental problems, but evidence synthesized across multiple observational and preclinical studies was convincing enough to result in policy changes to improve public health. Other examples in the pediatric literature include the association between prone sleep and sudden infant death syndrome and between aspirin use and Reye syndrome. The application of causal theory was proposed by Sir Austin Bradford Hill in 1965 in a seminal essay providing a framework of nine principles that could be used to guide the establishment of plausible causal relationships using observational data.[56] Although the Bradford Hill criteria remain one of the frequently cited frameworks for causal inference in epidemiologic studies, in recent years, calls have been made to modernize the criteria to take into account scientific and statistical advances.[57,58] For example, one specific criterion that has been criticized suggests that large effect sizes are more likely to be causal than small effect sizes. It is now recognized that most causal associations tend to have relatively modest effect sizes, arguing against the strength of the association itself determining causality.[59] An in-depth application of a causal framework to anesthetic neurotoxicity can be performed[60] but is a complex and multi-faceted endeavor. A lesson learned when searching for associations between genotype and disease is the need to replicate studies, and with the accumulation of well designed clinical and preclinical studies, the utility of using causal frameworks to evaluate anesthetic neurotoxicity will likely increase. However, while these methods may aid in establishing causality, as discussed above, the challenge that remains is that in most observational studies, the anesthetic exposure cannot be completely isolated from the rest of the perioperative experience, inflammation, and comorbidities associated with surgery.

Use of Intermediate Outcomes or Biomarkers

Another challenge in the design of anesthesia-related neurotoxicity studies is the significant latency between exposure and assessment, as early assessment of cognition and behavior in young children may not be predictive of long-term

Table 1. Advantages and Disadvantages of Potential Research Approaches

Approach	Advantages	Disadvantages
Further randomized controlled trials	Randomized controlled trials are the gold standard for proving causal effect of anesthetic exposure.	Randomized controlled trials are expensive and time consuming. Children cannot be randomized to no anesthetic, and what constitutes a "safe" anesthetic is unclear. A phenotype of injury and the ideal outcomes to evaluate are unclear. The optimal, feasible study design is not obvious.
Observational studies and causal theory	Randomized controlled trials may not be feasible in all situations. Causal theory is commonly applied to other areas such as environmental toxicity.	Observational studies are limited by the inability to distinguish effects of anesthesia from surgery and other perioperative factors, as well as issues with confounding by indication. Application of causal theory relies on well-designed observational studies.
Intermediate outcomes and biomarkers	Injury could be identified at an earlier stage. Objective pre- and postexposure testing could be performed. Endophenotypes could be identified. Translation between animal models and humans could be improved.	No consistent biomarkers have been identified. Any identified biomarker would need to be correlated to a long-term clinical outcome of interest. Intermediate outcomes and biomarkers would not be able to distinguish the effects of anesthesia from surgery and other perioperative factors.
Prenatal exposures	Children may be more vulnerable to prenatal exposures. Less confounding by indication in the child as exposure is typically due to a condition in the mother rather than underlying conditions in the child.	The effects of anesthesia from uterine perfusion, inflammation, and other fetal stressors cannot be distinguished. A randomized controlled trial is unlikely to be able to randomize a mother to general anesthesia or spinal anesthesia.
Nonhuman primate studies	This is the animal model that is most similar to humans. This model can be used to evaluate questions that cannot be studied in humans.	Translation between humans and animal models is challenging. The one study that evaluated the same outcome in humans and nonhuman primates found differences in nonhuman primates but not children, although nonhuman primates had significantly longer exposures.

function. Identifying suitable biomarkers associated with injury could be extremely valuable in (1) serving as intermediate outcomes that could facilitate clinical studies, (2) serving as "endophenotypes" of injury, and (3) providing insight into potential mechanisms of injury. Examples of biomarkers that have been explored in preclinical models in this context include brain imaging studies[61] and serum-based assays.[62,63]

Advantages and Challenges of Intermediate Outcomes

If the long-term neurodevelopmental effects of anesthesia are the ultimate outcome of interest, it can be argued that assessments cannot be made until adulthood because many neurodevelopmental domains may not fully mature until the late teens and early twenties. If an intermediate outcome such as a biomarker is found to be associated with long-term outcomes, injury could be identified at an earlier stage, reducing the time between exposure and assessment and thereby reducing the need for lengthy follow-up times. An objective biomarker could allow for the evaluation of children before and after exposure, which eliminates the confounding that occurs when comparing unexposed children with exposed children who may have higher rates of unrecognized underlying comorbid disease.

Endophenotypes

The identification of endophenotypes has also been raised as a method of identifying potential injury in children exposed to anesthesia. While a phenotype of a disorder is the associated collection of signs and symptoms that are immediately observable in that disorder, endophenotypes— "endo" meaning inside—has gained popularity with the development of advanced brain imaging and genetics. This concept applied to brain imaging has been used in environmental toxicology research as direct measures of the brain are in closer causal proximity to the genetic, environmental, and epigenetic determinants of clinical phenotypes than are cognition and behavior. In addition, the effects of putative genetic or environmental factors are often more robustly identified on brain imaging than with cognitive and behavioral testing. One relevant example is a study examining the relationship between prenatal exposure to polycyclic aromatic hydrocarbons (air pollution) and brain white matter, cognition, and behavior in children.[64] The authors found a dose–response relationship between prenatal exposure and specific regional reductions in white matter that mediated deficits in processing speed and attention deficit hyperactivity disorder severity, providing strong evidence for causality and potential insights into mechanism. These brain-based effects of prenatal exposure on white matter were much stronger and more robust in the presence of potential confounders than were the effects of exposure on cognition and behavior, presumably because many neural systems influence the performance on any

single cognitive or behavioral task, whereas the influence of prenatal exposure to neurotoxicants on brain development can be more directly evaluated by imaging of the brain. Discoveries of these direct effects on the brain have allowed identification of previously unknown cognitive and behavioral consequences of the causal factors that had not been appreciated clinically[65] or that were suspected but not yet proven.[66] Therefore, identifying the neural systems that constitute a brain-based endophenotype may in turn allow investigators subsequently to identify more precisely which cognitive and behavioral functions early anesthesia exposure most robustly affects, if a specific pattern of imaging findings associated with anesthesia exposure is discovered. Determining causality, however, will continue to be a challenge as exposure to anesthesia remains linked to surgery and other related perioperative insults.

Mechanisms of Injury

Identifying biomarkers such as imaging abnormalities associated with exposure can also suggest mechanisms of injury. To again use the example of polycyclic aromatic hydrocarbons, these compounds have known cytotoxic effects that may help explain specific structural changes seen in children with higher exposures.[64] In the context of anesthetic neurotoxicity, the preclinical literature has suggested an abundance of potential mechanisms for neural injury and also the utility of imaging-based biomarkers with measurable differences 6 h to 1 week postexposure.[67] The application of biomarkers, particularly imaging modalities, may help examine some of the proposed preclinical mechanisms and aid translation between humans and animal models.

Although available imaging data are currently limited, there is some evidence that deficits in certain domains associated with anesthesia exposure may be associated with magnetic resonance imaging changes. Two extant studies compared available magnetic resonance imaging structural imaging in children exposed and unexposed to anesthesia,[68,69] with another evaluating functional magnetic resonance imaging in nonhuman primates.[70] Although these studies have limitations, anesthetic exposure was found to be associated with imaging differences. Two ongoing National Institutes of Health–funded trials using prospectively collected magnetic resonance imaging images may provide further insight.

While biomarkers have the potential to allow for the development of new and more efficient study designs, identify unappreciated endophenotypes, and provide an improved mechanistic understanding of any injury, these methods also have limitations. The obvious limitation is that if these biomarker differences are identified, they still need to be correlated with a long-term outcome of interest, which is both a scientific and logistical challenge. Additionally, it may be difficult to determine whether a difference in a biomarker is due to the anesthesia or other non–anesthetic-related effects of the perioperative exposure.

Prenatal Exposures

Many studies have evaluated neurodevelopmental outcomes after early childhood exposure. Despite the presence of the Food and Drug Administration Drug Safety Communication warning against anesthetic exposures in pregnant women,[3] few clinical studies have evaluated children prenatally exposed to anesthetic agents outside the period of labor and delivery. In one small study of prenatal exposures,[71] children were found to have behavioral deficits similar to those found in the prospective assessment of postnatally exposed children,[29] with the effect size for a single prenatal exposure similar to that reported after multiple postnatal exposures.[36] Evaluation of prenatal exposures may assess a neurodevelopmental period during which children are thought to be particularly vulnerable to the effects of anesthetics. In addition, children with prenatal exposures are not subject to the same confounding by indication as children with postnatal exposure as the reason for exposure typically lies with the mother rather than the fetus. In addition, the fetus would not have any psychologic impact from the surgical experience. Nonetheless, these studies present unique challenges in interpretation because of the effects of anesthesia and surgery on uterine perfusion, inflammation, maternal infection, and other fetal stressors. However, in one preclinical study, surgery was not found to result in additional cell death compared to anesthesia alone.[72] Another limitation of this line of inquiry is that it is likely not amenable to a randomized controlled trial similar to the GAS study.[34] In pregnant women needing procedures when regional anesthesia is an option, a regional anesthetic is typically the standard of care, so it may not be ethical to randomize a woman to receive a general anesthetic.

Translation from Nonhuman Primate Models

While all animal models of anesthetic neurotoxicity have unique benefits, the nonhuman primate model is the most similar to humans and continues to be employed to address outstanding questions, including the critical window of vulnerability for the brain between birth and adulthood,[22] whether a similar effect is seen in all ages within this vulnerable period, dosing threshold effects (*i.e.*, mean alveolar concentration × time), and the effects of surgical trauma, inflammation, and pain on brain injury.[2] Additional questions that are currently being pursued include the optimal environment/socialization approaches to ameliorate brain injury and the effectiveness of various mitigating strategies, such as the use of alternative sedative drugs (*e.g.*, dexmedetomidine[73] and potential antidotes such as lithium, L-carnitine, and melatonin),[74–76] and the impact of compounding physiologic factors including shifts in body temperature, alterations in blood pressure, and optimum

ventilation strategies.[77] Because data concerning the effect of prenatal anesthetic exposure on fetal and neonatal behavioral and cognitive development are still limited,[78] as are data concerning sex differences in response to anesthesia in the developing brain,[79] nonhuman primate models may help address these gaps in knowledge. Because many studies needed to answer critical questions will be difficult or impossible to conduct in humans, robust nonhuman primate data may be important in informing future research in humans.

Which Children Are the Most Vulnerable?

While the most pressing question is to determine whether early anesthesia exposure causes any long-term neurodevelopmental effects in children, additional important questions remain, including determining which children are most vulnerable. Significant numbers of children require multiple and lengthy exposures, and these children report deficits larger than those found in children after single exposures.[36,40,41,80] Quantifying this increased risk is challenging, as these children tend to be more heterogenous in terms of illness level and comorbidities than children who require a single exposure. The consequences of lengthy exposures have also not been well studied. Children with congenital anomalies who undergo surgery and anesthesia, particularly those with congenital cardiac anomalies, typically have lengthy exposures and have significant neurodevelopmental deficits.[81,82] While these children may be more vulnerable to anesthetic agents, because they undergo major surgery and significant physiologic insult, isolating the effect of the anesthetic agent is difficult. Another potentially at-risk population is children of low socioeconomic status. In studies of neurotoxic substances such as pesticides, children of families of higher socioeconomic status show a reduced effect on IQ after exposure compared to children of families of lower socioeconomic status.[83] In children who were exposed to lead, compared to children of lower socioeconomic strata, those from higher socioeconomic strata required a higher level of lead exposure before seeing an effect on neurodevelopmental outcomes[84] and were able to more effectively recover from a toxic lead exposure.[85] While the MASK study did not find socioeconomic status to be a significant moderator,[36] children enrolled in some prospective studies such as the MASK and PANDA studies originated from significantly higher socioeconomic strata than the general population.[35,36] Children enrolled in the GAS study, however, were found to have mothers who more closely reflected the maternal education level of the general population.[42] While the Food and Drug Administration Drug Safety Communication defined the window of vulnerability as under 3 yr of age, as stated above, this threshold was an estimate based on translating the best preclinical data available. Since then, additional studies have identified similar associations between anesthesia exposure and neurodevelopmental deficit at all ages under age 5 yr,[38,86,87] and the critical window of vulnerability remains unclear.

Experience from Studies of Environmental Neurotoxins

Research on the neurotoxicity of environmental chemicals tends to evolve in a characteristic way. The initial studies tend to produce discrepant results due to a heterogeneity in design, population studied, exposure profiles, outcomes measured, and statistical approaches. Additional studies identify the key factors responsible for the discrepancies, and the public health community eventually arrives at a consensus about the magnitude of the risk in different populations. For some chemicals initially suspected of being neurotoxicants, subsequent research has suggested that the risk was modest, at best, at population levels of exposure. One example is ethyl mercury, a constituent of thimerosal, a vaccine preservative, that was considered by some to increase the risk of autism spectrum disorders in children.[88] This concern was shown to be baseless, and study of this chemical as a developmental neurotoxicant largely ended.[89] Lead, however, is an instructive example of a neurotoxicant on which the view on neurotoxicity has evolved dramatically as more data accumulated. In the 1980s, debate raged over whether "subclinical lead toxicity" existed or was solely the result of residual confounding by adversities associated with poverty. It was not until more than 30 yr later on the basis of many studies conducted in diverse settings that authoritative bodies such as the World Health Organization (Geneva, Switzerland) and Centers for Disease Control and Prevention (Atlanta, Georgia) agree that no level of lead exposure can be considered to be "safe."[90,91] Anyone who predicted in 1980 that this would be the eventual consensus would not have been taken at all seriously. Another line of inquiry that was important in drawing causal inferences about lead's neurotoxicity at low levels of exposure was the concordance between the human epidemiologic evidence, which was based solely on observational studies, and the experimental animal studies, most notably using nonhuman primates.[92]

Research focusing on the neurotoxic effects of early environmental exposure to pesticides and insecticides has followed a similar path as more data have accumulated but does provide some additional perspectives on risk assessment. For example, with respect to mechanism, the effects of exposure to organophosphate insecticides on brain development were initially assumed to derive from the same basic mechanism that produces acute systemic toxicity, in this case, inhibition of acetylcholinesterase and subsequent cholinergic hyperstimulation.[93] Subsequent evidence suggested additional noncholinergic mechanisms targeting events specific to the developing brain, and these events were likely to impair later performance on a range of cognitive and behavioral tasks.[94] This is worth noting because

in the case of pesticides and insecticides, noncholinergic mechanisms were found to dominate low-dose effects, while cholinesterase inhibition dominates toxic effects at higher doses. One caution suggested by this evolving research is that traditional methods of risk assessment based on classic monotonic dose–response relationships may not be appropriate for some neurotoxicants because of multiple mechanisms of action–mechanisms that may require elucidation through preclinical studies. In the case of some neurotoxic exposures, the timing of exposure during development likely determines the specificity of toxic effects at the cellular, neural systems, and neurobehavioral levels. The timing of assessment may also be important when assessing neurotoxins. More recently, with the advantage of extended longitudinal follow-up, accumulating evidence suggests longer-term effects of at least one insecticide on motor deficits and tremor in middle childhood—behavioral and neurologic domains that were not considered in the early studies assessing the safety of these chemical exposures.[95] Such experience argues for the consideration of new paradigms in the study of human health effects of early toxic exposures.[65]

Conclusions

As more clinical studies have been published, several have found anesthetic exposure to be associated with deficits in some neurodevelopmental outcomes but not others. No association with broad measures of intelligence have been identified, but deficits in specific outcomes including behavioral function have been more consistently reported and reflect findings from studies of nonhuman primates. While these results may help identify a phenotype of injury after anesthesia exposure, new approaches to research will still be needed to determine whether a recognized phenotype is caused by the anesthetic medications or other factors related to surgery or the perioperative experience. While several pathways for clinical research have been proposed, whether any of the proposed studies can answer the basic question of: "Do anesthetic agents cause neurodevelopmental deficits in children?" is still being actively debated. When comparing the arc of research of anesthetic agents to that of other chemicals that have been evaluated as potential environmental neurotoxins, the body of research on anesthetics appears at present to be in the middle of this evolution, as investigators are still developing novel strategies for addressing the methodologic problems that inevitably beset human neurotoxicity studies. Whether the end of the story will be more similar to that for lead or for ethyl mercury is uncertain until further research is done. There is, however, consensus that further research evaluating the long-term neurodevelopmental effects of anesthetic medications is necessary and will be valuable for clinical decision–making when caring for children who need surgery and anesthesia.

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