An Update on Preclinical Research in Anesthetic-Induced Developmental Neurotoxicity in Nonhuman Primate and Rodent Models

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The 2022 Pediatric Anesthesia and NeuroDevelopment Assessment (PANDA) Meeting included an update on preclinical research in the field of anesthetic induced developmental neurotoxicity (AIDN), with a focus on key developments since last meeting 3 years prior. The focus was on two areas: (1) The rapid growth in reports from nonhuman primate (NHP) studies, which play a pivotal role in understanding what elements of AIDN are likely to translate to human patients; and (2) The exploration of new targets, phenotypes, and exposure paradigms that have been conducted in rodent models. The purpose of this commentary article is to summarize this discussion and expand upon it both to update the reader on key developments in AIDN research and to highlight the importance of continued animal model research.

UPDATE ON NONHUMAN PRIMATE STUDIES OF AIDN

The nonhuman primate (NHP) model is of value to the field of AIDN as clinical approaches are limited due to the ethical considerations of pediatric studies. The NHP is phylogenetically close to the human condition, sharing similarities in the development and complexity of their brain, in their cognitive functions, and in behavioral repertoire. Both species show similarities in social cohabitation, and social signals in both species depend on visual cues. In contrast, small animals such as rodents strongly rely on olfactory signals. Studies exposing infant NHPs to anesthetics have greatly contributed to the field of AIDN.1 The most recent advances, including new knowledge about structural alterations and functional outcomes induced by early-in-life anesthesia exposure and the latest findings in neuroimaging studies and investigations of underlying mechanisms and mitigating strategies, are discussed.

Newly Identified Structural Alterations

Early histopathological studies in NHPs specified the complex gyrencephalic brain and expanded the understanding of acute neurotoxic effects on the immature brain caused by the exposure to diverse anesthetics that had been described from studies in rodent pups. 2–7 As little as 3 hours exposure to isoflurane on postnatal day 6 under control of physiological parameters and monitoring, caused widespread increase in apoptosis in the developing brain, affecting not only neurons but also causing apoptosis of oligodendrocytes.8 In addition, and in contrast to experiments in rodents, the ability to control

and maintain physiological parameters within normal ranges in the exposed NHP infant allowed the observed structural changes to be tied to anesthetic-induced neurotoxicity, and to exclude the possibility of them being the consequence of nonspecific effects, such as generalized hypoxia or low blood pressure. More recent studies in NHPs analyzed the animals' brains for anesthesia-induced structural changes after longer observation times. A recent histopathological analysis of brains of NHPs that were exposed to isoflurane during infancy discovered brain areas with in creased astrogliosis 2 years after the exposure.9 Increased expression of the glial fibrillary acidic protein, which identifies astrogliosis, was found in several brain areas including the primary visual cortex, the subiculum, the perirhinal cortex, and the amygdala. Astrogliosis indicates chronic astrocyte activation, a common residual of a previous brain injury also found in several neuro developmental or neurodegenerative diseases such as autism spectrum disorders or Alzheimer disease. Although in some areas the astrogliosis was only significantly increased in animals that had received a series of 3 early-in-life exposures to isoflurane, the increase in astrogliosis in the amygdala was also found 2 years after a single 5-hour isoflurane exposure during infancy, suggesting a potential sensitivity of this brain area to the neurotoxic effect of anesthetics early-in-life. Importantly, the amygdala is a major brain area for processing socioemotional behaviors, which are functions that have repeatedly been found to be affected by neonatal anesthesia exposure.9–12 Electron microscopic analyses of NHP brain tissue after neonatal exposure to sevoflurane suggest that early-in-life exposure causes alterations in the synaptic ultrastructure later in life.13 Four years after multiple 4-hour exposures to sevoflurane during infancy, the brains of exposed animals showed a significant reduction in synapse areas in the CA1 area of the hippo campus, while the synapse density was unaffected. Of note, the same animals had shown functional alterations such as heightened emotional reactivity to social stressors and impairments in visual recognition memory starting at the age of 6 months and 1 year, respectively.11,14 Future studies would be required to determine whether these structural changes in synapses represent the substrates for these functional impairments.

New Insights in Altered Neurobehavioral Outcomes

The high level of similarities in neurobehavioral development between NHPs and humans strengthens the translational validity of studies using the NHP for investigating infant anesthesia-induced consequences on long-term functional outcomes. Earlier studies that primarily focused on assessing cognitive function after early-in-life exposure to anesthetics have reported impairments using the operant test battery, an assessment tool that can be used in both humans and animals.15 While this study reported that exposed animals showed impairments in most of the tested categories at about 3 and a half years post-exposure, the field has critically questioned the clinical relevance of these results since they derived from extreme exposure conditions: NHPs had been exposed for an unusual length of 24 hours and with the less frequently used substance ketamine. In a more recent study, the same operant test battery was used to test 3.5-year-old NHPs after infant exposure to a more clinically relevant yet still lengthy regime.16 In contrast to the previous findings following neonatal ketamine exposure, 8 hours of exposure to a mixture of isoflurane and nitrous oxide showed no effects in most cognitive domains; significant differences between exposed and unexposed animals were largely limited to a measure of motivation. In human clinical studies, operant test battery evaluations have not found significant score differences between the children who received early-in-life anesthesia and the unexposed individuals.17 In contrast

to the findings for cognitive testing in NHPs, 2 independent studies of neonatal exposure to volatile anesthetics, such as sevoflurane or isoflurane, similarly found persistent increase in anxiety-like and inhibition behaviors.10,12,18 A recent article reported results from NHPs 2 years after single or multiple 5 hours of neonatal exposure to isoflurane during the first 2 weeks of life.18 In this study, the juveniles were assessed by a series of structured observations of the individuals in their social group within their home environment and without any provocation. These observations revealed that the 2-year-old animals that were exposed to anesthesia early-in-life withdrew themselves from the group more often and spent less time in close social contact. This effect was robust and significant for animals exposed multiple times; the individuals exposed a single time showed a similar trend towards spending less time close to peers as compared with control animals. Testing several cognitive domains in the same cohort of animals, including object permanence, executive function, stimulus-response learning, and spatial working, memory did not indicate impairments in the juvenile NHPs. These results share similarities with findings from the few clinical studies in the field with prospective assessments of children.19–21 A recent metanalysis that combined the results of the 3 studies confirmed that early-in-life anesthesia exposure was associated with increased behavioral problems in exposed children when behavior was observed by their caregivers, at home or at school.22 Similar to the above-described results in NHPs, the clinical studies did not find deficits in general intelligence or other cognitive domains in the exposed children.

Recent Neuroimaging Findings

There is great interest in the application of neuroimaging technology as it could have the potential to noninvasively detect pathologic processes in the brain associated with AIDN and, therefore, could serve as a biomarker to identify affected subjects, monitor treatment effects, or test protective strategies. However, while NHPs are frequently used in neuroimaging research, this approach has hardly been used in the field of AIDN. The studies of Zhang et al23,24 are some of the very few that had used in vivo micro-positron emission tomography/ computed tomography imaging as a minimally invasive method aiming to investigate its applicability for detecting AIDN in NHPs.23,24 The results of these studies revealed that early-in-life exposure to volatile anesthetics increased glial activation, and the authors proposed this as a surrogate for neurotoxicity in the NHP brain. More recently, a study examined the brains of early-in-life anesthesia-exposed animals for structural changes in the white matter using magnetic resonance imaging (MRI) technology.25 The reported findings, deriving from 2 primate cohorts at 2 separate centers with the animals' age either 12 or 18 months, showed that multiple short anesthesia exposures during early development were as sociated with alterations in white matter integrity. Animals in both cohorts had received 0 to 4 previous MRI sessions under short anesthesia with ketamine and/or isoflurane before undergoing diffusion MRI scans. Data were evaluated based on the calculation of a "total normalized exposure" index to ketamine and isoflurane, as there were no unexposed individuals included in the study. The authors found substantial disruptions in white matter integrity that correlated with this total normalized anesthetic exposure. They report a dose-dependent effect with greater total normalized exposure correlating with a stronger decrease in fractional anisotropy and greater diffusivity, both indicators of poorer organization of the white matter. These changes in the integrity of white matter support previous studies providing evidence of toxic effects of early-in-life anesthetics on oligodendrocytes.4,5,26 While there was variability in the ages at exposures and at the final scan and considerable procedural differences caused by the 2 cohorts residing at

separate primate centers, the congruency of the findings despite these differences make them valuable. In addition, this study provided novel data on the effect of multiple rather short exposures during infancy which resemble clinical situations of repeated early-in-life anesthetic exposures for consecutive diagnostic or therapeutic interventions. In addition, they suggest white matter architecture as a translatable imaging measure for AIDN. On the basis of these encouraging findings, future studies will be required to investigate the persistence of these measures until later in life and to address the potential implication of these alterations in white matter integrity for long-term neurobehavioral outcomes. Future studies will be required to link MRI-detectable white matter injury with impaired functional outcomes caused by AIDN.

Novel Insights into Mechanisms of Anesthetic-Induced Developmental Neurotoxicity

The underlying processes causing AIDN are still not fully understood, even though studies investigated various mechanisms including apoptosis, neuroinflammation, changes in lipid metabolism, or disruption of myelination.23,27,28 Only recently, the effect of neonatal anesthesia exposure on post transcriptional regulation of messenger RNA (mRNA) was investigated in the NHP.29 Methylation of adenosine at the N6-position resulting in the formation of N6-methyladenosine (m6A) represents one of the most abundant reversible chemical modifications of mRNAs. The formation of m6A modifications and their biological activity depends on a group of proteins, including m6A binding proteins that promote translation efficiency, methyltransferases, and demethylases, commonly referred to as readers, writers, and erasers, respectively. Using methylated RNA immunoprecipitation, prefrontal cortices of neonatal NHPs that had received 3 exposures of 5 hours sevoflurane within the first 4 weeks of life were found to have significantly more m6A peaks than control animals.29 Subsequent gene ontology analysis revealed links between genes with increased methylation of m6A sites and physiological functions critical for neurodevelopment, particularly for synaptic plasticity. In addition, the exposed animals had decreased mRNA levels of 2 m6A binding proteins, YTHDF 1 and 3 (YT521-B homology domain family 1 and 3), suggesting that the sevoflurane-induced dynamic regulation occurs on the m6A reader level. Of note, while these studies have not investigated long-term neurobehavioral outcomes, the exposure regime is closely comparable with one that resulted in long-term behavioral alterations and memory impairments in the exposed NHPs.12,14 Importantly, abnormalities in m6A methylation have been reported for other neurodevelopmental and -degenerative diseases, including autism and Alzheimer Disease, suggesting that this mRNA modification is involved in, and may even promote, the development of such diseases.30,31 Future studies will investigate the role of these epitranscriptomic alterations in impairments in neurobehavioral development caused by early-in-life anesthesia exposure.

Recent Advances in Mitigating Strategies in Nonhuman Primates

Over the past few decades, the number of studies aiming to identify mitigating or protective strategies against AIDN have risen considerably; however, the majority of those studies are limited to small animal models.32,33 A few studies investigated strategies to protect the immature brain against AIDN using the NHP model and reported amelioration of the acute neurotoxic effects of the anesthetic.34,35 Co-administration of lithium in neonatal NHPs that were exposed to 5 hours of isoflurane significantly reduced the apoptosis of neurons and oligodendroglia.34 However, clinical studies applying this strategy have not been conducted. A more recent study investigated

the potential of hypothermia being neuroprotective against AIDN.35 During a 5-hour sevoflurane exposure, the neonate's body temperature was regulated in either the normothermic (> 36.5°C), mild hypothermic (35 to 36.5°C), or moderately hypothermic (< 35°C) range, with the remaining parameters maintained within physiological ranges. Interestingly, only mild hypothermia, but not moderate hypothermia, led to a benefit with significantly reduced apoptosis of neurons and oligodendrocytes, shortly (8 h) after the exposure, as compared with the sevoflurane-exposed animals. The narrowness of the window of hypothermia being beneficial may be considered challenging, with loss of protection below 35 °C. While the ease of applying mild cooling during anesthesia in an already tightly controlled pediatric anesthesia setting may encourage additional investigations in NHPs to test whether the structural protection translates into benefits in neurobehavioral outcomes, this strategy may be limited to nonsurgical anesthesia exposures due to potential hypocoagulation, impairments in wound healing and increased risk for perioperative infections. With already existing experience in the use of hypothermia in neonatal medicine for other conditions, this protective strategy appears feasible, and, if turning out to be beneficial in subsequent NHP studies, it has the potential for translation into clinical practice.

Future Directions

The NHP model in AIDN research has generated profound new insights, as discussed above. For some of the remaining questions in the field, the approach using this translational model may be the only way to find the answers. With the phylogenetic proximity to the human, the size of the infants allowing for exposure conditions closely resembling those in the pediatric operating room and the ability to exclude confounders, such as surgery or pre-existing medical conditions, often impeding the evaluation of results from clinical studies, carefully designed NHP studies have strong potential to advance our knowledge about AIDN. Albeit expensive and requiring longer observation periods, compared with small animal models, studies in NHPs will facilitate characterization of the behavioral phenotype caused by early-in-life exposure to general anesthetics, identification of a noninvasive and specific biomarker, and help establish strategies to protect against AIDN.

UPDATE ON RODENT STUDIES OF ANESTHETIC-INDUCED DEVELOPMENTAL NEUROTOXICITY

The field of AIDN finds its origins in rodent research, and studies in rats and mice remain the largest source of information on how brief exposures to anesthetic agents can cause lasting alterations in neurologic function. Translation of rodent studies has obvious limitations related to the differences between rodent and human brain structure, the profound difference in the developmental timeline, and the difficulty in providing ideal physiologic conditions during anesthetic exposures in the absence of comprehensive monitoring and mechanical ventilation. However, much of what is known about human neuro science derives from rodents, and the relatively low barrier in comparison with humans and NHPs makes it plausible to use rodent models to explore the fundamental biology of AIDN. Studies in mice and rats are ideally designed to expand the boundaries of our understanding of AIDN, which is a necessary precursor to conducting more challenging translational studies. What follows below is a selective discussion at the 2022 PANDA meeting of advances in rodent research related to novel phenotypes, targets, and exposure models that have come to light in recent years.

Novel Phenotypes: Anxiety, Social Interactions, and Pain Thresholds

Driven by obvious changes in learning and memory testing in rodent models, AIDN studies have focused for some time on adverse outcomes related to reduced intelligence;

however, there is evidence in both human and NHP studies to suggest that other phenotypes, particularly those related to behavior, may be of greater clinical significance.36 Investigators have begun to explore new phenotypes beyond those reflective of intelligence that are measurable through behavioral testing, including changes in anxiety, social interactions, and pain thresholds. Several reports have explored the possible effects of early anesthetic exposure on anxiety, with mixed out comes. Diana et al37 found that early exposure to isoflurane, nitrous oxide, and midazolam in rats resulted in increased entries into the center zone of an open field test (OFT) and had fewer entries and traveled shorter distances in the open arms of an elevated plus maze (EPM). The authors interpret the OFT finding as an increase in risk-taking behavior and the EPM finding as an increase in anxiety. Sun et al38 examined the effects of early exposure to ketamine in mice and found avoidance in both the central zone of an OFT and in the open arms of an EPM with combined exposure to ketamine and dexmedetomidine and Turktan et al39 reported similar findings in mice with early exposure to dexmedetomidine. Two studies that examined EPM as part of a battery of behavioral tests reported evidence of increased anxiety resulting from isoflurane and sevoflurane exposures.40,41 In contrast, 2 studies of ketamine exposures did not observe significant differences in OFT and EPM between exposed and control animals.42 As is common in the rodent literature, the extant studies on anxiety-related outcomes use a range of anesthetics, doses, and exposure and assay paradigms, and thus it is not entirely unexpected that the results do not give a uniform outcome, but they certainly suggest that an anxiety phenotype is plausible. Despite the wide degree of variability in investigations of AIDN and social interaction, there is a clear signal that a diverse array of early exposures to anesthetics impairs social behavior in the rodent model. Diana et al37 employed a social novelty test and found that rats exposed to early postnatal isoflurane, nitrous oxide, and midazolam had an increased number of explorations of a novel rat compared with a familiar one, and Chen et al43 obtained essentially similar results in mice with an intrauterine sevoflurane exposure. Maloney et al40 replicated the finding of impaired response to social novelty, but only in male mice. The effects of ketamine were studied by Coronel-Oliveros and Pacheco-Calderón,44 who employed a social interaction test rather than a novelty paradigm and found impaired sociability and increased aggressive behavior. Taken together, these studies strongly suggest that further exploration of the effects of AIDN on social behavior is warranted. An entirely novel phenotype resulting from AIDN related to pain perception has recently been described. Li et al45 found that a single isoflurane exposure in early postnatal mice resulted in a decreased pain threshold as measured by tail flick, von Frey, and formalin tests. The authors speculate that early exposure to anesthetics could contribute to the development of chronic pain syndromes in adulthood, which is an intriguing possibility given that persistent chronic pain is a common outcome after pediatric surgery.46

Novel Targets: Gut Microbiome, DNA Methylation, Histone Acetylation, RNA Modification, and Neuroinflammation

Rodent models remain the most common tool to explore the fundamental question of how relatively brief exposure to anesthetics, which are designed to be short acting compounds, could have a long-lasting effect on the brain. As evidenced by previous reviews of the state of preclinical research resulting from the PANDA meeting, most work in the past has focused on effects on neuronal and glial cell death, neurogenesis, neuronal and glial growth, synapse formation, and molecular machinery best known to be associated with these processes.47–49 In the last several years, a new set of targets for study has emerged, including gut microbes, epigenetic/epitranscriptomic processes, and

inflammatory systems in the brain. It is now well-established that a healthy gut micro biome is important for normal neurodevelopment.50,51 Animal models have shown that a disruption of the gut microbiome during development, known as dysbiosis, is associated with neurobehavioral phenotypes in adulthood.48 Recently, inhalational anesthetic exposure was found to alter the gut microbiome in adult rodents,52,53 and an early postnatal isoflurane anesthetic exposure in rats was found to cause persistent dysbiosis.54 Liu et al55 have directly implicated the microbiome in AIDN by showing that microbiome-depleted mice that received fecal transplants from donor mice exposed to sevoflurane exhibited worse performance on the Morris water maze task as compared with untransplanted controls. Wang et al56 found that impaired performance of rats exposed prenatally to isoflurane on a novel-object recognition task and reduced levels of hippocampal brain-derived neurotrophic factor (BDNF) were both rescued by treatment with probiotics. Given that these compounds are designed to alleviate dysbiotic states, this finding implies that dysbiosis contributed to both behavioral and biochemical abnormalities in AIDN. While much study in this area remains to be done, it is clear that the gut microbiome and perhaps the microbial communities in other organs, such as the lung, represent a highly novel target in AIDN that is deserving of further study. DNA methylation at the cysteine base is a key mechanism of epigenetic regulation throughout organismal biology, including in the developing nervous system. The addition of 5-methylcytosine (5-MC) in cytosine–phosphate– guanine dinucleotides, which is mediated by DNA methyltransferases, results in gene silencing by turning the chromatin into a highly compacted and closed state. In an investigation of DNA methylation as a target for AIDN, Ju et al57 found that multiple sevoflurane exposures in rats downregulated the level of BDNF through hypermethylation mediated by increased expression of the DNA methyltransferases, DNMT-3A and DNMT-3B. Similarly, Wu et al58 reported a decrease in BDNF expression mediated by an increase in DNMT1 expression that resulted in increased 5-MC groups in the promoter region of Bdnf exon IV. Sevoflurane exposure has also been shown to act through DNMTs to cause hypermethylation and downregulation of Shank2, Psd95, Synapsin1, and Synaptophysin, which encode for key synaptic proteins.59 The effects of anesthetics may be cell-type specific, as in contrast to the reports above, a study of the effects of early anesthetic exposure on oligodendrocyte development showed that isoflurane exposure down regulated DNMT1 and decreased 5-MC exposure in oligodendrocyte precursors, resulting in inhibited progression to mature oligodendrocyte status.60 All of the studies on DNA methylation are targeted to specific genes and DNMTs, and the field would benefit from an unbiased, cell-type specific study of the effects of AIDN on DNA methylation. Histone acetylation, in which the addition of acetyl groups to histones opens chromatin structure to facilitate transcription, is another form of epigenetic regulation that has been studied as a possible target for AIDN. Acetylation is facilitated by histone acetyltransferase (HATs) and reversed by histone deacetylases (HDACs). Several studies of AIDN have found that early exposure to volatile anesthetics can cause an imbalance of HDAC and HAT expression and activity in rodent models.58,61,62 One study found that maternal exposure to propofol caused increased expression of HDAC2 and decreased levels of acetylated H3K14 and H4K12 in rat pups that correlated with worsened performance on tests of learning and memory.63 In a study of the effects of combined exposure to isoflurane, nitrous oxide, and midazolam, Dalla Massara et al64 found reduced HAT activity leading to a decrease in acetylated H3 at the c-Fos and Bdnf promoters. Intriguingly, in this study, a reversal of the effects on histone acetyltransferases by sodium butyrate treatment also resulted in improved outcomes in learning and memory tasks that were adversely affected by

anesthetic exposure, providing strong evidence of a causal link between alterations in histone acetylation and AIDN. As is the case with DNA methylation, our understanding of how histone acetylation may be a target of AIDN would benefit greatly from unbiased studies of anesthetic actions on this epigenetic mechanism. There are more than 170 RNA modifications that shape cellular and biological processes. Among them, m6 A is the most abundant RNA modification in mammalian systems and confers another layer of gene regulation, including splicing, mRNA stability, mRNA decay, localization, and translation.65 Several studies investigate the role of m6 A in mediating AIDN. Zhang et al66 reported that multiple sevoflurane exposures reduced the expression of synaptophysin, an important presynaptic protein in the prefrontal cortex of mice. This reduction was mediated by decreased m6 A methylation on synaptophysin mRNA and downregulated YTHDF1, a well-known m6 A reader mediating translation. In another study, Wu et al67 performed genome-wide screening of the m6 A modification in the mouse brain at P30-35 after multiple isoflurane or sevoflurane exposures and found that m6 A modification was altered in a substantial number of genes associated with metabolic, developmental, and immune functions. Future work should be directed towards understanding how these modifications may serve as mediators of AIDN and on exploring other RNA modifications beyond m6 A. Exposure to anesthesia during early childhood has been shown to induce a neuroinflammatory response in the brain. This is of special importance since perinatal inflammation has been linked to impaired neurological functions in humans.68 The hallmarks of neuroinflammation include upregulation of signaling molecules such as cytokines and chemokines, and the presence of activated microglia and reactive astrocytes. Microglia are the resident immune cells in the central nervous system that dynamically survey the tissue for dying cells and infections. Microglia play an important role in the postnatal neurogenic niche of the dentate gyrus, where they phagocytose microglia under homeostatic conditions and inhibit neurogenesis after injury.69,70 However, the mechanisms by which anesthesia-induced inflammation and microglial activation affect the developing human brain is not known. Under normal conditions, the central nervous system is an immunologically protected organ; however, studies of traumatic and anoxic brain injury in early life have shown that the induction of inflammatory changes in the brain has potentially severe consequences for neurodevelopment. Recent literature suggests that anesthetics may cause a pro-inflammatory state mediated either by cytokines, activation of microglia, or both, and this represents a novel potential mechanism of injury in AIDN. Early work by Shen et al71 found that repetitive exposures to sevoflurane caused upregulation of interleukin-6 and tumor necrosis factor-α and increased numbers of cells positive for the pan-microglial marker Ionized calcium- binding adapter molecule 1 (IBA1) in the hippocampus; these findings did not persist into adulthood. However, in a more recent study by Hogarth et al,72 early postnatal exposure in rats led to increased expression of pro inflammatory cytokines in cerebral cortex that was measurable at 10 months of age, indicating the possibility of a long-lasting effect. Zuo et al73 replicated the findings of both increased pro-inflammatory cytokine and activated microglial expression resulting from early postnatal exposure to both isoflurane and sevoflurane. The same report showed a co-incident suppression of neurogenesis in the hippocampus; given that microglia have been demonstrated to play a profound effect on the regulation of hippocampal neurogenesis,74 this finding is highly suggestive of a microglial contribution to the mechanism of AIDN. Finally, at least 1 recent study makes a compelling argument for a causal connection between AIDN and neuroinflammation. Two studies by Jiang et al75,76 investigated the effects of the isoflavone compound Genistein (4',5,7-

trihydroxyisoflavone), which has been reported to have antioxidant activity, in rats that underwent early postnatal isoflurane exposure. These authors found reduced levels of pro-inflammatory cytokines shown by qPCR and ELISA, a shift in the microglial population from the pro-inflammatory microglial state (M1) to the more anti-inflammatory microglial state (M2), reduced neuronal apoptosis, and an improvement in behavioral tests of learning and memory compared with rats that did not receive the antioxidant. Studies of the role of neuroinflammation in AIDN are very incomplete and would benefit greatly from comprehensive screening for a full array of both humoral and cellular mediators of inflammation, and from additional studies using a rescue approach to make a clear connection between inflammation and the evidence of poor outcomes in terms of both behavioral testing and histology.

Novel Exposure Paradigm: Sedation Toxicity

The Food and Drug Administration Advisory implicating sedative agents with similar mechanisms to general anesthetics as possible causes of harm to the developing brain begs the question of whether sedation in nonsurgical contexts, particularly in the intensive care setting, is a parallel or even greater cause of potential harm than AIDN. This is a challenging hypothesis to address as critical illness in and of itself is a likely cause of poor neurological outcomes. Nevertheless, notable parallels exist between AIDN and nonsurgical sedative exposures: (1) GABA agonists are common;77,78 (2) multidrug treatment includes opioids, N-methyl-D-aspartate inhibitors, and α2-adrenergic antagonists, and; (3) early childhood appears to be selectively vulnerable.79 Unfortunately, the generalization of findings from anesthetic neurotoxicity models is limited by the focus on inhalational agents and comparatively brief exposure in rodent models. Moreover, while anesthesia overwhelms neurotransmission for a discrete interval, intensive care sedation is typically administered in different patterns and doses, often including an incremental escalation over days to weeks and often requiring weaning, presumably because of receptor accommodation that alters pathways integral to brain maturation. Furthermore, while some patients undergoing general anesthesia have underlying conditions that would be expected to have implications for brain health, by contrast, essentially all children sedated for critical illness have substantial risk factors for poor neurological outcomes. Finally, it is unclear what the potential connections may be between the use of sedatives and the short-term outcome of delirium, and what the implications of delirium states are for long-term neurological outcomes. There are far fewer preclinical models representing prolonged pediatric intensive care sedation, and much of this work has investigated benzodiazepines. Xu et al80 performed daily 12-hour midazolam sedations for 5 days in P18-22 mice and reported not just significant dose-dependent changes in synapses and reduced hippocampal neurogenesis, but also impaired learning and memory. What is striking about this study, however, is that these pathologic observations were made after P60 when the ice had reached the rodent equivalent of early adulthood. Long-lasting suppression of neurogenesis is supported by Doi et al,81 who treated P7 mice for 3 days with midazolam and assessed hippocampal neural stem cells at several time points, including P10 and P84. Similar to Xu et al,80 Doi et al81 noted a learning and memory deficit in adult mice remotely treated with midazolam in early childhood that correlated with “quiescence-associated” genes and suppressed neurogenesis. While neuronal connectivity is often center stage, the importance of glia cannot be overlooked, particularly given the neuro physiologic resilience needed during illness and recovery that depends on glial integrity. To explore the glial effects of opioid-based intensive care sedation in the immature mammalian brain, Iqbal O’Meara et al82 treated P18 rat pups with morphine, midazolam, or combined

morphine and midazolam for 7 days. Brain homogenates were screened at P25 and showed that midazolam significantly increased the expression of 17 and 18.5 kDa myelin basic protein isoforms that are found in developing oligodendrocytes (17 kDa) and facilitated myelin compaction (18.5 kDa).83 There was also a significant increase in S100B calcium-binding protein, an astrocytic regulatory protein also found in glial progenitor cells and myelin.84–86 Assessment of the astrocytic glial fibrillary acidic protein in this study was notable for over 20% decrease in expression in all treatment groups compared with controls. Interestingly, co-treatment with morphine and midazolam seemed to mitigate the effect of midazolam alone on the limited proteins assayed, highlighting the need for multi-drug studies. Importantly, while these studies draw attention to the deleterious potential of sedation, they assume typically developing brains in otherwise healthy subjects. To leverage gains made in preclinical developmental anesthetic neurotoxicity, "reverse translation" of critical illness paradigms is imperative to identify therapeutic targets. It is unlikely that sedatives behave uniformly when superimposed on traumatic brain injury or meningoencephalitis, for example. Opioids have received less focus, but they are foundational in intensive care sedation and have been shown to affect synaptogenesis, astrocyte growth, and myelination.87–89 There has also been little to no investigation into the α2-antagonist dexmedetomidine despite its ubiquity in contemporary intensive care and conflicting evidence of neuroprotective and injurious effects.32,79,90 Notably, clonidine suppresses REM sleep and inhibits cortical growth in rat pups and is associated with functional decline after childhood critical illness, where it is frequently used to wean dexmedetomidine.91–93 Neurotransmitter and receptor concentrations are developmentally regulated, and epochs of brain development and refinement throughout childhood may be vulnerable. Disruption of foundational brain connectivity may have long-lasting impacts, and impaired neurogenesis or gliogenesis is salient to neurological recovery. Doi et al81 were able to rescue neurogenesis by providing an exercise wheel to promote physical activity, a finding that supports early intensive care unit mobility initiatives and has implications for post hoc rehabilitation. Taken together, these findings provide a highly compelling argument that sedative exposures in a critical illness model are far from benign and that further study should be conducted alongside and in comparison with investigations in AIDN models.

CONCLUSION

The NHP model in AIDN research has generated profound new insights, as discussed above. For some of the remaining questions in the field, approaches using this translational model may be the only way to find the answers. With the phylogenetic proximity to the human, the size of the infants allowing for exposure conditions closely resembling those in the pediatric operating room, and the ability to exclude confounders often impeding the evaluation of results from clinical studies, carefully designed NHP studies have a strong potential to advance our knowledge about AIDN. They will facilitate characterization of the behavioral phenotype caused by early-in-life exposure to general anesthetics, identification of a noninvasive and specific biomarker, and help establish strategies to protect against AIDN. Investigations in rodent models continue to serve as the leading edge of AIDN research. The sheer number and diversity of such investigations, as compared with human and NHP models, invariably leads to novel findings that may warrant further investigation. Future directions in rodent research should include better standardization of techniques across groups, which would allow for more direct comparisons between the work of different laboratories. In particular, it is of importance to begin to model the interplay between the putative effects of

anesthetics and those of surgery and comorbid disease in rodents. Furthermore, it is of key importance that rodent research continues to be informed by findings and trends in human and NHP research to support and extend scientific discoveries in these models that are obtained at the cost of very substantial resources. Conversely, researchers in the human and NHP model systems should stay abreast of key developments in rodent research to inform how best to direct their own investigations.

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