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Effects of Anesthetics on Brain Circuit Formation

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Abstract

The results of several retrospective clinical studies suggest that exposure to anesthetic agents early in life is correlated with subsequent learning and behavioral disorders. While ongoing prospective clinical trials may help to clarify this association, they remain confounded by numerous factors. Thus, some of the most compelling data supporting the hypothesis that a relatively short anesthetic exposure can lead to a long-lasting change in brain function are derived from animal models. The mechanism by which such changes could occur remains incompletely understood. Early studies identified anesthetic-induced neuronal apoptosis as a possible mechanism of injury, and more recent work suggests that anesthetics may interfere with several critical processes in brain development. The function of the mature brain requires the presence of circuits, established during development, that perform the computations underlying learning and cognition. In this review we examine the mechanisms by which anesthetics could disrupt brain circuit formation, including effects on neuronal survival and neurogenesis, neurite growth and guidance, formation of synapses, and function of supporting cells. There is evidence that anesthetics can disrupt aspects of all of these processes, and further research is required to elucidate which are most relevant to pediatric anesthetic neurotoxicity.

Keywords

Anesthetic; Neurotoxicity; Apoptosis; Neurogenesis; Neurite Growth

Introduction

The most fundamental question that can be addressed by preclinical studies of pediatric anesthetic neurotoxicity is how administration of general anesthetic agents (GAs) with a half-life measured on the order of hours could exert effects on brain function that continue for years. In spite of the thorough and careful work by several groups of investigators, the retrospective human studies that show a correlation between anesthetic exposure and subsequent learning disorders^{1–7} cannot establish a causal relationship between these

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events⁸. Even prospective studies, which are currently ongoing, remain confounded by the occurrence both of the surgery and the disease state which necessitated it. In contrast, research in animal models that address mechanisms of anesthetic neurotoxicity in the developing brain can be designed to ask whether the phenomenon is plausible by examining it in the absence of the confounding factors of surgery and co-morbid disease. Furthermore, insights into the mechanism of injury remain the best hope for developing prophylactic or treatment strategies.

Neurons are organized into circuits for the purpose of conducting the computations that underlie complex functions such as learning and cognition⁹. In order for these circuits to function, the correct populations of neurons must exist in anatomically appropriate locations, and they must communicate via precise connections established during development. Although brain circuits retain some degree of plasticity via changes in synaptic strength, their gross organization is created during critical periods of brain development and remains relatively fixed¹⁰. Even short toxic insults during these periods might be expected to have very far reaching consequences. In the seminal study on developmental anesthetic neurotoxicity Jevtovic-Todorovic and coworkers found that anesthetic treatment results in a chronic attenuation of long-term potentiation in the hippocampal circuit that is critical for learning, a finding that was recently confirmed by another group^{11–13}. The strongest evidence that anesthetics can interfere with the development of brain circuits come from the multitude of rodent studies from different laboratories in which early anesthetic exposure results in reduced performance on behavioral tasks at later ages^{11, 14–20}. The next challenge in preclinical studies of pediatric anesthetic neurotoxicity is to determine how anesthetics can have lasting effects on brain function. In this review, we will examine the mechanisms by which anesthetic agents can disrupt brain circuit formation.

Cytotoxicity of anesthetics

One of the most obvious mechanisms by which anesthetics could disrupt brain circuit formation is by eliminating neurons that are necessary for circuit function. There is a substantial body of evidence in animal models demonstrating that anesthetic exposure in early development leads to increased levels of apoptosis in the central nervous system (CNS). Increases in apoptotic markers have been observed after developmental exposures to a wide variety of anesthetic agents, including ketamine^{21, 22}, midazolam²², sevoflurane²³, desflurane¹⁴, isoflurane^{24, 25}, and propofol²⁶. Additionally, although nitrous oxide has not been demonstrated to be neurotoxic as a single agent, it can increase the apoptotic effect of other anesthetics when used in combination²⁷. Enhanced apoptosis has also been demonstrated in rhesus monkeys following prenatal or neonatal exposure to ketamine²⁸, isoflurane²⁹, and propofol³⁰, suggesting that the phenomenon may be relevant to species with more complex brains and a longer developmental timeline.

The precise significance of the anesthetic-induced increase in apoptotic markers that has been demonstrated by several laboratories remains unclear. While some studies have shown acute increases in markers of neurodegeneration or lasting reductions in cell density in the cerebral cortex (e.g.^{11, 31}), other studies have assayed only for levels of activated caspases acutely after anesthesia exposure. Recent work in the field of neuronal apoptosis has led to a

new understanding that this approach may not always give an accurate assessment of actual apoptotic cell death¹. There is some question as to whether cognitive deficits in mice exposed to isoflurane are caused by apoptotic cell death. Stratmann and coworkers showed that isoflurane and hypercarbia both caused similar patterns of apoptosis, but only isoflurane exposure was correlated with reduced performance on the Morris water maze task³². Furthermore, apoptosis is not necessarily pathologic in all cases. The elimination of neurons that fail to make appropriate functional synaptic connections is an important part of normal CNS development³³. It is even possible that an increase in apoptosis following anesthetic exposure represents a protective mechanism that eliminates neurons with inappropriate connectivity which might otherwise disrupt circuit function. Alternatively, the physiologic neuronal apoptosis that normally occurs during early CNS development may, in fact, be the link between anesthetic drugs and increased apoptosis in juvenile, but not mature animals. Anesthetic exposure in postnatal day (PND) 7 rat pups resulted in early activation of the intrinsic pathway of apoptosis followed by late activation of the extrinsic pathway, while such effects were not observed in PND 14 animals²⁷. As anesthetic drugs effectively 'silence' developing neurons, apoptotic pathways may be inappropriately activated, leading to the cell death of not only redundant neurons, but also of correctly integrated, synaptically active neuron as well³⁴.

Although the significance of enhanced apoptosis remains unclear, research on this putative mechanism in animal models has led to the identification of several promising strategies to mitigate the neurotoxic effect of anesthetic agents on the brain development. The alpha-2 adrenergic agonists dexmedetomidine³⁵ and clonidine³⁶ reduce levels of apoptotic markers and rescue behavioral deficits in rodents treated with isoflurane and ketamine respectively. Concomitant administration of an adrenergic antagonist with dexmedetomidine eliminates the protective effect, implicating the alpha-2 receptor as critical to this phenomenon³⁵. Xenon, an inhalational anesthetic gas that is not widely available, has also been shown to prevent anesthetic mediated increases in apoptosis and rescue behavioral outcomes^{25, 37, 38}. Models of hypoxic injury suggest that xenon's neuroprotective properties may arise from up-regulation of pro-survival factors such as brain-derived neurotrophic factor and Bcl-2³⁹, both of which have been implicated in anesthetic neurotoxicity. The improvement in behavioral outcomes seen with dexmedetomidine and xenon demonstrate that these strategies preserve neuronal circuits, although it is unclear whether they do so by preventing apoptosis or via other mechanisms. Further studies testing the putative protective properties of these agents are needed to evaluate their capacity to act on axon guidance, synapse formation, and other critical processes in circuit development.

Effects of anesthetics on neurogenesis

Anesthetics have the potential to disrupt circuit formation not only by eliminating cells, but also by interfering with the production of new neurons. A preponderance of neurons in the dentate gyrus of the hippocampus in mice are generated in the early postnatal period⁴⁰. In non-human primates 25% of dentate neurons are generated within the first three months after birth⁴¹, indicating that neurogenesis occurs at peak levels during the window of vulnerability for pediatric anesthetic neurotoxicity. The dentate gyrus is a critical component of the hippocampal circuit that is required for spatial learning across species⁴². Early

postnatal exposure to anesthetics leads to reduced performance on dentate dependent tasks such as the radial arm maze and Morris water maze¹¹, and thus one potential mechanism of anesthetic toxicity is via a disruption of neurogenesis in the dentate gyrus.

Effects of anesthetics on neurogenesis might be manifested either as a loss of critically important neuronal populations through inhibition of proliferation or more potently as a loss of precursor populations leading to a chronic reduction in neurogenesis. In the field of developmental alcohol toxicity, it has been shown that a single developmental exposure to ethanol reduces dentate gyrus neurogenesis well into adulthood⁴³. Thus it is not surprising that several studies have found evidence that anesthetic exposures in early postnatal life may cause a lasting impairment of neurogenesis. Stratmann and coworkers showed that isoflurane causes a persistent reduction in proliferation of dentate neurons in the rat hippocampus after anesthetic exposure⁴⁴. This effect was seen in PND 7, but not PND 60 mice, suggesting a window of developmental vulnerability. Furthermore, the decrease in neurogenesis was correlated with reduced performance on fear conditioning and spatial reference memory tasks^{45, 46}. Zhu and coworkers found that early isoflurane exposure in mice resulted in a reduced stem cell pool and a decrease in the total number of dentate gyrus granule cells. Interestingly this phenotype correlated with deficits in object recognition and reversal learning tasks and performance relative to controls on these tasks worsened as the animal aged⁴⁷. Deficits were found with exposure at PND 14, a later age than previously studies, suggesting the possibility of a distinct mechanism of toxicity. Reductions in neurogenesis have also been observed following early exposure to other anesthetic agents, including sevoflurane and propofol^{48, 49}. Experimentation in cell culture models using neuronal precursors also suggests that isoflurane and sevoflurane exposure can inhibit neurogenesis^{45, 50}. Interestingly, isoflurane treatment did not eliminate neuronal precursors, but rather slowed their rate of proliferation, whereas sevoflurane treatment both inhibited proliferation and caused apoptosis^{45, 50}. Taken together these studies suggest that anesthetics could disrupt brain circuit formation by interfering with neurogenesis at young ages, but it is difficult to rule out contributions from other mechanisms of toxicity that were not evaluated in these studies.

Neurite growth and guidance

In order for functional brain circuits to form, axons and dendrites in the developing brain must grow towards each other according to a stereotyped pattern that creates the appropriate patterns of connectivity between brain regions. This highly complex process depends not only on neurite initiation, differentiation, and growth⁵¹, and also on guidance systems that steer developing axons towards their dendritic targets⁵². Even brief off-target effects of anesthetics on the molecular mechanisms of neurite growth and guidance at critical time points might result in lasting disruptions of brain circuit formation given that guidance cues are only present for limited periods of time^{52, 53}.

Anesthetics might interfere with the establishment of connectivity by inhibiting axonal or dendritic growth, disrupting axon guidance, or by causing degeneration of axons or dendrites. The earliest evidence that anesthetic agents can interfere with neurite growth come from studies showing that rats exposed chronically to low dose halothane in utero

exhibit reduced dendrite growth and branching on subsequent histopathological analysis^{54, 55}. These data are consistent with the observation that isoflurane can inhibit actin based motility in dendritic spines and in fibroblasts, a finding that suggests that the volatile anesthetics may have effects that generalize to many forms of actin-based motility⁵⁶. More recent study in developing hippocampal neuron cultures has shown that isoflurane can cause a RhoA mediated depolymerization of actin that is accompanied by a loss of neurite processes⁵⁷. This finding suggests a degenerative mechanism of action which may or may not fall along the same spectrum as growth inhibition. One functional consequence of neurite growth inhibition which has been demonstrated is a delay in axon specification, the process by which one of the pre-polarized neurites acquires characteristics that allow it to assume an axonal fate⁵⁸. Given that the cues which direct axons to establish appropriate connections are presented transiently, delays of this nature might result in failures of circuit formation⁵³. Finally, a broad range of anesthetics have been shown to interfere directly in axon guidance by interfering with growth cone sensing of both netrin and semaphorin guidance cues⁵⁹. Thus, anesthetics interfere with critical processes in axonal and dendritic growth and guidance, but it remains to be seen whether these effects translate into an actual loss of circuits.

Synapse formation and maintenance

Anesthetic exposure could also disrupt circuit formation during development by interfering with the formation or maintenance of synapses, the structures which allow electrochemical neurotransmission. Disruptions in synapse development can have profound effects on subsequent function, as demonstrated by the role of dysfunctional synaptogenesis in devastating developmental neuropsychiatric disorders such as autism⁶⁰. Using electron microscopy, Lunardi and co-workers showed both a loss of synapses in the subiculum in PND 21 rats that had been exposed to midazolam, nitrous oxide, and isoflurane on PND 7⁶¹. Both excitatory and inhibitory synapses appeared to be lost in equal numbers suggesting a generalized toxicity, but it is not apparent from this study whether synaptic loss may simply be a byproduct of neuronal cell death which is seen with the triple anesthetic cocktail. In contrast, the Vutskits lab has demonstrated an increase in dendritic spines, the post-synaptic entity associated with many excitatory synapses, in rats exposed to a variety of anesthetic compounds including midazolam, propofol, ketamine, and the potent volatile anesthetic agents^{62, 63}. The discrepancy between the findings of these studies has been partly reconciled by more recent work showing that propofol treatment resulted in a loss of spines if administered prior to the second postnatal week and a gain of spines if administered between the second and fourth postnatal week⁶⁴. Of great interest, the alterations in synapse number observed in this study persisted into early adulthood. Thus it appears clear that anesthetics could have a lasting impact on circuit formation through effects on synaptogenesis, although the cognitive consequences of this finding have not been elucidated.

Effects on glia

Finally, it is possible that anesthetics could act on the development of brain circuits through effects on non-neuronal cells. Brambrink and coworkers showed that isoflurane exposure in

the neonatal primate brain induced apoptosis of oligodendrocytes, the glial cells that generate myelin wrapping to speed conduction along axons ⁶⁵. A loss of oligodendrocytes could be expected to broadly disrupt brain circuit function if it was accompanied by decreases in myelination, as occurs in demyelinating disorders like multiple sclerosis. Anesthetics also appear to have effects on astrocytes, a glial cell type that supports and promotes neuronal growth and synapse formation. Lunardi and co-workers demonstrated that anesthetic exposure delayed astrocyte maturation and disrupted actin based morphology ⁶⁶. A second report confirmed this finding, but failed to show any effects of isoflurane on the capacity of neurons to form synapses ⁶⁷. However, in an accompanying manuscript Ryu and coworkers demonstrate that astrocytes treated with isoflurane have a reduced capacity to support neurite development. Relatively little study has been devoted to the effects of anesthetics on non-neuronal cells that are critical for brain function, and thus the significance of these findings is not yet clear.

Conclusion

Due to its overwhelming complexity, brain development has many possible points of vulnerability to toxic exposures, and it appears that anesthetic agents could disrupt circuit formation through several discrete mechanisms, none of which are mutually exclusive. Future research should be directed towards determining which of these mechanisms are most relevant. This will require careful testing of dose-response relationships, comparative studies between different anesthetic agents, and simultaneous evaluation of more than one mechanism of toxicity within the same study. While studies of this nature are more complex and costly, their results represent the best hope of understanding pediatric anesthetic neurotoxicity.

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