Anesthetic Toxicity in the Pediatric Brain
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For over 150 years of anesthetic practice, it was believed, that, as a general anesthetic wears off, the brain would return to the same state as before the anesthetic. We are now beginning to understand, that this basic premise of anesthetic pharmacology is false. In 2003 Jevtovic-Todorovic(1) presented her sentinel findings, that a combined anesthetic (midazolam, nitrous oxide and isoflurane), administered to 7-day old rats for 6 hours, kills neurons in the developing brain and causes long-term impairment of brain function. They showed that long-term potentiation (LTP) in the hippocampus was impaired in anesthetized rats(1). LTP is a form of synaptic plasticity, often considered the electrophysiologic correlate of learning and memory and the hippocampus is a brain structure important for learning and memory. More importantly yet, these authors demonstrated a progressive deficit in spatial recognition tasks administered both 4 weeks and 4 1/2 months after anesthesia(1). Immediately concern mounted, within the anesthesia community(2-5) and also within regulatory authorities(6), about whether or not these phenomena might apply to humans. Subsequently, it became clear that the histologic data were reproducible, not only in rodents but in virtually every species tested (for review see(7)), including primates(8-10) further heightening the degree of concern about anesthesia in the immature human brain. An FDA advisory committee meeting in 2007 concluded, that no change in clinical practice is justified based on available data(6) and a follow up meeting in March of this year upheld this recommendation.

It is uncertain if it will ever be feasible to test, if anesthesia kills neurons in the brain of children. However, this may not be entirely necessary. A focus on anesthesia-induced neurodegeneration seems only appropriate, if some aspect of brain function in humans were changed permanently by anesthesia, and if a causal link between neurodegeneration and long term brain function could be demonstrated in animals. Let us examine these two premises in more detail.

Anesthesia and brain function in humans

Until recently, speculation as to whether or not developmental anesthetic neurotoxicity might exist in humans, occurred mostly on the basis of studies that were not specifically designed to address this question(2-5; 7; 11). Since 2009, seven publications appeared, that were designed to shed light on whether or not anesthesia in humans might impair brain function long term(12-18). Unfortunately, for a number of reasons discussed next, the issue remains far from being resolved.

Wilder and colleagues(12) studied, if anesthesia at less than 4 years of age was associated with learning disabilities between age 5 and 19. A cohort of 5357 children born in Olmsted County, Minnesota between 1976 and 1982 was assessed for the presence, type and duration of anesthesia prior to age 4. Anesthesia administered both for surgical and diagnostic procedures was included in the analysis. The school district, in which the study was performed, routinely administered reading, writing and math aptitude tests as well as intelligence tests. In this study, learning disability was defined as a performance on standardized achievement tests below a certain predicted score,
that was based on the child’s IQ. If any of three different definitions, used by the school district to identify disabled learning, applied, the primary outcome of this study -- learning disability -- was considered to be present and study follow up ceased at this point. Eleven percent of children underwent at least one anesthetic prior to age 4, of which 24% underwent more than one anesthetic. Learning disabilities were more common in those children that underwent more than one anesthetic, and cumulative anesthetic duration of > 2 hours was a risk factor for learning disability. Learning disability was not more common if only one anesthetic exposure occurred before age 4. Since children requiring more than one anesthetic were sicker than those requiring only a single anesthetic, the authors performed a subgroup analysis of children requiring more than one anesthetic with an ASA physical status 1 and 2 and excluding those with ASA physical status 3 and 4. Despite including only less sick children with multiple anesthetic exposures, the association between learning disabilities and anesthesia persisted. Methodologic advantages of this study include, that studying a birth cohort does not bias surgical procedures and comorbidities in the same way, that recruitment of a cohort of patients from an academic center might. Further, controlling for IQ seems like an elegant approach to controlling for one of the strongest confounders of a child’s ability to learn. General methodologic drawbacks include retrospective analysis of a retrospective cohort, forcing to study an outcome variable, which is available, rather than one, which is chosen prospectively. Learning disability is a very nonspecific outcome and many underlying pathologies may impair a child’s ability to learn, for example motivation, attention, intelligence, sensory neural problems, or other, more specific functional abnormalities, all of which may have relevance to anesthetic developmental neurotoxicity. Other drawbacks include that the anesthetic almost uniformly administered to the study cohort was halothane / nitrous oxide, which is now an outdated anesthetic in most pediatric anesthetic practices. Reporting the cumulative incidence of learning disabilities requires that follow up is stopped when learning disability is detected. In other words, once a child meets the criterion for learning disabilities, it is assumed that learning disabilities persist and never resolve. This makes it impossible to comment on the true prevalence of the outcome. It is possible that children with learning disabilities at some point may have change in performance that places them back in the normal range, an event, which cannot be captured by the current study design. On the other hand, it might be possible that anesthesia-associated learning disability maybe progress, as has been suggested for anesthesia-induced neurocognitive dysfunction in animals(1; 19-21). The current study design would not be able to detect progression of cognitive disability. Likewise, this methodologic would not capture spuriousness of the outcome. For example, a low aptitude score in any one of the tested domains, for whatever reason, would trigger the diagnosis of learning disability, as would a spuriously high IQ score, resulting in a predicted aptitude score, that might render an otherwise normal aptitude to be classified as meeting the learning disability criterion.

The same group(16) reported, later that year, that general anesthesia for cesarian delivery does not increase the cumulative incidence of learning disabilities in the same(12) birth cohort of children. This is consistent with their earlier study(12), since cesarian delivery required one single, rather short, anesthetic. Surprisingly, children born by cesarian delivery under regional anesthesia had a lower cumulative incidence
of learning disability than those born by vaginal delivery(16). The significance of this finding is unclear and requires further study. However, this study suggests that a brief general anesthetic during late fetal life is not associated with later cognitive problems. The retrospective nature of this study confers the same limitations to interpretation of the data that apply to their previous study(12).

Kalkman and coworkers(13) approached the problem from a different and interesting angle. They argued, that anesthesia is mostly administered to tolerate a surgical procedure. Therefore, in order to draw conclusions about the effects of anesthesia versus surgery on cognitive outcome, an unanesthetized control group, undergoing surgery, would be required or anesthesia would have to be administered to children who don’t need it, neither one of which is ethically feasible. The authors further assumed, that there is a distinct period of vulnerability to the effects of anesthesia on neurodevelopment, as suggested by animal studies using histologic outcomes(8; 20; 22-24). Based on this assumption, the authors hypothesized that children anesthetized during the period of vulnerability (earlier in life) should have a worse cognitive outcome than children anesthetized after the period of vulnerability. They defined the period of vulnerability in humans as less than 2 years of age(13). This design circumvents the issue of requiring an unobtainable control group and allows children anesthetized later in life to serve as controls. The authors used scores from the Child Behavioral Checklist to identify behavioral abnormalities and found that children anesthetized at < 2 years of age tended to have a higher incidence of clinically deviant behavior than children anesthetized at > 2 years, undergoing the same (urological) procedures. The difference was more pronounced yet between children undergoing anesthesia at < 6 months of age compared to > 2 years. However, neither effect was pronounced enough to reach statistical significance. A sample size calculation revealed that more than 6000 children would have to be studied in order to show the difference, given the effect size comparing children < 2 years with those of > 2 years at the time of anesthesia and more than 2200 patients if the sample size calculation were based on the effect size comparing children < 6 months and > 2 years of age at the time of anesthesia(13).

Although the authors chose an innovative and logical approach to a difficult ethical dilemma, the validity of the observation is based on the assumption that the period of vulnerability in humans is limited to 2 years of age. This assumption may or may not be correct. It has been perpetuated, over the years, that the period of vulnerability coincides with the peak of synaptogenesis, which is also known as the brain growth spurt. The publication frequently cited in support of this is a scholarly article by Dobbing and Sands(25), that is silent to synaptogenesis. Instead, it synthesizes knowledge from various brain weight studies and proposes a hypothesis of how to relate vulnerability to environmental and nutritional challenges, amongst different animal species. Appropriately, notes of caution regarding the limitations for interpreting their hypothesis abound, for example, that the term brain growth spurt is an oversimplification, because different areas of the same brain develop at a different paces(25). Indeed, the peak of synaptogenesis in many structures of the rodent brain, including the cortex and the dentate gyrus of the hippocampus, does not occur until postnatal day 11-16 and synaptogenesis seems to persist until at least postnatal day 32(24; 26-30). Even within a given cortical neuron, synaptogenesis is not a uniform phenomenon(28). The period of vulnerability to anesthesia-induced neuronal apoptosis occurs prior to postnatal day...
10 - 14(8; 22; 23) and is thus not well aligned with the peak of synaptogenesis. Most importantly, the period of vulnerability to the long-term behavioral effects of anesthetic agents extends to at least postnatal day 14 - 17 in the rat(20). Rats reach sexual maturity at postnatal day 50(31). It must be concluded that the period of vulnerability to the outcome of interest -- the long term cognitive effects of anesthesia -- may extend way past 2 years of age in humans. Consequently, Kalkman’s estimate of the anesthetic effect on behavior might, if anything, be an underestimate.

The advantages and disadvantages of various study designs had been discussed in an editorial by the third contributors to the current human literature on long term cognitive effects of anesthesia(11). According to these authors, the power of studying a prospective cohort must be balanced against the lead time for data to become available. For example, if enrollment of a randomized, controlled trial of regional versus general anesthesia for pediatric surgical procedures were completed today, data of remote neurobehavioral outcomes would not be available for years, maybe decades. Given the urgency, with which data on developmental neurobehavioral endpoints after anesthesia in humans are sought, a long lag time is, arguably, unacceptable. Thus, the authors(11) concluded, that an ideal combination of lag time and design strength would be prospective analysis of a retrospective cohort. The same group(14). later studied if hernia repair at age 3 or less is associated with subsequent behavioral and/or developmental disorders. A set of 383 medicare records listing procedure codes related to hernia repair was compared to a control set of 5050 age-and sex-matched medicare records not listing these procedure codes. Children younger than age 3 were included. The behavioral outcome was defined as a diagnostic code for unspecified delay or behavioral disorder, mental retardation, autism and language and speech disorder. If the behavioral outcome preceded the surgery, the record was excluded. After controlling for age, sex, race and the presence of confounding diagnoses at birth, procedure codes indicating hernia repair were more than twice as likely to be associated with the behavioral outcome codes as when procedure codes for hernia repair were absent. The study design did not allow for assessing the type, frequency and duration of the anesthetic in either the hernia repair or the control group. It was not possible to exclude, that children in the control cohort did not have an anesthetic for surgeries other than hernia repairs. Perhaps the most interesting finding of this study is the delay, with which the behavioral outcome presented, in this case 3-4 years after the surgery. This is reminiscent of animal studies, suggesting a progressive nature of the deficit(1; 19-21).

Recently, the academic performance of a national cohort of Danish 15-16 year old children (n=2689) who had undergone inguinal hernia repair between 1986 and 1990 at the age of 1 year or less was compared to a random sample of 14,575 age-matched controls(17). When important confounders like gender, birth weight and parental age and education were controlled for, there was no evidence that the relatively brief (presumed by the authors to be 30-60 min) general anesthetic had affected academic achievement scores. All of the above confounders more strongly affected academic achievement than surgery plus anesthesia(17). This is despite the fact that children were less than 12 months of age at the time of surgery, and thus may be considered more sensitive to the effects of anesthesia than older children(13). The authors appropriately conclude that these reassuring results cannot exclude deficits in more
particular cognitive domains. It is understood that the effects of longer anesthetic durations are likewise not detectable with this study design.

Another human trial was designed to test if a causality exists between anesthesia at less than 3 years of age and between 3 and 12 years of age and cognitive performance in children. The authors studied 1143 pairs of monozygotic twins, hypothesizing that if anesthesia, and not the underlying disease, caused cognitive disabilities, then the exposed twin of a pair should have a higher incidence of underachievement than the unexposed twin of a pair. Most pairs of twins in this study consisted of twins that were either both exposed or both not exposed to anesthesia. However 71 twin pairs (15%) were discordant (one twin exposed, the other not exposed to anesthesia). Anesthesia was administered mostly for surgical procedures. Exposed twins had similar achievement scores on a nationwide test at 12 years of age as unexposed twins, and a similar incidence of cognitive problems, as assessed by a teacher questionnaire. The authors conclude that the comorbidity but not the combination of anesthesia and surgery is the cause of the cognitive problems. If these results can be duplicated, they would make a convincing argument that neither anesthesia nor surgery are a problem for the cognitive development of children.

DiMaggio and coworkers(18) subsequently came close to doing just that by identifying 10,450 twins of unknown zygocity (i.e.”siblings”), 306 of which had been exposed to anesthesia during a surgical procedure prior to age 3 and 10,146 of which had not. Of the 138 discordant pairs, in which only one of the two twins was exposed to anesthesia, in 107 pairs neither sibling had ICD-9 diagnostic codes that would suggest a problem with brain development and in 11 pairs both siblings had such ICD-9 codes subsequent to the procedure of the exposed twin.

When only one twin of a pair discordant for anesthetic exposure had an ICD-9 code suggesting a problem with brain function (n=20), there was an even split of these codes between exposed (n=9) and unexposed twins (n=11). This supports the findings by Bartels(15), that there is no causal relationship between anesthetic exposure and brain dysfunction as measured by occurrence of ICD-9 codes subsequent to the surgical procedure. A similar conclusion can be drawn from another finding from that same study(18), namely that the hazard ratio of behavioral/developmental diagnosis was 1.6 when anesthesia occurred prior to the first occurrence of the ICD-9 code but 1.3, when the ICD-9 code appeared prior to the anesthetic exposure. In this latter case, anesthesia could not possibly have caused the behavioral/developmental diagnosis.

The fact there is nonetheless an association between anesthetic exposure and behavioral/developmental diagnosis in this case highlights the existence of confounders, which is unavoidable given the study design. The authors also found an increasing likelihood of an ICD-9 code of a behavioral/developmental diagnosis with multiple anesthetics. Whether this represents an anesthetic dose response or a greater burden of disease is unclear.

In summary, the human literature is controversial as to whether or not anesthesia in infancy causes cognitive problems later in life. Furthermore, it is unclear what the period of vulnerability to anesthetic neurotoxicity is. We do not know if there is a safe anesthetic technique or duration. The specific cognitive deficit caused by anesthesia, if any, that may underlie such outcomes as learning disabilities, has not been identified.

None of the studies, alone or in combination, form a basis for informing clinical practice.
Animal studies
As discussed above, it is not entirely clear, if long-term cognitive dysfunction, the most worrisome feature of developmental anesthetic neurotoxicity, occurs in humans. In the meantime, animal models of anesthesia are important in furthering our understanding of the phenomenology, pharmacology and the mechanism of anesthesia-induced neurocognitive dysfunction. To that end a recent study(21) in monkeys demonstrating a persistent and progressive decline in cognitive domains following ketamine anesthesia is the latest in a series of alarming studies suggesting that anesthesia given to immature mammals impairs brain function later in life.
Understanding the mechanism, by which anesthesia impairs brain function months after anesthesia in infancy would allow to develop rational preventive strategies, and is thus a very important, yet currently elusive, milestone in this field. Implicit in the concept of a mechanism is the concept of causality. Although causality might be impossible to prove, it is usually accepted on the basis of (good) enough evidence for and insufficient or bad evidence against such a link(32). If anesthesia caused cognitive dysfunction, the mechanism by which anesthesia caused cognitive dysfunction would be causally linked to both anesthesia and cognitive dysfunction. The following discussion suggests that the mechanism of anesthesia-induced cognitive dysfunction or decline, as the case may be(1; 19-21; 33), is a lot less clear than previously thought. Specifically we will discuss evidence for each of three cellular phenomena to qualify as a mediating mechanism of anesthesia-induced cognitive decline -- neurodegeneration, synaptogenesis and hippocampal neurogenesis.

Neurodegeneration
It is now accepted, on the basis of overwhelming experimental evidence (for review see Loepke and Soriano(7)), that anesthesia causes neurodegeneration in a variety of animal species, including primates(8; 9). Few would dispute that the possibility of anesthesia causing neurodegeneration in humans is real although it will be very difficult to prove this definitively. Furthermore, whether or not anesthesia-induced neurodegeneration happens in humans is not nearly as important as whether or not anesthesia impairs cognition in humans. What is important, however, is to define the role of anesthesia-induced neurodegeneration in causing anesthesia-induced cognitive decline. Unless anesthesia-induced neurodegeneration mediated the anesthesia-induced cognitive outcome, it would merely be an epiphenomenon with little significance to cognitive function. When anesthesia was first shown to cause both neurodegeneration and cognitive decline in rats(1), a causal link between the two outcomes must have appeared so plausible, that it was not as rigorously scrutinized as other, less intuitive potential mechanisms may have been. In order to address this question in more detail, we must consider the evidence for and against such a causal link.

It would be difficult to conceive how an isolated event, like acute anesthesia-induced neurodegeneration, can have far reaching consequences for brain function without persisting until the time, at which brain function is assessed or without causing sequelae, that persist until the time, at which brain function is tested. In other words, if months after anesthesia the brain of a formerly anesthetized person or animal were
indistinguishable from a brain that was not exposed to anesthesia it would be hard to argue that anesthesia caused the brain to be dysfunctional. Applied to neurodegeneration this means that several months after anesthesia a causality between anesthesia-induced neurodegeneration and anesthesia-induced cognitive dysfunction would be hard to accept unless neurodegeneration had altered the brain of anesthetized animals somehow. If neurons wiped out by anesthesia left a detectable gap in the brain or if the neuronal number were different from unanesthetized animals, a reasonable argument could be made that neurodegeneration qualifies as a potential mediating mechanism for the cognitive outcome. Rizzi and coworkers (34) used pregnant guinea pigs to show that a triple anesthetic cocktail consisting of 0.55% isoflurane, 75% nitrous oxide and 1mg/kg midazolam for four hours, but not fentanyl 15mg/kg/h, causes acute neurodegeneration, in the offspring. They also found that the neuronal density in the first postnatal week is reduced by 30-50% in the offspring that had been exposed to anesthesia (34) The authors concluded that the observed degree of anesthesia-induced neuronal deletion far exceeds the roughly 1% neuronal deletion observed in their prior studies and therefore suggests that neurons are permanently lost (34). It could be argued however, that the observed anesthesia-induced neuronal deletion is well in line with the normal rate of developmental apoptosis, which is usually at least 50% (for review see (35; 36)) That being said, neurons dying during development are immature (37-39) but the postmitotic age of neurons killed by anesthesia is not yet known. Nonetheless, the authors (34) observed a significant difference between neuronal deletion after the triple anesthetic versus the fentanyl infusion or no anesthetic. The absence of a behavioral assessment with concomitant assessment of neuronal density does not allow to conclusively determine if the neuronal density that was abnormal in the first postnatal week (34) would also have been abnormal at the time of neurocognitive testing. This study is interesting also in that guinea pigs, like humans and unlike rats, have a relatively mature brain at birth. Anesthetizing the pregnant mother with agents that cross the placenta is elegant in that it allows for hemodynamic monitoring or even hemodynamic control of the mother and thus indirectly of the fetus, which is very difficult in neonatal or infantile rodents. Further, temperature and nutritional status can be more easily controlled than in newborn rodents. However, if anesthesia in utero is administered too close to delivery, maternal oxytocin might change neuronal vulnerability to anesthesia, by temporarily shifting the chloride reversal and causing immature neurons to be inhibited by GABA, which is usually characteristic of mature neurons (40). This peripartum model of anesthesia is reminiscent of a human trial discussed above (16), in which no adverse cognitive sequelae could be demonstrated by general anesthesia for delivery after adjusting for important confounders. The discrepant results do not allow to conclude that neurodegeneration is not important for cognitive outcome, partly because the anesthetic durations differed dramatically between the two studies. It is not known if a triple anesthetic cocktail administered for the duration required to perform a cesarian delivery (presumably an hour or less), causes neurodegeneration or permanent neuronal deletion in an animal model. The most compelling evidence that acute neurodegeneration causes lasting neuronal deletion comes from two rat studies by the same group (41; 42). Rats that were anesthetized on postnatal day 7, had neuronal deletion at postnatal day 30 (41) and ultrastructural abnormalities suggestive of ongoing cell death on day 21 (42). This is
getting close to the age, at which the same group previously demonstrated learning and memory deficits in rats(1). These results would be strengthened if it could be demonstrated that the total number of neurons were decreased long term. This requires assessment of the volume of the structure of interest, which did not occur in the above studies. The results would be stronger yet if animals with a proven learning and memory deficit suffered neuronal deletion, and strongest, if those animals with the worst brain function were those with the greatest degree of neuronal deletion. Another group(43) did not find a long-term effect of isoflurane during infancy on neuronal density in two brain regions most severely affected by acute cell death in mice. Since the volume of these structures was not assessed, it is impossible to know what the total number of neurons in these structures was. Interestingly learning and memory was not affected in this study, which would either suggest that isoflurane does not affect long term neurocognitive function or that the degree of acute cell death does not determine long-term neurocognitive outcome(43). The latter possibility was suggested by another study(44), demonstrating that 2h of isoflurane but not 1h of isoflurane at 1 MAC causes neurodegeneration. However, despite extensive neurodegeneration, mainly in the thalamus and cortex, no long term neurocognitive sequelae were demonstrated by 2h isoflurane. From a functional standpoint 2h isoflurane appears to be a safe dose in rats. This conclusion is supported by the finding that in the same study(44) 4h of isoflurane caused long term learning and memory problems. Hence the absence of neurocognitive deficits after 2h isoflurane was not due to a general inability to demonstrate neurocognitive dysfunction. MAC 1 of isoflurane given to rats at the peak of vulnerability to developmental anesthetic neurotoxicity (postnatal day 7), causes respiratory depression and hypercarbia. Hypercarbia alone for 4h caused a similar degree and distribution of cell death in the brain as 4h isoflurane but instead of impairing brain function long term, rats exposed to 4h hypercarbia at P7 outperformed all other groups, including the control group. Hypercarbia alone caused robust improvement in long term neurocognitive function despite causing extensive cell death in the developing brain. Collectively these findings suggest that the degree, to which an intervention causes acute neurodegeneration does not always determine long-term cognitive outcome. An important prediction required by the concept that anesthetic neurodegeneration is responsible for later cognitive dysfunction is that interventions preventing anesthesia-induced neurodegeneration also prevent anesthesia-induced long-term neurocognitive sequelae. Examples of such interventions include melatonin(45), lithium(46), dexmedetomidine(47), inhibitors of the p75 neurotrophin receptor (TAT-conjugated Pep5 or fc-p75NTR)(48; 49), hypothermia(50) and bumetanide(51), all of which have been shown to prevent anesthesia-induced neurodegeneration. The rationale for using melatonin to counteract the effect of anesthesia is the demonstration that anesthesia causes neuronal apoptosis via a mitochondria-dependent pathway, amongst others, which is associated with biochemical changes that melatonin had previously shown to counteract(45). Furthermore, melatonin has several other, nonspecific, protective effects in the brain(45). Melatonin was found, in a dose-dependent manner, to reduce anesthesia-induced neuronal apoptosis in rats(45). The authors suggested, that its bioavailability, lipophilicity, ability to cross the blood brain barrier, absence of toxicity, sleep-inducing and analgesic effects make it an ideal
adjuvant for anesthesia(45). Surprisingly, it is not known if melatonin reverses the long-term behavioral effects of anesthesia. Another group(46) showed that lithium protects against anesthetic neurotoxicity in the developing brain. They argued that lithium is known to counteract extracellular signal-regulated protein kinase inhibition and neurodegeneration caused by alcohol. Alcohol acts via antagonism of NMDA receptors and by facilitating GABA-ergic receptor transmission. Hence they hypothesized that a combination of an NMDA antagonist anesthetic -- ketamine -- and a GABA-ergic agent -- propofol -- should cause similar effects as alcohol on the developing brain, and these effects should be preventable by coadministration of lithium. This hypothesis was largely confirmed and the authors concluded, that lithium may be an effective adjuvant to anesthesia, so long as it can be demonstrated that the inhibition of naturally occurring apoptosis, which is also caused by lithium, has no ill effects(46). This may be an important caveat, since naturally occurring neuroapoptosis is critically important for brain development(36) and when this process is inhibited, learning and memory is impaired(52). It is not known if lithium can prevent anesthesia-induced neurocognitive decline.

Developmental anesthetic neurotoxicity has largely been attributed to the combination of GABA-ergic and NMDA antagonist actions of anesthetic agents. Dexmedetomidine is neither a GABA-ergic nor NMDA-antagonist and has therefore been hypothesized to be free of developmental anesthetic toxicity(47). Furthermore, it has a number of antiapoptotic effects, and thus Sanders and colleagues(47) hypothesized that it might protect against anesthesia-induced neuronal apoptosis. Dexmedetomidine reduced neuronal apoptosis caused by a subanesthetic dose of isoflurane for 6h in a dose-dependent manner, which was reversed by blocking the α-2 adrenoceptor, indicating that the protective effect is mediated by this receptor. Furthermore, dexmedetomidine prevented an isoflurane-induced impairment in trace fear conditioning at 40 days of age. This is the only study, to date, of an intervention that nonspecifically protected from anesthesia-induced cell death that also protected from anesthesia-induced neurocognitive dysfunction. The behavioral outcome was virtually devoid of interindividual variability, which is unusual for behavioral experiments when using rats from different litters(53). Even the rate of neuroapoptosis is usually subject to substantial litter variability(54; 55). Either way, these results require confirmation both in animal and human studies prior to considering a change in practice. An important mechanistic finding of this study(47) is that the neurodegeneration caused by isoflurane was not prevented by a GABA-A-receptor antagonist, indicating that this receptor does not mediate the neurodegeneration caused by GABA-ergic agents(47).

Olney and coworkers(50) advanced an interesting hypothesis on the basis of a two-part assumption: Anesthesia decreases neuronal activity in the developing brain with subsequent withdrawal of trophic support and neurodegeneration. They argued that another intervention known to decrease neuronal activity -- hypothermia -- should therefore cause neurodegeneration, and found the exact opposite. Hypothermia (30 degrees C) protected against isoflurane-induced and ketamine-induced neurodegeneration(50). This indicates that either neuronal inactivity does not cause neurodegeneration or that anesthesia does not cause neuronal inactivity. This latter possibility is actually a given for GABA-ergic agents, which cause neuronal excitation in immature neurons, rather than neuronal inhibition, as is true in mature neurons. The
mechanism of neuronal excitation in immature neurons is immaturity of a chloride transporter prior to the second postnatal week in rats (for review see(56)) and the first 3-12 months in humans(57). Isoflurane being a predominantly GABA-ergic anesthetic, should cause neuronal excitation in immature brains or immature parts of the brain. It may therefore be no surprise that hypothermia failed to reproduce the isoflurane-induced neurodegeneration. Sevoflurane, has been shown to cause neuronal excitation in the immature brain, which was actually postulated to be the mechanism underlying sevoflurane-induced neurodegeneration(51). It is not known if hypothermia protects against anesthesia-induced neurocognitive dysfunction.

Substantial insight into what does mediate anesthesia-induced developmental neuroapoptosis comes from two elegant studies(48; 49). In the first, Head and coworkers(48) show that inhibitors of the p75 neurotrophin receptor prevent isoflurane-induced cleaved-caspase3 expression \textit{in vitro} and loss of dendritic spines and synapses \textit{in vivo}. Brain derived neurotrophic factor (BDNF) is excreted as pro-BDNF and cleaved by proteases, such as plasmin, to BDNF, which interacts with the TrkB receptor to signal survival. If pro-BDNF remains uncleaved it interacts with the p75 neurotrophin receptor and acts as a cell death signal. Plasmin is cleaved from plasminogen by tissue plasminogen activator (tPA), which is released from the presynaptic terminal when neurons are firing. The authors(48) interpret their findings as confirmation that neuronal silencing caused a shift in the balance of BDNF signalling to preferentially occur via proBDNF as opposed to mature BDNF. The authors(48) went to great lengths to elegantly exclude alternative interpretation of their findings. However, they did not show that isoflurane actually decreases neuronal firing in immature neurons. As stated above, isoflurane is not expected to decrease neuronal firing in immature neurons. In fact the opposite is the case, in that a isoflurane, or any other GABA-ergic anesthetic, for that matter, should cause neuronal excitation(56). A possible explanation for these discrepancies comes from the observation that in a cell culture model, such as the one used by Head and coworkers(48), glucose is commonly used as an energy substrate, whereas the predominant energy substrate of the developing brain is ketone bodies(58; 59). Glucose causes a shift in the chloride reversal potential of neurons in culture, that makes them act like mature neurons(58; 59). Mature neurons are indeed silenced by isoflurane. The authors(48) also found that isoflurane decreases the number of immature dendritic spines in vitro and the number of synapses in 5-7 day old mice. This reduction in synaptic density was attenuated by the p75 neurotrophin receptor blocker TAT-Pep5(48). Importantly, these authors(48) demonstrate that their intervention is nontoxic and does not cause an unwanted suppression of naturally occurring neuronal apoptosis. This is an advantage over nonspecific modalities that ameliorate anesthesia-induced neurodegeneration, such as lithium, melatonin, dexmedetomidine or hypothermia.

In a second study, the same group(49) showed that the effect of isoflurane-induced p75 neurotrophin receptor signalling on synaptogenesis and neurodegeneration is mediated via activation of RhoA, a kinase causing actin depolymerization. This causes growth cone collapse, loss of immature dendritic spines, and, presumably, the loss of synapses observed in their previous study(48). The authors also observed expression of cleaved caspase 3, a marker for apoptotic cell death. When signaling via the p75 neurotrophin receptor was inhibited or when the cytoskeleton was stabilized, isoflurane-induced loss
of dendritic spines and expression of cleaved caspase 3 was attenuated. This suggests that anesthesia causes actin depolymerization via RhoA activation, which in turn causes loss of dendritic spines and apoptotic cell death. It is unknown if p75NTR antagonism or cytoskeletal stabilization can prevent anesthesia-induced neurocognitive dysfunction.

In another elegant study, Vutskits’ group confirmed that propofol, either as a single shot of 40mg/kg or given repeatedly over 6h at half that dose, decreases synaptic spine density in 5 day old rodents and increased spine density in 15 - 25 day old rodents(30). Amazingly, both the 6-hour duration as well as the single shot of propofol caused persistent changes into adulthood, indicating that a single, brief anesthetic to an anesthetic depth, which would not permit a surgical procedure, is sufficient to permanently alter cortical synaptic spine densities. This work confirms results of decreased synaptogenesis at around postnatal day 5(48; 49) and their own previous results(24; 60) of rapid increase in synaptogenesis after postnatal day 15 in the cortex and the hippocampus. Consistent with previous results(22), no neurodegeneration occurred in the cortex of 16-day old rats(24), confirming that the period of vulnerability to anesthetic apoptotic cell death is limited to postnatal day 10. Importantly, it was recently shown that the period of vulnerability to anesthesia-induced neurocognitive decline extends to at least postnatal day 17 in rats(20), a time at which neurons are no longer sensitive to the apoptotic effects of anesthesia(20; 22; 24). If these results(20) can be confirmed, the causal link between anesthesia-induced neurodegeneration and anesthesia-induced neurocognitive decline would be further weakened. Also it would need to be explained how anesthesia-induced decrease(30; 48; 49), or increase in synaptogenesis(24; 30; 60) could both be responsible for the same outcome -- anesthesia-induced neurocognitive decline.

One interesting feature of the age-dependent switch in anesthetic effect on synaptogenesis(24; 30; 48; 49; 60) is that it nearly parallels the age-dependent switch in the chloride reversal potential and thus the excitatory to inhibitory switch in GABA phenotype(56; 57). However, mechanistically linking the developmental switch in GABA phenotype with the switch from anesthesia induced decrease to increase in dendritic spines, although possibly accounting for the ill effects of GABA-ergic agents in the immature brain, would not readily account for cellular or behavioral phenomena caused by NMDA antagonists, for example the progressive cognitive decline in of monkeys treated with ketamine during early postnatal development(21).

It has been assumed that anesthesia-induced neuronal silencing is responsible for anesthesia-induced effects on synaptogenesis and apoptosis, which is difficult to consolidate with the switch in the GABA-ergic phenotype from excitatory to inhibitory at exactly the time, at which vulnerability to anesthesia-induced neuronal apoptosis ceases. This assumption was formally challenged in a recent study(51) demonstrating that sevoflurane causes global brain excitation in rats, which is entirely compatible with a motionless animal. These nonconvulsive seizures were associated with neuronal cell death. Bumetanide, which inhibits the immature chloride transporter responsible for the excitatory action of GABA during early development, prevented both the sevoflurane-induced seizures and sevoflurane-induced neurodegeneration(51). Interestingly, bumetanide did not prevent the functional consequences of sevoflurane, namely a reduction in hippocampal long-term potentiation, the electrophysiologic correlate to learning and memory(51). Anesthesia-induced neurodegeneration had previously been
associated with reduced hippocampal long term potentiation(1). The fact that prevention of anesthesia-induced neurodegeneration did not prevent the functional sequelae of anesthesia(51) again draws into question the assumption that one causes the other. If anesthesia-induced neurodegeneration does not cause anesthesia-induced neurocognitive decline, then what does? It is possible that the age-dependent anesthetic effects on synaptogenesis(24; 30; 48; 49; 60) can have functional relevance independent of whether or not they cause neuronal apoptosis. One prerequisite to this claim -- persistence of these effects until the time of neurocognitive testing -- has been met(30). Now it must be demonstrated that an intervention that prevents the anesthetic effects on synaptogenesis also prevents the anesthetic effect on cognitive function.

Another possible mechanism is an anesthetic effect on postnatal hippocampal neurogenesis(19; 20). Postnatal neurogenesis occurs only in two brain areas, one of which is the hippocampus(61-63). Inhibition of dentate neurogenesis is sufficient to impair learning and memory, in a manner, similar to anesthesia(64; 65). Of particular interest is the time course of the deficits. Neurogenesis is exquisitely sensitive to brain irradiation(66-71) and children who underwent brain irradiation develop progressive cognitive decline over a number of years(72). The deficit caused by anesthesia is hippocampus dependent and appears to progress(1; 19-21). Isoflurane has been shown to impair neurogenesis(19; 20), as does phenobarbital(73). These effects persist until the time of neurocognitive testing(20; 73). If an anesthetic effect on neurogenesis mediated anesthesia-induced neurocognitive decline, interventions that restore neurogenesis should rescue the behavioral phenotype. Such interventions include environmental enrichment, voluntary exercise, caloric restriction or antidepressant agents(74-80). We have shown that environmental enrichment reverses the behavioral effects of anesthesia, even when instituted with a 3 week delay after anesthetic exposure (unpublished observation). The treatment efficacy of environmental enrichment may or may not be due to its effects on neurogenesis.

Which anesthetic is the safest?

This question is slowly beginning to be addressed in comparative toxicity studies in animals. Human studies have not addressed this issue at all and given the controversy as to whether or not functional sequelae of anesthesia in infancy even exist in humans, the argument might be made that comparative studies are not quite yet indicated. In animals models, where anesthetic developmental neurotoxicity has been clearly demonstrated, these studies can be carried out relatively easily, with the caveat that anesthetic equipotency is vitally important for interpretation of results of comparative studies. If an anesthetic results in both greater anesthetic depth and greater anesthetic toxicity than another anesthetic, then interpretation of the data becomes difficult. For example, when it was determined that an anesthetic combination of three agents causes a greater degree of neurodegeneration than two or one anesthetic agent, the three agents were simple added to one another, which would have resulted in a greater anesthetic depth than the two anesthetic combination or the single anesthetic. (1).

Specifically, the single GABA-ergic volatile agent isoflurane caused mild cell death at 0.75% atm, which was aggravated by an otherwise nontoxic dose of midazolam (9g/kg), and made worse yet, by an otherwise nontoxic dose of nitrous oxide (75% atm)(1). This has been interpreted as greater toxicity when GABA-ergic and NMDA antagonist agents are combined but it is unclear if this does not also reflect an effect of anesthetic depth.
In anesthetic practice as well as in research minimum alveolar concentration (MAC) is used to express anesthetic potency and anesthetic depth(81). Unlike in adult rodents, MAC in immature rodents is not a stable anesthetic concentration but decreases steadily with increasing duration of anesthesia(82; 83). The reason for this phenomenon is unclear, as is, whether or not this occurs in humans. This steady decrease in anesthetic requirements make comparative studies of volatile anesthetic agents a challenge(84-87). An example of a good study comparing isoflurane, sevoflurane and desflurane is in press as of the time of this writing(83). In this study, desflurane caused greater neurotoxicity in the immature mouse brain than near equipotent doses of isoflurane or sevoflurane(83). Sevoflurane had been reported to have a more favorable neurotoxicity profile than isoflurane(85-87). Two recent studies dispute these findings(83; 84). However, in both of these studies less sevoflurane was used than isoflurane, despite attempts to achieve equipotency. This is a primarily a result of our incomplete understanding and agreement about how equipotency should be achieved in immature rodents. The simplest way would be to give a fraction of a published constantly decreasing MAC(82; 83; 88). Alternatively, a constant anesthetic concentration can be expressed as % MAC over time and the area under the curve of this plot can be calculated(88). If the areas under the % MAC over time curve are within a certain limit of agreement (e.g. within 10% of each other), the anesthetic agents were used at equipotency. The situation becomes more difficult when an inhaled agent is to be compared with an intravenous agent. While MAC determination for volatile agents are possible in immature rodents, the same would be much more difficult for intravenous agents, since a constant plasma and brain concentration would have to be achieved in order to do so. This would require insertion of an intravenous cannula and infusion of drug with subsequent sampling of blood and/or brain tissue, a difficult experimental preparation. Furthermore, since it is conceivable that MAC for intravenous agents also decreases over time in immature rodents, the above would have to be done at various time points. Hence, comparative studies between inhaled and intravenous agents are currently very difficult to interpret. For example, it has been shown that sevoflurane has a favorable neurotoxicity profile over propofol but it is entirely unclear what the anesthetic depth of these animals was(89).

Conclusion
Knowledge of developmental anesthetic neurotoxicity is rapidly accumulating but clarity about the mechanisms or the significance of this phenomenon for human pediatric anesthesia is not emerging. A change in clinical anesthetic practice is unwarranted, based on the currently available human literature and should probably not be based on animal studies. Most importantly a change in clinical practice requires a superior alternative to current practice, and no evidence guides us as to what this might be. More research is urgently needed to determine if anesthesia impairs brain function in humans, what the specific deficit is, how it can be prevented and / or treated. This will require both human trials, good translational animal models and mechanistic studies. The Smart Tots initiative, a joint effort of the International Anesthesia Research Society and the Food and Drug Administration, through funding such research, may go a long way towards meeting this important goal.
Bibliography


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