Anesthetics and the developing brain: Time for a change in practice?

A Pro/Con Debate

Laszlo Vutskits M.D., Ph.D.*, Peter J. Davis M.D. #, Tom G. Hansen M.D., Ph.D. §

* Senior Lecturer and Staff Anesthesiologist, Department of Anesthesiology, Pharmacology and Intensive Care, University Hospital of Geneva; Department of Fundamental Neuroscience, University of Geneva Medical School, Geneva, Switzerland

# Professor of Anesthesia and Pediatrics, University of Pittsburgh School of Medicine, Anesthesiologist-in-Chief, Children’s Hospital of Pittsburgh of UPMC, Pittsburgh, Pennsylvania, USA

§ Consultant Pediatric Anesthesiologist, Department of Anesthesia and Intensive Care, Odense University Hospital, Clinical Institute, University of Southern Denmark, Odense, Denmark

Introduction

Early clinical observations, approximately 60 years ago, raised the possibility of a causal link between anesthesia exposure and central nervous system (CNS) dysfunction in young children (1). This issue, however, only gained widespread interest following the publication of experimental data less than 15 years ago demonstrating neurotoxic effects of commonly used general anesthetics in the developing rodent brain (2-4). These pioneering laboratory observations initiated a never-ending academic and often emotional debate as to the extrapolation of these results into clinical practice (5-7). During the past 10 years, this debate has
continuously been fueled with the ever-increasing number of laboratory data showing the potential for general anesthetics-induced neuronal death even in higher order species (8). More recently, emerging clinical epidemiological data also support possible association between anesthesia / surgery exposure in children and altered neurocognitive outcome (for review see (9)). The goal of this debate review is to highlight both experimental and clinical arguments regarding the association of anesthesia-related neurotoxicity as well as to note the limitations of the published studies.

**Pros of anesthetics-related neurotoxicity**

Multiple lines of evidence, extending from bench to bedside, support the hypothesis that general anesthetics adversely affect the developing brain. First, to set the stage for the possibility of toxicity, it is now generally recognized that the immature central nervous system, similar to the majority of developing organs, is extremely sensitive to any kind of stimuli. Pharmacological interventions of even short duration could thus interfere with the highly orchestrated molecular, cellular and systemic mechanisms underlying proper assembly of neural networks. Second, to put increased vulnerability in the context of exposure to general anesthetics, there is a well-established functional role of neurotransmitter systems during brain development. Since general anesthetics are powerful modulators of neurotransmitter signaling, impairment of neural circuitry development by exposure to these drugs is an intriguing possibility. Third, and in line with the two aforementioned fundamentals, there is now extensive laboratory evidence, extending from worms to primates, supporting anesthesia neurotoxicity. Fourth, and probably the most important to clinical relevance, epidemiological studies
demonstrate association between early life anesthesia exposure and altered long-term neurocognitive outcome in humans. In the next few paragraphs, we will briefly review all these items with the goal to provide a comprehensive framework for considering the reasonable possibility of anesthetics-related neurotoxicity.

Enhanced sensitivity of the developing brain to stimuli: a role for critical periods

Developing organisms are highly receptive to environmental influences. This increased vulnerability implies that compromised fetal and postnatal development can set the stage for many chronic adult pathological conditions (10). In this context, a well-established developmental principle is that there are species- and organ-specific critical time windows when developing systems are most accessible to challenge. A classic example to reveal this concept is that exposure of female rats to a single dose of androgen in the first 5 days of postnatal life results in impaired reproductive cycling at puberty while no such effects are observed when androgen was administered after 15 days of age (11). These experiments, together with many others, demonstrate how even brief stimuli, when applied during critical periods of development, can condition long-term organ function.

The concept of critical period plasticity in the developing central nervous system has been elegantly demonstrated by the seminal work of Konrad Lorenz revealing how incubator-hatched greylag geese imprint on the first suitable stimulus they saw within a well-defined temporal window between 13-16 hours shortly after hatching (12). Since these pioneering experiments, an overwhelming amount of laboratory and clinical data accumulated and suggest evidence for periods of extreme receptivity in the majority of developing cognitive, motor and sensory systems (13). It is important
to realize that this extreme receptivity does not solely imply vulnerability but rather a highly increased modulability and thus heightened level of plasticity in developing neural circuitry. Critical periods of brain development can thus be defined as region-specific, temporally-confined time windows when brain circuits that subserve a given function are particularly receptive to acquiring certain kinds of information, or even need that instructive signal for their continued normal development (13).

In all species, critical period plasticity coincides with the highly intense phase of synaptogenesis in most cortical regions. In the human cerebral cortex, the majority of synaptic contacts are established between the third trimester of pregnancy and the first few years of postnatal life; the most intense synaptogenic phase, with several fold increase in synapse number, being situated between birth and 6 months of age (14-16). The length of the synaptogenic period is significantly shorter in experimental species. In primates, up to a 17-fold increase in the number of synaptic contacts occurs within a few months during the perinatal period (17), while a comparable extent of synaptogenesis is limited to a period situated between second and fourth postnatal weeks in rodents (18, 19). This intense synaptic growth leads to an initial overproduction in the number of synapses and then to a selective pruning of synaptic contacts extending from puberty to adolescents and beyond (16, 17).

General anesthetics are powerful modulators of neural activity

General anesthetics bind to and modulate neurotransmission through a large variety of ligand-gated ion channels (20). Of particular relevance to anesthesia exposure during the brain growth spurt, these drugs potentiate signaling through the GABA, receptor complex and / or inhibit glutamatergic neurotransmission principally through
blockade of the NMDA receptor (20). These two neurotransmitter systems are central in determining excitation / inhibition activity balance underlying experience-dependent sculpting of developing neural networks during critical periods of development (13). Through drug-induced targeting of GABAergic and glutamatergic neurotransmission, both hyperexcitation and intense inhibition of actively developing neural circuitry have been shown to disrupt critical period plasticity, leading thereby to markedly altered information processing in the brain (21-23). Indeed, impaired excitation / inhibition balance have been hypothesized to underline neurodevelopmental disorders such as autism, epilepsy or Rett syndrome (24, 25).

Experimental data demonstrating anesthetics-related neurotoxicity

In line with the aforementioned fundamentals of developmental neurobiology, extensive experimental evidence indicates that exposure to general anesthetics during the brain growth spurt interferes with physiological patterns of brain development. Original series of experiments initially revealed that exposure to drugs that either block NMDA receptors or potentiate GABA, receptor-mediated signaling induce apoptosis in the immature rodent brain in a developmental stage-dependent manner (2, 3). As a significant extension to these initial observations, exposure of 7-day-old rat pups to a mixture of nitrous oxide / isoflurane / midazolam anesthesia for 6 hours have been shown to induce not only widespread apoptosis but also permanent functional impairments of neural networks and associated persistent cognitive deficits in these animals (4). Since these seminal works, the issue of anesthetics-related neurotoxicity has been the subject of intense experimental investigations during the past ten years (8, 26). The obtained laboratory data not only confirmed initial findings but also revealed that (i) all general anesthetics, with the possible exception of xenon,
can induce apoptosis during critical periods of development; (ii) the anesthetics-induced cell death can be observed not only in rodents but also in higher order species such as primates and can correlate with durable cognitive deficits; and that (iii) in addition to induce apoptosis, these drugs are powerful developmental stage-dependent modulators of synaptogenesis and thus neural circuitry assembly during the brain growth spurt.

Association between early childhood anesthesia exposure and altered long-term neurocognitive outcome in humans

The most significant issue regarding anesthetics-induced CNS toxicity during development is to determine whether it occurs in humans. While it is clearly difficult, if not impossible, to provide compelling evidence, there are human clinical data available arguing that this might in fact be the case. As early as in the 1950’s, a prospective questionnaire study reported a 17% incidence of behavioral changes in children following anesthesia / surgery, and this incidence reached nearly 60% in children less than 3 years of age (1). Since this early report, a large number of observational studies have demonstrated behavioral abnormalities in up to 50% of children in the postoperative period, the majority of these nevertheless significantly decreased during the first postoperative months (reviewed in (8)). There are also case series and case control studies where, using prospective validated assessment tools, there is suggestive evidence for a negative impact of anesthesia /surgery on long-term neurocognitive outcome (reviewed in (8)).

In the past few years, several retrospective studies have been published on large-scale epidemiological datasets of well-characterized and followed birth cohorts. Analysis of
a population-based birth cohort from Minnesota revealed that children who received multiple anesthesia before 4 years of age had an increased risk of developing learning disabilities compared with those undergoing only one surgery or none even after correction for a multitude of potential confounding factors (27). More recent data from the same birth cohort also reveal an association between repeated anesthesia exposure before the age of 2 and the development of attention-deficit / hyperactivity disorders (ADHD) in later life (28). Investigation of the New York Medicaid database revealed that, following adjustment for potential confounding factors, children undergoing hernia repair before the age of 3 were twice as likely to be subsequently diagnosed with developmental or behavioral disorders then those in the age- and population-matched comparison group (29). Analysis of a sibling birth cohort from this same database demonstrates that there is a 60% greater risk of subsequent diagnosis of behavioral or developmental disorders in children undergoing anesthesia / surgery compared to that of a similar group of siblings without procedure (30).

**Cons of anesthetics-related neurotoxicity**

**Limitations of experimental data**

Although, the animal data on this topic are indeed impressive, overwhelming and disturbing, caution should be undertaken when translating these data into a human clinical context. First of all, if it exists, the clinical manifestations of anesthesia-induced neurotoxicity must be vague and subtle, otherwise it would be easy to demonstrate and it would have been suspected many years ago. The animal studies were never driven by any clear or well-defined associations between general anesthesia and subsequent specific neuro-cognitive deficits. Furthermore, it is
unknown when and how the supposed neurotoxicity will be revealed clinically in humans.

Apoptosis and neuronal pruning are part of normal mammalian development. Animal models of neurodegeneration suffer as well from significant difficulty determining human equipotent dosing for anesthetics. With inhaled anesthetics the difference in the ratio of pulmonary surface area to body mass, differences in circulatory flow, differences in the relative size of body compartments and differences in non-shivering thermogenesis may all alter the pharmacokinetics and pharmacodynamics of anesthetic agents (8). Additionally, MAC of inhalational anesthetics in immature rodents is not constant during an anesthestic, but decreases steadily with increasing duration of anesthesia (31), i.e. there appears to be exist a context sensitive pattern of MAC in immature rodents in that MAC depends very much on the duration of exposure.

In animal models, the method of sacrifice may also play a role in apoptosis. Numerous studies (e.g. (32, 33)) euthanized experimental animals with intraperitoneal pentobarbital 100mg/kg. Although both control and experimental animals were sacrificed with the same method, it is possible that the degree of neuroaptoptosis reflects the interaction between pentobarbital and the anesthetic as opposed to the direct effect of the anesthetic itself. Pentobarbital is known to inhibit neuroapoptosis (34), and thus these studies may underestimate the effects of anesthetic agents, depending on the time course of the apoptosis.

Extrapolating neurodevelopmental data from animals is also difficult because different animal species develop and mature at varying rates. The period of peak
synaptogenesis (P7) (brain growth spurt) stated in most animal studies to be where the vulnerability of anesthetic-induced neurotoxicity coincides may be based on the false premise that the rates of development between somatic and neural structures or between all brain regions during normal development happen uniformly. In addition, studies frequently refer to brain weight, water content, ganglioside, cholesterol and total DNA to hypothesize how these factors relate to vulnerability regarding environmental and nutritional influences among different animal species (35-37).

Knowledge about early human brain development is derived from data extrapolated from various animal models, where a uniform sequence of events has been demonstrated between different species of mammalians (38). Integrating data from core developmental events from many mammalian animals into a statistical model (corrected for differences in growth rates in the cortex and limbic system) has allowed for events in developing brain regions across different species to be estimated (39). A free available website has been constructed (www.translatingtime.net) with the aim e.g. to improve accuracy in extrapolating vulnerable periods between different animals. Unfortunately, this new bioinformatics approach has never been implemented in animal studies on anaesthetic-induced neurotoxicity. Recent studies using this approach suggest that this developmental stage of the rodent cerebral cortex corresponds to the maturational state of the human brain at the very beginning of the third trimester of pregnancy (39, 40). Thus, experiments with 7-day-old rodents would correlate with providing anesthetics to extreme premature babies.

Another major issue is that, in the vast majority of experimental studies assessing anesthetic-related toxicity, drugs were administered in the absence of surgical stimuli. Perioperative stress and painful stimuli during surgery can activate NMDA receptors
and other excitatory pathways in the immature brain, thereby creating a non-physiologic level of excitation (41, 42). In fact, anesthetics might be protective through reduction of the extreme levels of excitation, by pushing the excitation/inhibition balance towards inhibition. Ketamine has also been shown to reduce inflammatory pain-induced cell death in the newborn rat brain (43). Moreover, in a recent animal study it was shown that nociceptive stimulation and prolonged anaesthesia produced significantly more apoptosis than prolonged anesthesia alone when administered to neonatal rats during the synaptogenic period (44).

There are also important pharmacokinetic and probably pharmacodynamic differences between different animal species and humans. Indeed, compared with humans, up to 100-fold higher doses of general anesthetics are needed in rodents to provide a surgical plane of anesthesia. These dosing regimens obviously result in plasma, and probably cerebral, concentrations of these drugs that are much higher than those observed in human clinical practice. In contrast, lower dosing of anesthetics, resulting in plasma concentrations comparable to those in human practice, did not induce cell death or cognitive disabilities (45-47). Thus, it is difficult to conclude whether the observed neurotoxicity in experimental studies is linked to the higher concentration of general anesthetics in the brain of experimental animals compared with humans, or merely to the ability of these drugs to induce anesthesia.

Finally, despite the existence of near-physiological blood gas values in some experimental studies (4, 48), the level of physiological monitoring in animal studies does not correlate to the human clinical setting, and it is not known to what extent that e.g. hemodynamic, metabolic or respiratory changes might contribute to neurotoxicity. Indeed, some of these animal studies have been associated with an up
to 25-30% mortality (48).

Limitations of clinical data

Studying the effects of anaesthesia on neurocognitive outcome in children is obviously difficult, because the anesthetic is confounded by both the surgery and the patient’s co-morbid disease(s). Ideally, the necessary information needed to answer these important methodological questions should be gathered from multiple randomised controlled trials (RCT). However given the inherent design difficulties, RCTs require a kind of trial-and-error approach and they will most likely be inconclusive. The information can also be gained more rapidly and at significantly less cost via epidemiological studies. Again, ideally, these would be based on well-defined prospective data gathering efforts, but similar to an RCT, the need for a very long follow up time after surgical and anaesthetic exposure is a significant problem. An alternative approach is observational epidemiological studies. When the biases and their potential effects are recognized both in the analyses and in the interpretation of the results, observational studies can often complement RCTs (49, 50).

Since 2009, several cohort studies from various institutions around the world have been published. In 2009, Kalkman and coworkers found that children undergoing urologic surgery at age less than 2 years showed more behavioural disturbances than children in whom surgery was performed after age 2 yr (51). However, this study was significantly underpowered and thus the results were not statistically significant.
Wilder and coworkers from the Mayo Clinic in the US studied whether anaesthesia administered at children younger than 4 years was associated with learning disabilities between 5 and 19 years (27). They used a birth cohort of 5357 children born between 1976 and 1982. The children were assessed for the presence, type and duration of anaesthesia before 4 years of age. Their primary endpoint was the presence of learning disabilities defined as performance on standardised achievement tests below a certain predicted score based on the child’s IQ. Learning disabilities were more common in those children who had more than 1 anaesthetic, whereas children having only one anaesthetic did not show any signs of learning disabilities. Using the same cohort the Mayo Clinic group recently showed that repeated exposure to anesthesia before the age of 2 years was a significant independent risk factor for later learning disabilities (52) and that children who had more than one anesthetic before age 2 years are at increased risk for later development of ADHD (28).

Main limitations of these studies are that, the authors were unable to disentangle the effects of surgery from that of the anaesthetic itself, the demographics of the birth cohort is somewhat “protected” and does not reflect the diversity of the overall US population. Migration was huge in this cohort; almost 1/3 of the original cohort moved and information from these individuals could not be retrieved. Thus, selection bias may have occurred (If you have a significantly ill child and you live in close proximity to the Mayo Clinic you are probably more likely to stay than move). The age span was rather large in this cohort, which included were few neonates and infants.
The impact of exposure for anaesthetics prenatally has also been investigated by the Mayo Clinic group. For instance, they studied a cohort of 5320 children. 4823 had vaginal delivery, 197 had cesarian delivery under general anaesthesia and 304 had cesarian delivery under regional anaesthesia. Fetal exposure to general anaesthesia did not increase the risk for learning disability (53).

The primary endpoint in these studies has been criticized in a recent editorial (54). Learning disability (LD) is not a specific neuropsychological outcome measure, but a categorical determination based on inconsistencies between a child’s educational expectations (IQ) and his or her actual achievement. In the Mayo Clinic studies, 3 different methods for determining LD were used. None of these examines exclusionary factors for LD (e.g. sensory impairments, social-emotional difficulties, weak academic instructions, non-English proficiency or cultural differences). Thus, LD is subjected to local variation, and it is possible that the incidence of LD may have been overestimated in these studies.

DiMaggio and Sun from New York have published 2 cohort studies. Using the New York state Medicaid dataset they first constructed a birth cohort of 383 children who underwent inguinal hernia repair during the first 3 years of life as the exposure group (29). The unexposed cohort for comparison comprised a sample of 5050 children who were frequency-matched on age and gender with no history of hernia repair before age 3 years. Behavioural outcome was defined as the presence of a diagnostic code for unspecific delay or behavioural disorder, mental retardation, autism and language and speech disorders following anaesthesia and surgical exposure. After controlling for age, sex, race and birth mode, hernia repair was performed twice as often in
children who subsequently had a developmental delay disorder than in those children without hernia repair. The primary outcome in this study was not standardized and as such it could easily be subjected to variation due to differences in local practice patterns and misclassification from diagnostic coding.

In recent study, they constructed a retrospective cohort comprising 10,450 siblings (twins of unknown zygocity) (30). Three hundred and six of those had been exposed to anaesthesia during a surgical procedure before age 3 years. Overall, they found an increased risk for later diagnosis of developmental and behavioural disorders (ICD-9 codes) in the exposure group. However, more interestingly, of the 138 discordant pairs in which only 1 of the 2 twins was exposed to anaesthesia, neither sibling of 107 pairs had ICD-9 diagnostic codes that would suggest a problem with brain development, and both siblings of 11 pairs had such ICD-9 codes subsequent to the procedure of the exposed twin. When only 1 twin of a pair discordant for anaesthetic exposure had an ICD-9 code suggesting a problem with brain function (n=20), there was an even distribution of these codes between exposed (n=9) and unexposed twins (n=11), suggesting no causal relationship between anaesthesia exposure and brain dysfunction. These results support the findings by Bartels and coworkers (55). They studied a total of 1143 monozygotic twin pairs and found that exposure to anaesthesia before age 3 years significantly reduced educational achievements at age 12 years and reports of behavioural problems by teachers. However, similar to the previous study, there were no differences between twins when they were discordant for anaesthesia exposure.

In a nationwide unselected follow up study of the Danish birth cohorts from 1986 to 1990 comprising 2,689 children who had undergone inguinal hernia repair in infancy,
a single relatively brief anesthetic exposure in connection with hernia repair in infancy did not reduce academic performances at age 15-16 years after adjusting for known confounding factors (56).

As mentioned above in all these studies the effects of anesthesia per se cannot be disentangled from factors associated with anesthesia, such as surgery and pathology. Bias from potential unmeasured confounders is troublesome as well. Given the retrospective nature of these studies, subtle but important neurocognitive deficits may have been missed. Since the majority of the observations were made on population-based cohorts with relatively homogenous ethnic and/or socioeconomic distribution, extrapolation of the results to other populations can also be questioned. Importantly, anesthesia protocols were not consistent across studies, and today, they are clearly different from those used clinically 20 years ago. In the majority of studies the anesthetic technique used were outdated or even unknown (e.g. types of drugs, doses, routes of administration and duration of exposure) by today’s standards and did not address improvements in multiparameter monitoring (e.g. pulse oximetry, capnography, hemodynamics, end-tidal inhalational anesthetics). Most of these cohort studies include many different types of surgeries and procedures, the age of the child at anesthesia exposure extend well beyond infancy, and only a small proportion of neonates and infants are included. In fact, only one study focused on infants <1 year of age.

**Time for a change in practice**

It is clear from laboratory data that anesthetic agents are associated with neurotoxicity in the developing animal. Because of the retrospective study designs as
well as problems associated with database mining, the published studies in humans regarding neurotoxicity and anesthetic agents makes the association in humans less clear cut.

Though it is unclear how the data from animal species with different neurodevelopmental gestations can be applied as models to human development and synaptogenesis, nonetheless, the anesthesiologist must be open to the possibility of anesthetic associated neurotoxicity and therefore open to the possibility of change in the practice of anesthesia. However, before change can occur, evidence for change needs to be compelling. The evidence also needs to be insightful so that change can be directed. At present, there is no evidence to suggest (with the possible exception of xenon) that one anesthetic agent is less neurotoxic than another. At present, there is no human data that defines the window of vulnerability of anesthesia neurotoxicity. At present, there is no data that defines a safe or toxic anesthetic length of exposure or dose that causes neurotoxicity in humans. At present, there is no human phenotype that is associated with anesthetic neurotoxicity. Without a better understanding of what agents are best, what is the threshold toxic dose, the threshold length of exposure (maximum dose, area under the curve) and what is an appropriate alternative, it becomes difficult to support a change in practice.

Though the above questions still need to be clarified, the effects of anesthesia and the stress response and/or inflammatory response of surgery on the patient’s well being cannot be dismissed. It is known in experimental animals that the apoptic effects of anesthetic agents are mitigated when the animals are also exposed to pain. Thus, as changes in anesthetic practice are contemplated, so must changes in surgical practice.
Do we operate on babies when it is safe from a hemodynamic perspective or should surgery be performed when the risk of neurotoxicity has been minimized? These answers may not be simple or straight forward, but the answers will require input from surgeons, pediatricians, neonatologists and neuropsychologists.

Although we may not have all the answers, we do know a few things:

1. It is ethically unacceptable to subject infants to invasive procedures without the benefit of anesthesia and analgesia.
2. Any change in anesthesia practice must be evidence-based.
3. There are things we know we know and things we know we don’t know.
4. When we know what we don’t know, all we are left with are opinions, emotions and little evidence.

Though much has been published in this area, we are still connecting the dots but are a long way from knowing the answers.

References

4. Jevtovic-Todorovic V, Hartman RE, Izumi Y et al. Early exposure to common

5 Todd MM. Anesthetic neurotoxicity: the collision between laboratory neuroscience and clinical medicine. *Anesthesiology* 2004; **101**:272-273.

6 Anand KJ, Soriano SG. Anesthetic agents and the immature brain: are these toxic or therapeutic? *Anesthesiology* 2004; **101**:527-530.


11 BARRACLOUGH CA, GORSKI RA. Evidence that the hypothalamus is responsible for androgen-induced sterility in the female rat. *Endocrinology* 1961; **68**:68-79.


16 Petanjek Z, Judas M, Simic G et al. Extraordinary neoteny of synaptic spines in


24 Gatto CL, Broadie K. Genetic controls balancing excitatory and inhibitory synaptogenesis in neurodevelopmental disorder models. *Front Synaptic Neurosci* 2010; **2**:4.

25 Chattopadhyaya B. Molecular mechanisms underlying activity-dependent GABAergic synapse development and plasticity and its implications for

26 Vutskits L. Anesthetic-related neurotoxicity and the developing brain: shall we change practice? *Paediatr Drugs* 2012; **14**:13-21.


31 Stratmann G, Sall JW, Eger EIn et al. Increasing the duration of isoflurane anesthesia decreases the minimum alveolar anesthetic concentration in 7-day-old but not in 60-day-old rats. *Anesth Analg* 2009; **109**:801-806.

32 Shu Y, Patel SM, Pac-Soo C et al. Xenon pretreatment attenuates anesthetic-induced apoptosis in the developing brain in comparison with nitrous oxide and hypoxia. *Anesthesiology* 2010; **113**:360-368.


40 Clancy B, Darlington RB, Finlay BL. Translating developmental time across mammalian species. *Neuroscience* 2001; **105**:7-17.


