Review

General anesthesia and neurotoxicity on the developing brain

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Abstract

With the evolution of surgical techniques and technology an increasing number of infants, neonates, and fetuses are exposed to general anesthesia.

Despite the acknowledged safety of general anesthesia, a considerable amount of preclinical evidence shows that the developing brain is highly vulnerable to anesthetic drugs. Early-age anesthesia may impair the fine tuning of neurotransmitters and growth factors that orchestrate the replication, differentiation and organization of neural cells into functional networks.

In order to translate these insights from animal models to human patients, large trials and observational studies have been published or are currently ongoing.

The aim of this narrative review is to provide an update on the pathophysiologic mechanisms and published evidence of anesthesia-related neurotoxicity in pediatric patients.

Keywords

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The human brain as a complex dynamic system

Anesthesia-related neurotoxicity on the developing brain is gaining a great interest in both the clinical and academic medical community. We summarize the pathophysiologic mechanisms and the results of large clinical studies investigating the behavioural and cognitive findings associated to early-age anesthesia.

The human brain is a complex, dynamic system of neural and non-neural networks, made of billions of highly specialized cells [1]. Its development is an ongoing process spanning from prenatal life over several years of postnatal life. The ultimate goal of this process is building a highly efficient functional architecture, capable of fast information processing with the minimal expenditure of energy and "wiring" volume [2].

Any noxious agent affecting this delicate process may compromise the whole system capabilities.

The main microscopic events during the early phases of central nervous system (CNS) development are:

1. neurogenesis and gliogenesis, the differentiation of precursor multipotent cells into neurons and glia;
2. migration of neural cells to develop a stratified tissue;
3. synaptogenesis, through the branching of dendritic spines, axonal pathfinding and synaptic cell adhesion;
4. pruning, the controlled reduction of neural cells and synapses via apoptosis [3].

A fine tuning of these cellular processes is necessary for the development of small and large scale brain networks and general anesthesia may affect them at multiple target sites.

Brain development: the basics

Most of the published evidence on anesthesia-related neurotoxicity derives from animal studies on different species such as rats, guinea pigs, piglets, non-human primates. As a general caveat, the timing of damaging events in relation to brain development is critical for any generalization or translation to clinical practice. The peak of synaptogenesis is considered the most vulnerable period to anesthesia toxicity in all mammals [4].

Different species display a different distribution of brain maturation between prenatal and postnatal life, so in most animal studies, a specific age is chosen because of histologic evidence of a specific development phase, such as the peak of synaptogenesis in the 7-day-old puppy rats [4, 5].

In humans most of these events are concentrated in the brain growth spurt, lasting from the 6th month of gestation to 3 years of postnatal age [3, 6].

Neurogenesis is a process largely limited to intrauterine life up to the 29th gestational week [7]. Neural stem cells (NSC) derive from the ectodermic neural plate in the area called ventricular zone [8]. NSC are the multipotent precursors of both neurons and macroglial cells (astrocytes, oligodendrocytes, and Schwann cells). They undergo both symmetric divisions (proliferation) to increase the pool of stem cells and asymmetric divisions (differentiation) to beget more committed precursor cells [9].

Differentiated neural cells migrate from the ventricular zone under the guidance of radial glial cells, who can be both a scaffolding type of glia and a multipotent progenitor cell [8].

The cellular milieu, made by growth factors, neurotransmitters, respiratory gases may modulate neurogenesis. Simplistically, fibroblast growth factor, transforming growth factor-alpha, insulin-like growth factor-1, the monoamine neurotransmitters and the physiological intrauterine "hypoxia" (25-30 mmHg) increase the proliferation of neural precursors while gamma-aminobutyric acid and opioid peptides negatively affect it [7, 10].

Glutamatergic signaling is critical for the survival, proliferation and differentiation of neural cells precursors [11]. The embryonic extracellular fluid has a really high concentration of glutamate in comparison to most of adult nervous tissue, mainly because of glutamate release during axon elongation and immaturity of glial reuptake system [11]. The effects of glutamate on neurogenesis are complex and may depend on receptors, phase of development and permeability to calcium of sampled cells [11].

Ionotropic receptors seem to induce a proliferative effect and to trigger the switch from proliferative to asymmetric cellular division of NSC [11]. Metabotropic receptors may modulate neurogenesis in multiple ways such as regulating gene expression via MAP kinase and protein kinase A and influencing cell cycle via oscillating intracellular calcium spikes [11].

Glutamate is also a key regulator of dendritogenesis and synaptogenesis, perhaps because NMDA-associated calcium entry is necessary for dendrite branching in radial glial cells and

glutamate neurotransmission increases the rate of dendrite growth [12, 13].

Anesthesia-related neurotoxicity in the developing brain: a preclinical perspective

All inhaled and intravenous anesthetic drugs administered to the pregnant woman readily cross the placental blood barrier and may exert injurious effects on fetus' brain [14].

In utero and early postnatal exposure to potent volatile anesthetics, such as isoflurane, desflurane, sevoflurane and intravenous drugs, such as ketamine, propofol, midazolam results in a widespread neuronal apoptosis in several animal models, including rats, piglets, rhesus monkeys [4, 15-21].

General anesthesia with midazolam, N_2O and isoflurane induces the intrinsic pathway of apoptosis during the peak of synaptogenesis, as shown by a down-regulation of bcl-xL, up-regulation of cytochrome c and the activation of caspase-9 in 7-day-old rats, but not in 14-day-old rats, i.e. at the end of synaptogenesis [5].

A similar regimen of anesthesia promotes the neurotrophin-mediated apoptotic neurodegeneration in 7-day-old rats, due the disruption of brain-derived neurotrophic factor (BDNF) survival pathways [22].

The mitochondria are critical organelles for energy homeostasis and apoptosis, so they may represent a subcellular target of anesthesia-related neurotoxicity [23].

Anesthesia may also promote inappropriate mitochondrial fission in the developing brain and induce the production of reactive oxygen species (ROS), leading to lipid peroxidation, swelling, cristae disruption, dysfunctional morphogenesis and oxidative damage [24, 25].

The role of ROS in anesthesia-related neurotoxicity is confirmed by the evidence that scavengers, such as inhaled molecular hydrogen and EUK-134, significantly suppress anesthesia-related neuronal apoptosis [26, 27].

The hindrance to mitochondrial morphogenesis and migration at the subcellular sites of metabolic activity may account for synaptogenesis failure, increased oxidative stress and, ultimately, cellular death [23].

Inhaled anesthesia may also promote an excessive calcium release by endoplasmic reticulum through inositol 1,4,5-trisphosphate receptors, a known pro-apoptotic signaling pathway [28].

Eukaryotic cells isolate dysfunctional organelles or subcellular debris in a selective way through autophagocytic vesicles, whose contents are cleared by the lysosome enzymes [29].

General anesthesia with propofol and isoflurane may exert a dose-dependent modulation of autophagy through the mammalian target of rapamycin (mTOR) pathway: low-dose short exposure results in a cytoprotective enhancement of autophagy, while high-dose prolonged exposure impairs autophagy flux causing cell death [30].

In cultured rat hippocampal neurons, propofol-related apoptosis and oxidative stress were significantly attenuated by transfection-induced overexpression of PTEN-induced kinase 1 (Pink-1), a key regulator of autophagic turnover of dysfunctional mitochondria (mitophagy) [29, 31].

General anesthetics are pleiotropic drugs affecting cellular function at multiple levels, so neurotoxicity arises from a disturbance of both signaling and subcellular survival mechanisms.

Clinical evidence of anesthesia-related neurotoxicity in human patients

Translation of these non-human animal findings is of uttermost complexity (see **Tab. 1** for a summary of the main clinical studies).

The evidence of human neurotoxicity may arise from preclinical studies on human tissues or cells, or observational studies on patients undergoing general anesthesia, or randomized controlled trials comparing regional neural blockades and general anesthesia. These studies may investigate the same phenomenon, i.e. neurotoxicity, measuring different outcomes, such as biomarkers of neuronal apoptosis, neuroradiology, cognitive performance (i.e. academic success or standardized neuropsychological assessment).

A Multi-site Randomized Controlled Trial Comparing Regional and General Anesthesia for Effects on Neurodevelopmental Outcome and Apnea in Infants (GAS) is the first multicenter (28 hospitals in 7 countries) randomized trial on pediatric anesthesia-related neurotoxicity [32]. GAS is currently comparing general and neuraxial anesthesia in a population of infants up to 60 weeks (post-menstrual age), scheduled for unilateral or bilateral inguinal hernia repair (with or without circumcision), without any previous or *in utero* exposure to volatile anesthesia or benzodiazepines, without any chromosomal abnormality or neurological injury [32, 33]. The

Table 1. Summary of data provided by the main clinical studies on pediatric anesthesia-related neurotoxicity (continues on the next page).

Study	Design	Groups (sample size)	Inclusion and exclusion criteria	Outcome(s)	Exposure to GA, mean ± SD or median (IQR), in minutes	Results	Surgery	Age at exposure, mean ± SD or median (IQR)	Main limitations
Davidson et al. (2016) [32]	Multicenter RCT	• Awake-RA (ITT 361); • sevoflurane-based GA (ITT 359).	Inclusion criteria: • any infant scheduled for unilateral or bilateral inguinal hernia repair (with or without circumcision); • any infant whose gestational age is ≥ 26 weeks or more; • any infant whose postmenstrual age is up to 60 weeks. Exclusion criteria: • any contraindication to general or spinal/caudal anesthesia; • pre-operative ventilation immediately prior to surgery; • congenital heart disease that required therapy; • known chromosomal abnormality or any other known acquired or congenital abnormalities which are likely to affect development; • follow-up difficult for geographic or social reasons; • English is not the primary language spoken at home; • known neurological injury such as cystic periventricular leukomalacia, or grade 3 or 4 intraventricular hemorrhage; • previous exposure to volatile anesthesia or benzodiazepines as a neonate or in the third trimester *in utero*.	Bayley Scales of Infant and Toddler Development III at age 2 years	54.0 (41.0-70.0)	No difference	Inguinal herniorrhaphy	GA 0.19 (0.089), RA 0.19 (0.086)	• Interim analysis of an ongoing study; • per-protocol analysis and significant loss to follow-up; • 74/361 ITT RA group received GA (major protocol violation); • assessment at age 2 may miss impairment in higher functions.
DiMaggio et al. (2011) [35]	Retrospective cohort	• GA before age 3 (304); • unexposed controls (10,450).	Inclusion criteria: • sibling births in New York State Medicaid population. Exclusion criteria: • selected neurosurgical, cardiac, palatal, and diaphragmatic procedures.	ICD-9 diagnosis code for: • autism, • unsocial and social conduct disorders; • developmental delay; • reading and language disorders; • attention deficit and hyperkinetic disorders; • other emotional or conduct disorders.	NR	• Increased risk for multiple procedures; • matched siblings' analysis inconclusive.	Miscellaneous (isolated circumcision, and selected neurosurgical, cardiac, palatal, and diaphragmatic procedures excluded)	< 3 years	• Analysis of administrative data (Medicaid database); • Medicaid uneven coverage of U.S. general population; • small sample size for matched analysis; • outcome is relevant but not sensitive for subtle cognitive impairment.
Glatz et al. (2017) [38]	Retrospective cohort study	• Single GA (33,514); • multiple GA (3,640); • unexposed controls (159,619).	Inclusion criteria: • children born between 1973 and 1993 who had any hospital admission for surgery at age < 4 years. Exclusion criteria: • ineligible condition occurring up to age 16 years, neurosurgery, cardiovascular surgery, eye surgery, significant plastic surgery, significant peripheral vascular surgery procedures.	• Mean school grades at age 16 years; • IQ test scores at military conscription at age 18 years.	NR (the authors estimated that 55% of procedures did not exceed 60 min)	Minimally lower scores in exposed.	Miscellaneous (neurosurgery, cardiovascular surgery, eye surgery, significant plastic surgery, significant peripheral vascular surgery procedures excluded)	< 4 years	• Outcome not sensitive for subtle impairment; • no data on duration of GA.

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Hu et al. (2017) [36]	Retrospective propensity-matched cohort	• Single exposure to GA (457); • multiple exposure to GA (116); • unexposed controls (463).	Inclusion criteria: • children born from January 1, 1994 to December 31, 2007, in Olmsted County, Minnesota. Exclusion criteria: • children who moved from Olmsted County before their 3rd birthday; died before their 5th birthday; and were not enrolled in the local school district at age 5 years.	• Learning disabilities; attention-deficit/hyperactivity disorder; • group-administered ability; • achievement tests in medical and school records.	• Single GA: 52 (26-90); • multiple GA: 125 (87-234).	• Increased risk for multiple procedures; • single procedure associated with reduced scholastic performance.	Miscellaneous	< 3 years	• Higher ASA status in multiply exposed (but not different incidence of outcome between ASA I-II and ASA III-IV); • missing data in 21% of cohort members; • 70 children exposed to GA were excluded from the study cohort because an appropriate propensity-matched control could not be identified.
Sun et al. (2016) [34]	Sibling-matched cohort study	• GA (105); • matched controls (siblings, 105).	Inclusion criteria GA group: • single GA before age 36 months for elective inguinal hernia surgery during 2000-2010; • ASA I-II; • 36 weeks' gestational age or older at birth. Inclusion criteria control group: • biologically related siblings (half or full) closest in age (within 3 years) to the exposed child with no anesthesia exposure before age 36 months and 36 weeks' gestational age or older at birth.	• Wechsler Abbreviated Scale of Intelligence; • domain-specific neurocognitive functions and behavior (assessed at age 8-15 years).	84 ± 33	No difference	Inguinal herniorrhaphy	1.44 (0.91)	• Gender unbalance in sibling matching; • GA exposure after 36 months occurred in 18 exposed and 23 unexposed siblings in control group; • selection bias of families.
Wilder et al. (2009) [37]	Retrospective cohort study	• GA (593); • unexposed controls (4,764).	Inclusion criteria: • birth cohort of children born in Rochester, Minnesota, between January 1, 1976, and December 31, 1982, to mothers residing at the time of delivery in the five Olmsted County, Minnesota, townships. Exclusion criteria: • children who left Olmsted County before age 5 years.	Learning disabilities in medical and school records	75 (45-120)	GA was risk factor for the development of learning disabilities in multiply exposed children for a cumulative duration of 120 min or greater	Miscellaneous	< 4 years	• Possible selection bias due to migration of 8,548 birth cohort members; • halothane as most common GA; • "old" anesthetic management.

ASA: American Society of Anesthesiologists physical status classification; GA: general anesthesia; ICD: International Classification of Diseases; ITT: intention to treat; IQR: interquartile range; min: minutes; RA: regional anesthesia; RCT: randomized controlled trial; SD: standard deviation; U.S.: United States.

primary outcome is the *Wechsler Preschool and Primary Scale of Intelligence Third Edition Full Scale Intelligence Quotient* score at age 5 years and its data collection will be accomplished in 2018 [33]. The secondary outcome data, i.e. the composite cognitive score of the *Bayley Scales of Infant and Toddler Development III*, assessed at 2 years were recently published [32].

The authors collected the cognitive composite score for 238/363 children in the awake-regional

anesthesia group and 294/359 in the general anesthesia group. The statistical analysis of mean scores resulted in similar performance (mean [SD], 98.6 [14.2] vs 98.2 [14.7]) [32].

The strength of this study lies on its solid block randomized design stratified by site and gestational age at birth, the outcomes assessor's blinding, and the quite even distribution of potential confounding factors such as antenatal exposure to nitrous oxide and perinatal adverse events. The main factors affecting the quality of evidence are related to the age of assessment, which may miss subtle higher function impairment, such as reading and math skills, and the intrinsic methodological limitations of a per protocol interim analysis of secondary outcomes [32].

The Pediatric Anesthesia NeuroDevelopment Assessment (PANDA) investigated the hypothesis that a single exposure to general anesthesia for hernia repair in otherwise healthy children younger than 3 years during the period 2000-2010 was associated with a reduced Wechsler Abbreviated Scale of Intelligence (IQ) score or domain-specific performance later, at age 8-15 years [34]. The authors screened the electronic registries for eligible participants and retrieved all the clinical records related to the surgery and anesthesia [34]. In order to reduce the bias related to genetics and nurture, it was designed as a sibling-matched cohort study, which analyzed the data of 105 pair of siblings from the 130 enrolled pairs [34]. Enrolled participants with their matched control were assessed with neurocognitive tests, whose results were quite similar with differences between the anesthesia and control groups in mean IQ scores for full-scale IQ, 0.2 (95% CI, -2.6 to 2.9), performance IQ, 0.5 (95% CI, -2.7 to 3.7), and verbal IQ, -0.5 (95% CI, -3.2 to 2.2) [34]. The findings of this study are relevant because they investigate a typical infant anesthesia procedure (herniorrhaphy), which is really representative of the large majority of pediatric surgery and is not frequently associated with a significant burden of residual disability, but it is also a brief procedure [34]. Another point of strength is the use of a standardized neuropsychological assessment, useful for reproducibility and comparability with the published literature [34]. The limitations of this study are related to the unequal gender matching between the groups (males were 90% vs 59% in anesthesia and control group, respectively), exposure to anesthesia in 23 subjects of control group after age 3, selection bias of middle-to-high income families, and the really brief exposure to general anesthesia (mostly under 1 hour) [34].

Recent retrospective cohort studies with moderate to large sample size seem to confirm an association between multiple exposure to anesthesia during the vulnerable period up to 4 years of age and learning disabilities, attention-deficit/hyperactivity disorder and reduced academic achievements [35, 36]. The strength of the association is clearly exposure-dependent and is concordant to previous retrospective studies, whose findings were criticized because of the significant differences with modern anesthesiological management, such as the use of isoflurane and the non-standardized instrumental monitoring [37].

The largest retrospective cohort study in the published literature compared academic success of 33,514 children exposed to a single general anesthesia and 3,640 multiply exposed before age 4 with 159,619 matched unexposed control children [38]. Their findings revealed a reduction of 0.41% (95% CI, 0.12%-0.70%) of school grades at age 16, a negligible association in comparison to other factors such as gender, maternal education, month of birth [38].

The interpretation of the statistical associations provided by the published literature still carries the fundamental issue of causality attribution. The evidence about a causative link between early-age anesthesia and cognitive impairment in later life is still inconclusive because early-age anesthesia and neurodevelopmental adverse events may share the same risk factors.

According to a newly published retrospective cohort study on 20,922 subjects born in Olmsted county, as the cohorts studied by Wilder et al. and Hu et al., prematurity and low weight at birth were associated with a higher risk of multiple surgical procedures [36, 37, 39].

The United States Food and Drugs Administration (U.S. FDA) has recently added a warning label to anesthetic drugs addressing the topic of neurotoxicity in the developing brain [40]. The label's contents are really agreeable because the limitations of the available evidence are recognized, while it is offered a sensitive clinical guideline in choosing which surgery is really needed under age 3. For example, any surgery for trauma, for life-threatening conditions or acute illness has a clear benefit-risk ratio and should not be postponed [40, 41].

As techniques and instrumentation evolve, the emerging field of fetal surgery is blossoming.

Birth defects that compromise early survival or lead to end-stage organ damage may be amenable to antenatal surgical treatment (e.g. congenital diaphragmatic hernia, urinary tract obstruction, twin-twin transfusion syndrome, myelomeningocele, amniotic band syndrome, critical aortic stenosis with evolving hypoplastic left heart syndrome) [42, 43].

Minimally invasive procedures are carried out under regional anesthesia with or without maternal sedation, while open fetal surgery requires general anesthesia and fetal administration of opioid and neuromuscular blocking agents [43].

In these cases, we suppose that anesthesia-related neurotoxicity may exert the most detrimental effects on the developing brain, as shown by preclinical animal models, but we lack large observational studies or randomized controlled trial to support this hypothesis. Furthermore, the burden of residual morbidity could bias the results of neurocognitive assessment on fetal surgery survivors.

Conclusions

The clinical evidence of a long lasting cognitive impairment after general anesthesia during a vulnerable period of brain development is still weak, with many conflicting findings and the intrinsic limitations of higher functions assessment. Many confounding factors may affect the results, such as the residual burden of disability after surgery, the age of exposure, the total dose of exposure, environmental factors (family income, maternal education, nurture), genetic factors, unknown determinants of vulnerability to anesthesia-related neurotoxicity, the impact of surgical experience (e.g. pain). The majority of preclinical studies on anesthesia-related neurotoxicity focused on the neurotoxic effects of anesthesia without noxious stimulation. We also have evidence of a pain-induced neurotoxicity in neonates and infants, so analgesia and anesthesia must retain a role in management of painful procedures [44].

Furthermore, neurotoxic effects of drugs may be different according to the clinical setting, such as a surgical intervention with significant nociception or sedation for imaging without any noxious stimuli. For example, ketamine was shown to induce less neuroapoptosis in rat pups undergoing intrapalmar injection of complete Freund's adjuvant [45].

Size effect of long lasting behavioral and cognitive disparities may be clinically unimportant, but any degree of subclinical reduction of cognitive skills may hindrance personal self-realization and achievements. Furthermore, the increasing awareness of anesthesia-related neurotoxicity may distress both parents and caregivers in the diagnostic and therapeutic process. In conclusion, as already stated by regulatory agencies, the decision of surgery under general anesthesia in a vulnerable period must be cautiously weighted, because postponing emergent or urgent surgeries may increase both mortality and morbidity (disability), while for minor surgeries, such as circumcision or inguinal herniorrhaphy, regional anesthesia may be preferred.

Declaration of interest

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