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Anesthesia and neurotoxicity in the developing brain : A non-systematic review

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Abstract : In recent years, increasing experimental evidence has suggested an association between exposure to anesthesia in early life and subsequent poor neurodevelopmental outcome. Retrospective and follow-up studies have also suggested anesthesia-related neurotoxicity in the developing human brain. The present non-systematic review summarizes the available evidence, depicts the current knowledge on the potentially harmful effects of anesthesia and will discuss whether this knowledge urges us to implement changes in clinical practice.

Key words : Anesthesia ; developing brain ; neurotoxicity.

Introduction

In late fetal and early postnatal life, the mammalian central nervous system undergoes a growth spurt with massive synaptogenesis, making the brain particularly vulnerable to exogenous noxious stimuli during this period. Detrimental effects on early neurological development have already been suggested in 1991, when a link was proposed between the use of general anesthesia (diazepam, ketamine, methoxyflurane or enflurane, nitrous oxide and pethidine) for delivery and autistic disorders, developmental disturbances and mental retardation in the offspring (1). Experimental data demonstrating neuroapoptosis after exposure to anesthetics was first reported over a decade ago (2, 3). Since then, a large number of studies have demonstrated noxious effects of anesthesia in rodent and non-human primate studies. So far in humans, results are available only from retrospective and follow-up studies. Of note, the results of these human studies are inconclusive as they are subject to numerous potential confounders which are difficult to identify and virtually impossible to be accounted for. Further research is warranted to uncover the exact mechanisms of toxicity through which anesthetics induce damage to the developing brain. Prospective randomized trials will be extremely difficult to realize in this area, mainly due to ethical concerns and owing to the problem that study participants have to be followed-up for at least two decades. Meanwhile, the project 'Strategies for Mitigating Anesthesia-Related NeuroToxicity in Tots' (SmartTots) was initiated as a partnership between the US Food and Drug Administration and the International Anesthesia Research Society to support research, both fundamental and clinical, aimed at improving the safety of anesthesia in the early years of life (4). In order to identify, support and coordinate research in the preclinical and clinical field, the EUROSTAR (EUROpean Safe Tots Anaesthesia Research, EUROSTAR) platform was launched by the European Society of Anaesthesiology (5).

Neurotoxic potential of various anesthetics

Ketamine : The N-methyl-D-aspartate (NMDA) receptor antagonist ketamine is a well-known product in pediatric anesthesia. Although accidental overdose of up to 100 times the intended dose in patients between 24 days and 5 years old did not cause adverse neurological outcome in the long term (6), laboratory studies reveal a strong potential for harm of this popular anesthetic. Chronic or repeated administration of ketamine at subanesthetic and anesthetic levels induced apoptosis of neurons up to 34 times the normal range in seven day old rat pups (2). Studies on the effects of single or low dose ketamine administration yielded inconclusive results with respect to neurodegenerative effects in rodent models (7-9). One study described detrimental effects of a single exposure to ketamine, with the maximum of apoptotic damage reached 72 hours

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post anesthesia (9). Even when used in concentrations not leading to apoptosis, ketamine induced damage to γ-aminobutyric acid (GABA)-ergic neuron cultures through branching point elimination, dendritic retraction and impaired development of dendritic arbor architecture (10, 11). In both rodents and rhesus monkeys, neuronal damage caused by ketamine appears to be dependent upon the administered dose and the developmental age during exposure. Different damage patterns have been demonstrated at different ages (12-15), with the frontal cortex and hippocampus being the most sensitive areas (12). Rhesus monkey fetuses showed a 2.2 times higher rate of neurodegeneration after exposure to ketamine than neonatal animals, suggesting reduced sensitivity with aging (13). Of note, ketamine exposure in the early developmental stages was demonstrated to result in long-term effects, as it induced neurocognitive defects in adult mice (16) as well as long-term problem solving accuracy and psychomotor speed deficits in rhesus monkeys (17). It has been suggested that neurons in the mammalian brain undergo a phase during neurodevelopment in which they are vulnerable to deprivation of trophic NMDA receptor mediated glutamate stimulation, resulting in programmed cell death (2). In contrast, ketamine has been demonstrated to exert a protective effect on cell death following inflammatory pain and to ameliorate the cognitive outcome of rats when used as an analgesic for the treatment of experimentally induced repetitive neonatal pain (18). The authors suggest this might be due to the analgesic or anti-inflammatory effects exercised by ketamine (18).

Propofol (2,6 diisopropyl phenol) : Supraclinical concentrations of propofol, a $GABA_A$ receptor agonist, lead to rapid cell death of cultured GABA-ergic neurons and glial cells (19). In addition, concentrations used in clinical practice have been reported to exert dose- and time-dependent effects on neuronal differentiation *in vitro*. At culture concentrations as low as 1 μg/ml, propofol significantly alters dendritic development of immature neonatal rat GABA-ergic neuron cultures *in vitro*, with the developmental changes remaining detectable in the long term (20). In an infant mouse model, it was demonstrated that propofol doses as low as 25% of those required during a surgical plane of anesthesia, induce significant neuroapoptosis (21). The combination of low-dose propofol with low-dose ketamine proved to have a synergistic effect on the apoptotic rate in the brain of neonatal mice, which was associated with a behavioral, learning and memory assessment deterioration (22). These findings are similar to the well-known detrimental effects of an intrauterine exposure to ethanol (both an NMDA antagonist and $GABA_A$ agonist) during pregnancy, and the resulting Fetal Alcohol Syndrome (3, 22). Propofol exposure during early stages (day 5 or 10) of development in the neonatal rat brain led to a decrease in dendritic spines, while later exposure (day 15, 20 or 30) increased these (23). These findings were suggested to be related to the developmental switch of the electrochemical chloride potential rendering GABA agonism excitatory in early developmental stages, and inhibitory after maturation (24). In a rhesus monkey model, propofol induced not only neuronal apoptosis, but also an increase in oligodendrocyte apoptosis, at a stage where neurons begin to achieve myelinisation (25). These effects were however less pronounced than with exposure to isoflurane (25).

Nitrous Oxide (N_2O) : the NMDA receptor antagonist nitrous oxide has been shown to induce neurotoxicity as evidenced by *in vivo* extensive swelling of mitochondria and endoplasmic reticulum and other cell organelles in mature rats (26). Animal testing has so far revealed that N_2O amplifies the neurotoxicity of isoflurane (27).

Volatile anesthetics : In rodents and non-human primates, the administration of volatile anesthetics during early neurodevelopment increased neuronal apoptosis and caused a decrease in dendritic spine density (28-35). These detrimental alterations were associated with short and long term memory deficits when isoflurane was used, but only long term memory deficits in the case of sevoflurane use (36). In a neonatal mouse model, abnormal social behavior and deficits in fear conditioning were observed after exposure to sevoflurane on day 6 (37). It was demonstrated that female mice, anesthetized with sevoflurane 3% 6 days after birth showed deficits in maternal behavior, with a severely increased pup mortality as a consequence (38). Exposure to nociceptive stimuli (surgical incision of the left hind paw) during combined isoflurane and N_2O anesthesia even aggravated the rate of apoptosis in rat pups (39). Both intrauterine and neonatal exposure of rhesus monkeys to isoflurane induced severe apoptosis in neurons and oligodendrocytes during myelinogenesis, most pronounced in the cerebellum, caudate nucleus, putamen, amygdala and several cerebrocortical regions (40, 41). When comparing isoflurane, desflurane and sevo-

flurane, studies have yielded conflicting evidence, suggesting either no difference in toxic potency (33), or reporting desflurane to be more detrimental than isoflurane and sevoflurane to be least toxic (28, 32, 36). Of note, these effects were already present at sub Minimum Alveolar Concentration (MAC) levels (29, 32).

Xenon : The noble gas xenon is a non-competitive glutamate antagonist at the NMDA-receptor. Due to the low potency of xenon, general anesthesia with xenon can only be achieved in rodents when using hyperbaric conditions. Interestingly, when exposing developing hippocampal neurons to 1 MAC xenon in a pressurized chamber (60% xenon at 2.67 atmosphere), xenon caused neuronal death (42). In contrast, xenon at subanesthetic concentrations did significantly reduce the neurotoxic effect induced by isoflurane (27, 43). Newborn rat pups exposed to 75% xenon and 25% oxygen for two hours, had increased JNKK1 (c-Jun N-terminal kinase kinase 1) mRNA and decreased Akt mRNA levels in forebrain neurons, a transcription level indicator of apoptosis (44). Noteworthy, subanesthetic concentrations of xenon activate and enhance pro-survival mechanisms in neurons, which may result in overall neuroprotection (45, 46).

Barbiturates : Thiopental has been demonstrated to exert the same effects as other $GABA_A$ agonists, potentiating the neurotoxicity of NMDA antagonists in rodents and causing apoptosis in a dose dependent manner in ten day old mice, although less pronounced than propofol (22).

Benzodiazepines : Midazolam, a commonly used benzodiazepine, has been shown to exert toxic effects on the differentiation of neurons *in vitro*, mainly through interference with calcium currents (47). In rodent studies, midazolam (either used as sole agent in the context of long term administration or administered in combination with other anesthetics) had detrimental effects on neurodevelopment (3, 48). Likewise, three days of diazepam or clonazepam administration were shown to produce apoptotic effects in a rat model when investigating the safety of their use as anticonvulsants (49).

An overview of the properties of these anesthetics is given in Table 1.

Table 1

Anesthetics, their molecular targets and properties (adapted from DE GRAAFF *et al*., Ned. Tijdschr. Geneeskd., 2013)

Anesthetic	**GABA-agonism**	**NMDA-antagonism**	**Effects**
Ketamine (6–17)	+	+++	– Dose-dependent neuroapoptosis from chronic or repeated exposure to (sub) anesthetic doses in rodents – Oligodendrocyte apoptosis – Synergistic neurotoxicity with propofol – Neuroprotective when used as analgetic in a rat model of repetitive neonatal pain
Propofol (18–24)	+++	+	– Neurotoxic in subanesthetic doses – Synergistic neurotoxicity with ketamine – Less toxic than isoflurane, more toxic than ketamine – Causes oligodendrocyte apoptosis
N_2O (25, 26, 38)	+	+++	– Toxic effects in cell organelles – Potentiates neurotoxicity of volatile anesthetics in rodents – Isoflurane and N_2O toxicity aggravated by noxious stimuli
Volatile anesthetics (3, 27-40, 57)	+++	+	– Neurotoxicity at sub MAC levels – Toxicity : sevoflurane < isoflurane < desflurane ? – Isoflurane and N_2O toxicity aggravated by noxious stimuli – Cause oligodendrocyte apoptosis
Benzodiazepines (3, 45-47)	+++		– Toxic effects on neuronal differentiation – Prolonged administration as anti-epileptic drug induces neuroapoptosis
Barbiturates (21)	+++		– Induces dose dependent neuroapoptosis, but is less neurotoxic than propofol – Potentiates NMDA-antagonist neurotoxicity
Xenon (26, 41-44)		+++	– Neurotoxic at MAC concentrations – Neuroprotective properties at sub MAC concentrations against isoflurane neurotoxicity

GABA, gamma-amino butyric acid ; MAC, Minimal Alveolar Concentration ; NMDA, N-methyl-D-aspartate.

Neurotoxic potential of opioid analgesics

Given its important place in modern anesthesia, the role of opioid analgesics requires further investigation in terms of neurotoxic potential. The natural presence of opioid receptors on neurons suggests that these receptors may play a role in neuronal development.

An in vitro study demonstrated that 3 days of morphine exposure led to increased apoptosis of human fetal microglial cells and neurons, but not astrocytes. It was also demonstrated that neuronal apoptosis occurred earlier than microglial apoptosis, that this effect was mediated by opioid receptors, and that it was reversible by naloxone administration (50). Rodent studies demonstrated that prenatal heroin exposure induces neuroapoptosis (51). A study investigating the effects of buprenorphine and methadone treatment in prenatal and newborn rats found that Nerve Growth Factor mRNA decreased strongly in cholinergic neurons, but that this window of vulnerability ended after the fourth post-natal day (52). Newborn piglets who were given a fentanyl treatment on day 5 only displayed a minor increase in apoptosis, failing to reach statistical significance (53). Single dose morphine administration (10 mg/kg intraperitoneally) on day 7 or 15 did not produce neuroapoptosis in neonatal rats. Even repeated exposure (between day 7 and 15 or between day 15 and 20) did not induce dendritic arbor abnormalities (54). Morphine as a treatment for early life stressors such as maternal separation/isolation, injections, gavage feeding, cold exposure and brief hypoxia and hyperoxia episodes in rodents (mimicking neonatal intensive care) has been found to produce varying results, with one study suggesting more severe cognitive impairment (55) and another study providing evidence for ameliorated neurocognitive outcome (56). Chronic morphine exposure early in life has been shown to increase nociceptive behavior in rats. In adult rats, it was demonstrated that through sustained activation of opioid receptors, morphine has the potential of promoting excessive apoptosis through the upregulation of the Fas-receptor and the downregulation of the anti-apoptotic Bcl-2 protein (57). Also in humans, the evidence on potential harm of morphine analgesia in ventilated critically ill neonates is conflicting. For pre-emptive morphine-infusions, the "Neurologic Outcomes and Pre-emptive Analgesia in Neonates (NEOPAIN)" trial could not demonstrate a reduction in the frequency of severe intraventricular hemorrhage, periventricular leukomalacia, or death in ventilated preterm neonates, while intermittent boluses of open-label morphine were associated with an increased rate of the composite outcome (58). A Cochrane analysis found only insufficient evidence to recommend routine use of opioids in mechanically ventilated newborns (59). Even more important, de Graaf *et al*. found that morphine, received during the neonatal period, exerts negative effects on the child's cognitive functioning at the age of 5 years (60). Conversely, the same group found that continuous morphine infusion at a rate of 10 μg/kg/h during the neonatal period had no negative effects on general functioning and may even have a positive influence on executive functions in children aged 8 to 9 years (61).

Mechanisms leading to anesthetic injury

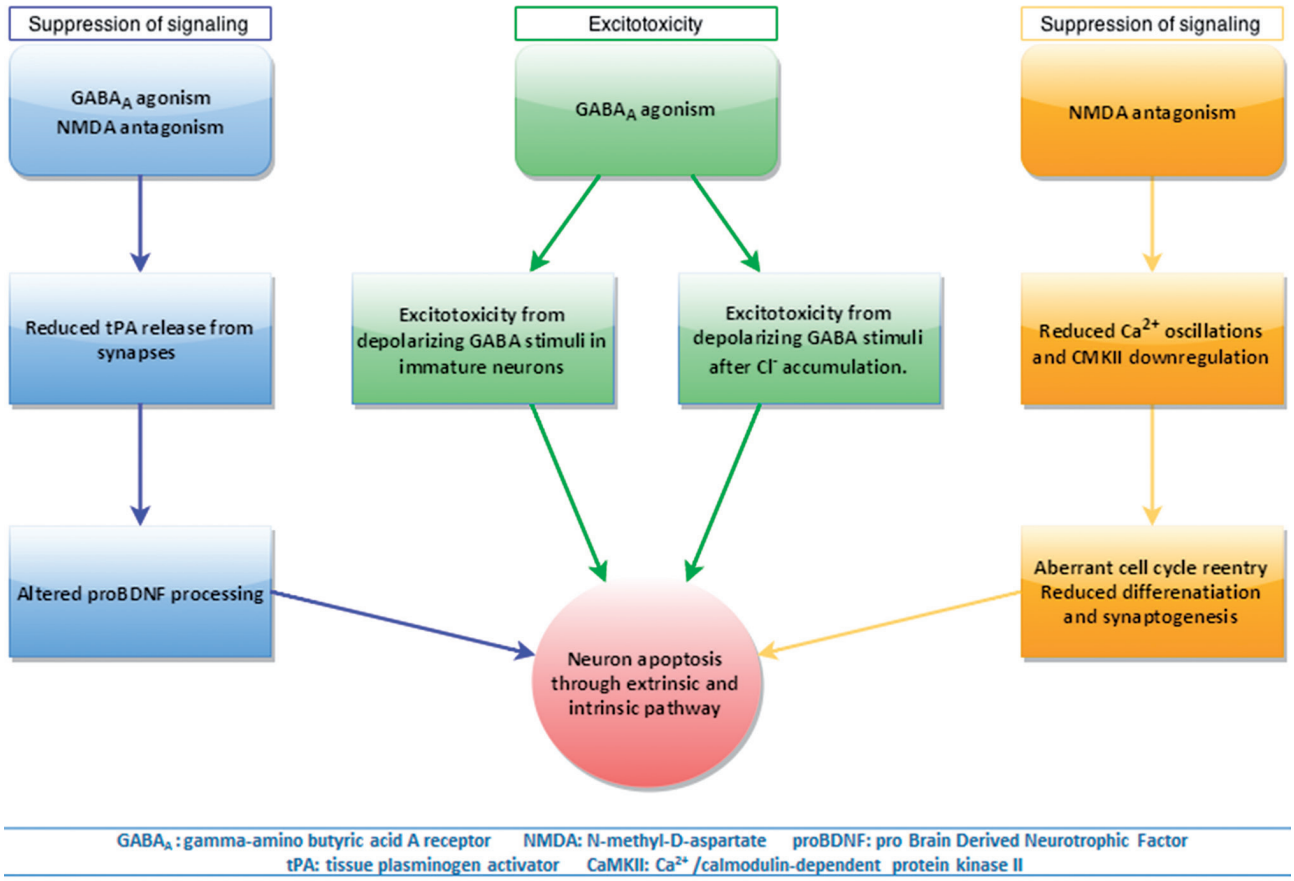
$GABA_A$ receptor potentiation and NMDA receptor antagonism seem to be a key factor in causing neuroapoptosis. These mechanisms appear to produce a synergistic effect, resulting in widespread programmed cell death, which has been demonstrated for the administration of a combination of anesthetics, or for the intrauterine exposure to ethanol, which possesses both NMDA antagonistic and $GABA_A$ agonistic effects (3, 62).

Two main mechanisms have been proposed to explain anesthetic-induced neurotoxicity, as well as some mechanisms contributing to overall toxicity (Fig. 1 and Table 2).

Suppression of synaptic activity by anesthetics

$GABA_A$ agonism and NMDA antagonism deprive the neuron from neurotrophic signaling and thereby induce neuronal apoptosis. This mechanism is mediated by decreased tissue plasminogen activator (tPA) release from synapses, altering pro Brain Derived Neurotrophic Factor (proBDNF) processing. This alteration induces the apoptosis promoting form of the neurotrophin, causing loss of dendritic philopodial spines and synapses via the p75 neurotrophic receptors ($p75^{NTR}$) (35). This process leads to programmed cell death through the intrinsic pathway, involving Bax (a pro-apoptotic protein) mobilization, cytochrome c release, caspase-9 activation and caspase-3 cleavage (35, 63, 64). The extrinsic apoptotic pathway contributes to neuronal apoptosis through upregulation of Fas, caspase-8 activation and caspase-3 cleavage (64). Of note, the early apoptotic injury appears to be mainly modulated by the intrinsic pathway. More mature neurons

Fig. 1. — Proposed mechanisms of neurotoxicity.



demonstrate a protective upregulation of the Bcl-2 protein (64).

For NMDA antagonists, a variety of neurotoxic mechanisms have been described : reduced differentiation and synaptogenesis by Ca^{2+}/calmoduline dependent kinase II (CaMKII) down-regulation, induced by suppression of Ca^{2+} oscillations (65) ; interference with proteins involved in the brain maturation process (16) ; and aberrant cell cycle reentry leading to apoptosis (14).

Excitotoxicity caused by anesthetics

The activation of $GABA_A$ receptors in the early stages of neurodevelopment acts as an excitatory rather than an inhibitory stimulus (66). Immature neurons express the cation-chloride cotransporter NKCC1 (Na^+-K^+-Cl^--cotransporter isoform 1) rather than KCC2 (K^+-Cl^--cotransporter isoform 2). NKCC1 is a transporter that enhances Cl^--accumulation within the neuron and whose activity is regulated by the Na^+ gradient. During maturation, the brain down regulates the NKCC1 co-transporter, and up regulates the KCC2 co-transporter in a caudal-rostral progression. KCC2 is known to promote Cl^--efflux which has been found to change the Cl^--reversal potential. This converts GABA from a depolarizing neurotransmitter in early neurodevelopment to a hyperpolarizing neurotransmitter later, during postnatal neuronal maturation.

It is therefore likely that early anesthetic neurotoxicity is – at least in part – caused by excitotoxicity through extensive agonism at $GABA_A$ receptors. Intracellular accumulation of Cl^- ions, resulting from potent GABAergic stimulation in neurons expressing adult levels of KCC2, causes GABAergic stimulation to induce depolarization as well (67), which could induce excitotoxicity in later developmental stages (68).

Other mechanisms

Additional factors that have been implicated in anesthetic neurotoxicity include oxidative stress (mitochondrial apoptosis induced by reactive oxygen species) (69, 70), impaired mitochondrial morphogenesis (71), inositol 1,4,5-triphosphate receptor overstimulation leading to dysregulation of intracellular Ca^{2+} levels (72), impaired cortical neuron polarization (73), disturbed axon guidance (74) and

Table 2

Mechanisms of neurotoxicity

Suppression of neurotrophic signaling leading to apoptosis via the intrinsic pathway
Apoptosis via extrinsic pathway
Aberrant cell cycle reentry leading to apoptosis
Impaired differentiation and synaptogenesis by suppression of Ca^{2+} oscillations
Excitotoxicity
Oxidative stress and mitochondrial apoptosis induced by radical oxygen species
Impaired mitochondrial morphogenesis
Impaired cortical neuron polarisation
Disturbed axon guidance
Dysregulation of intracellular Ca^{2+} levels by inositol 1,4,5-triphosphate receptor overstimulation
Neuroinflammation
Oligodendrocyte apoptosis

neuroinflammation (75). Experimental data also provide evidence of widespread oligodendrocyte apoptosis in a fetal and neonatal rhesus monkey model exposed to isoflurane or propofol (25, 40, 41), which may have a severe impact on the development of neural networks. It may be interesting to note that not all neurons are (equally) susceptible to anesthetic injury. In a rat model, exposure at the age of 7 days to N_2O and isoflurane resulted in apoptosis of glutaminergic, GABAergic and dopaminergic neurons, while cholinergic neurons appeared unharmed (76).

It remains unclear which effects nociceptive stimuli have on anesthesia-induced neuroapoptosis. Data from a neonatal rat study, in which anesthesia was provided with an isoflurane-N_2O mixture, suggest attenuated neuroapoptosis in both brain and spinal cord. Noxious stimulation was surgical incision and formalin injection, and a mechanism mediated by pro-inflammatory cytokines was proposed (39). Tissue damage, induced by tail clamping under sevoflurane anesthesia did however not influence neurocognitive performance. Interestingly, delayed environmental enrichment reversed the spatial short-term memory impairment the tested rats suffered from 8 weeks post exposure to isoflurane anesthesia (77). Ketamine-induced neuroapoptosis has been shown to be attenuated by simultaneous noxious stimulation through plantar injection of complete Freund's adjuvant (78). The differing outcomes of these studies are probably caused by the varying nature of anesthetics and noxious stimulations used.

Protective strategies

Several strategies have been proposed to counteract/ameliorate anesthesia-induced neurotoxicity. $Alpha_2$- adrenergic agonists including clonidine and dexmedetomidine were found to improve both histological and functional outcome in a rodent model of ketamine and isoflurane neurotoxicity (79-81). $Alpha_2$-adrenergic agonists appear to exert a dose dependent neuroprotective effect. For dexmedetomidine however, a ceiling effect was described. Upregulation of the postsynaptic norepinephrine-mediated trophic system was proposed as the involved protective mechanism, which, coupled with the protein kinase RNA-like endoplasmic reticulum kinase (PERK)-Bcl-2 pathway, provided an anti-apoptotic effect (80). As mentioned above, xenon was demonstrated to have a protective effect on neuroapoptosis caused by isoflurane 0.75% when simultaneously administered at sub-anesthetic doses (30% or 60% at 1 atm) (27). Interestingly, in rat pups, 2 hours of pretreatment with 70% xenon 1 day prior to a 6 hour exposure to 70% N_2O and 0.75% isoflurane provided neuroprotection as well, while apoptosis and neurodevelopmental outcome were aggravated by pretreatment with hypoxia (2 hours in 8% oxygen) (45). Several agents have been demonstrated to protect, at varying degrees, the developing neonatal rodent brain against detrimental effects of anesthetics, including melatonin (82), β-estradiol (83), lithium (84), L-carnitine (85), pramipexole (70, 86), erythropoietin (87) and bumetanide (88). Adding 1.3% hydrogen gas to the anesthetic gas mixture containing 3% sevoflurane also provided neuroprotection (60%) by reducing oxidative stress during a 6 hour exposure to sevoflurane anesthesia in neonatal rats, significantly reducing negative behavioral outcome (38, 89). Carbon monoxide (CO) levels of 5ppm and 100 ppm in the anesthesia gas mixture were shown to reduce apoptosis induced by isoflurane 2% partially and completely respectively in neonatal mice (90). Hypothermia (mean body temperature 29.7°C) has been demonstrated to fully counteract isoflurane- or ketamine-induced neuroapoptosis in a neonatal mouse model (91). In vitro, the concept of preconditioning as a protective strategy was demonstrated by pretreating immortalized neuroprogenitor cells with 2.4% isoflurane for 1 hour prior to 12 or

Table 3

Retrospective human studies

Author	Year	Age*	Cases	Results
Wilder *et al*.	2009	4	593	Multiple but not single exposures to anesthesia were a significant risk factor for the later development of LD.
Bartels *et al*.	2009	3	71 discordant MTP 77 concordant MTP	Discordant twins had equal levels of learning-related outcomes. Authors suggest genetic vulnerability predisposes for both LD and likelihood of needing anesthesia.
DiMaggio *et al*.	2009	3	383	Undergoing hernia repair increased the risk of being subsequently diagnosed with behavioral or developmental disorders.
DiMaggio *et al*.	2009	3	304	When compared to their siblings, children who underwent surgery had a 60% greater risk of being diagnosed with a developmental and/or behavioral disorder.
Kalkman *et al*.	2009	6	243	Children who underwent a urological procedure prior to age 2 had increased risk of behavioral disturbance, although this difference was not statistically significant. Adequatley powered study would require 2.268-6.020 patients.
Hansen *et al*.	2011	1	2.689	After correction for confounders, there was no evidence that undergoing hernia repair surgery reduces academic performance at age 15-16.
Flick *et al*.	2011	2	350	Single exposure did not represent a risk factor for the development of LD, multiple exposures however significantly increased the risk, but without need for individualized education programs.
Sprung *et al*.	2012	2	350	After adjusting for comorbidities, repeated exposure to anesthesia increased the risk of ADHD development.
Ing *et al*.	2012	3	321	Exposure to anesthesia resulted in a higher risk of language and abstract reasoning deficits.
Hansen *et al*.	2013	3 months	779	After correction for confounders, no correlation was found between surgery for PS and lower educational scores. There was however a lower attainment rate among case patients.

LD, Learning Disabilities ; MTP, monozygotic twin pair ; *, age before which anesthesia exposure took place ; ADHD, Attention-Deficit/Hyperactivity Disorder ; PS, Pyloric Stenosis.

24 hour exposure to 2.4% isoflurane, mitigating the harmful effects of the 12 or 24 hour exposure (92).

Human studies

Until now, the impact of exposure to anesthesia in early childhood on neurodevelopmental outcome has been investigated exclusively in retrospective or follow-up studies (Table 3). Wilder *et al*. analyzed a cohort of children who were born between 1976 and 1982 in five townships of Olmsted County, Minnesota, and remained in the community until 5 years of age. Out of 5357 children included, 593 had been exposed to anesthesia prior to the age of 4 years. In this cohort, a correlation was found between a significantly increased risk of diagnosis with learning disabilities (LDs) and multiple, but not single exposures to anesthesia and surgery before the age of 4 years (hazard ratio (HRs) 1.59 (95% confidence inter-val 1.06-2.37) for two exposures, 2.60 (1.60-4.42) for three or more exposures, and 1.0 (0.79-1.27) for a single exposure, after adjustment for birth weight, lower gestational age and gender (93). Flick *et al*. performed a matched cohort study in children, drawn from the same population, who were exposed to anesthesia prior to the age of 2 years, and confirmed a significantly increased risk of LDs after exposure to anesthesia. Multiple exposures were confirmed to be a risk factor for LDs and speech/language impairment (HR : 2.12 (1.26-3.54)), while this was not the case for the need for requiring individualized education programs for behavioral or emotional disorders (94). The most frequently used combination of anesthetics in this cohort was nitrous oxide (88.1%) and halothane (87.5%) (94). Sprung *et al*. demonstrated a correlation between Attention-Deficit/Hyperactivity disorder (ADHD) and multiple, not single, exposures to anesthesia in the same cohort (HR 1.18 (0.79-1.77) and 1.95 (1.03-3.71) for single and multiple exposures, respectively), after adjustment for confounders such as gender, birth weight, health status and gestational age. This correlation had already been suggested in a rodent model with neural injury associated with hyperactivity, induced by N-methyl-D-aspartate (NMDA)-antagonists and ameliorated by the use of dextroamphetamine (95).

Another trial demonstrated an increased risk (HR 2.3 (1.3-4.1)) of diagnosis with behavioral or developmental disorder after anesthesia for hernia repair in the first three years of life. In this study, anesthesia was administered in a cohort that underwent this procedure between 1999 and 2002. Unfortunately, type, administration route or doses of anesthetics used were not reported (96). KALKMAN *et al*. studied a cohort of 243 patients who underwent a urological procedure in 1987, 1991, 1993 or 1995 prior to the age of 6. The used agents were predominantly a combination of volatile anesthetics (halothane, isoflurane and enflurane with or without nitrous oxide) and an opioid (fentanyl or sufentanil). A caudal block was sometimes used as a supplement. An increase in behavioral disturbances was observed, but this did not reach statistical significance, for which a cohort of 2.268 up to 6.020 patients would be required (97). A sibling birth cohort study of patients born between 1999 and 2005 analyzed the number of exposures to anesthetic procedures and the risk of being diagnosed with subsequent developmental or behavioral impairment. Single exposure yielded an HR of 1.1 (0.8-1.4), two exposures raised the HR to 2.9 (2.5-3.1), while three or more exposures produced an HR of 4.0 (3.5-4.4) (98). Language, cognitive function, motor skills and behavior were examined at age 10 in children included in the Western Australian Pregnancy Cohort, in which 321 out of 2608 children were exposed to anesthesia prior to the age of 3. Motor function domains and behavior did not differ significantly between the exposed and unexposed group. However, receptive, expressive and total language scores did differ, as did abstract reasoning (99). These findings indicate that neurotoxic damage may be limited to specific neurocognitive domains. Hazard ratios were of the same magnitude as those previously reported. In Denmark, a nationwide cohort study analyzed a birth cohort from 1986 to 1990 with respect to differences in academic performance. Two thousand six hundred and eighty nine children, who underwent inguinal repair surgery during infancy, were compared to a randomly selected control population of the same age. Before correction for known confounders such as gender, birth weight and parental education, the exposed group performed worse than the control group, but no statistically significant difference remained after correction for these factors. However, test score nonattainment rate in the anesthesia group was higher than in the group without exposure to anesthesia (100). Similar results were found in a study by the same group investigating the effects of anesthesia and surgery for pyloric stenosis prior to the age of 3 months (101). A Dutch study evaluated a monozygotic twin population born between 1986 and 1995 and found a correlation between exposure to anesthesia and learning-related outcomes. The study has been heavily criticized for the inaccurate assessment of educational achievement scores. Interestingly however, the study was unable to demonstrate within-pair differences in educational achievement in twins discordant for having undergone anesthesia. The authors suggested that rather than anesthetic-induced neurotoxicity, underlying vulnerability, genetically and/or otherwise determined, makes children prone to undergo surgery and contributes to learning disabilities in children who have to undergo surgery (102). As an example, middle ear effusion, causing hearing impairment, has been associated with language-related developmental delay prior to insertion of tympanostomy tubes (103). Likewise, sleep apnea, caused by tonsillar and adenoid hypertrophy, is associated with learning problems prior to tonsillectomy and adenoidectomy (104). Both these procedures represent an important portion of indications for anesthesia in the pediatric setting (99, 105).

Many of the abovementioned studies were included in a systematic review with meta-regression by WANG *et al*., who suggest that the number rather than the timing of anesthesia exposures forms a risk factor for neurodevelopmental delay (106).

LIMITATIONS OF RETROSPECTIVE AND FOLLOW-UP STUDIES

Many confounders may remain undiscovered and unaccounted for in research concerning neurotoxicity of anesthesia in the developing human brain. Since the developmental age of the brain may have an influence on the vulnerability or susceptibility of different brain regions when undergoing anesthesia, cohorts including patients with exposure prior to age 1 (100), 2 (94, 97, 107), 3 (96, 98, 99, 102) or 4 (93) may yield different results, correlating with different stages in neural and neuroarchitectural development. The neurocognitive and neurodevelopmental domain has many different subdivisions, correlating with the various aspects of mental capacity, not all of which are covered and thoroughly examined in the neurocognitive tests in use today. As mentioned earlier, the indication for surgery and anesthesia itself may and very often does predestine the patient for learning or other disabilities. Differences in use of anesthetics may also yield different results due to dissimilar neurotoxic properties of various drugs and their combinations

in humans. Unfortunately, information on the anesthetics used is not always available. Duration of anesthesia itself and cumulative doses of anesthetics differ between indications for surgery and may influence the neurodevelopmental outcome for these patients. Moreover, effects of anesthesia cannot be dissected from effects of surgery itself, underlying pathology necessitating anesthesia, comorbidities and socio-economic status. Furthermore, only limited information about perioperative care (anesthetic drugs, hemodynamics, postoperative care, complications and comorbidities) is available. The relevance to modern practice is unclear, since most studies date back decades, when halothane was used predominantly, and both pulse oximetry and capnometry were not yet available. Different outcome measures make it difficult to compare results of these studies, and upon review, neuropsychological testing demonstrated greater sensitivity to determine cognitive difference than academic achievement scores (108).

Ongoing studies

The General Anesthesia and Apoptosis Study (GAS) is an ongoing international, multi-site, randomized controlled trial that has been designed to investigate neurodevelopmental outcome after hernia repair surgery under spinal versus general anesthesia in newborns born with a gestational age of 26 weeks, and no older than 60 weeks post-menstrual age. With final analysis results not expected before 2017, the aim is to collect data at age 2 and 5 years from 660 patients and compare results for apnea, neurodevelopmental and intelligence testing (109). The Pediatric Anesthesia NeuroDevelopment Assessment (PANDA) Study aims at assessing neurodevelopment and cognitive outcomes in a retrospectively obtained sibling pair cohort, discordant on having undergone anesthesia for hernia repair prior to the age of 3. Results of a pilot and feasibility study were published in October 2012 (110). Lastly, a cooperation between the National Center for Toxicological Research (Jefferson, Arkansas, USA) and the Mayo Clinic (Rochester, Minnesota, USA) performs the Mayo Safety in Kids (MASK) study, evaluating children who underwent no, one or multiple anesthesia procedures with a number of neurocognitive tests, including the Operant Test Battery (111, 112). In the coming years, these studies will hopefully enrich the body of knowledge concerning neurodevelopmental and cognitive outcome after anesthesia during the early stages of life.

Conclusion

There is strong evidence from experimental data that exposure to anesthesia during the brain growth spurt may have detrimental effects on neurodevelopmental outcome. Toxicity from all commonly used anesthetics has been repeatedly described in mammalian models, including rodents, piglets and non-human primates, such as rhesus monkeys. The majority of effects were observed in a dose dependent manner. The exact mechanisms underlying anesthesia-induced neurotoxicity remain to be elucidated, but suppression of trophic stimuli, oxidative stress, activation of intrinsic and extrinsic pathways of apoptosis, pro-inflammatory response, altered neurotrophic factor signaling and excitotoxicity have been proposed as harmful molecular mechanisms. These noxious mechanisms result in neural and oligodendrocytic apoptosis, dendritic spine alterations, axon guidance disturbances, cytoskeletal impairment and cortical migration interference. Several pharmacological agents have been described to protect the developing brain from harm. The clinical relevance of these experimental findings is difficult to determine, since translation from observations in animals to clinical practice remains complex. As an example, brain development including the brain growth spurt lasts much longer in humans than in rodents. Therefore, when trying to translate experimental findings into the human setting, the duration of exposure to anesthesia should be considered in relation to the duration of the brain growth spurt. As an example, several hours of anesthesia in a neonatal rodent correspond to several days of anesthesia in a human neonate. Moreover, it is very challenging to distinguish between developmental impairment caused by the underlying disease leading to surgery/diagnostic procedures, by the impact of the interventions themselves and the potential neurotoxicity from anesthesia. Several retrospective and follow-up studies in humans have been conducted and yielded inconclusive results, yet the majority of these trials suggest an association between exposure to anesthesia in early infancy and deterioration in neurodevelopmental outcomes. Prospective randomized controlled trials are currently being conducted. Unfortunately, the results of these trials will only be available within years. Current knowledge does not support major modifications to clinical anesthesia (24, 68, 113-115). Of note, indications for anesthesia, both for surgical and diagnostic purposes, must be the result of a well-considered risk benefit balance, weighing a real added risk of delay

against a risk of toxicity that is still dubious (116). In some cases, combining two minor surgical procedures to avoid repetitive exposure may be a safe way to minimize risks, as would postponing purely elective surgery, some authors suggest until the age of 3 (117).

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