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Review Article

REVIEW ARTICLE

Safety of general anaesthetics on the developing brain: are we there yet?

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Summary

Thirty years ago, neurotoxicity induced by general anaesthetics in the developing brain of rodents was observed. In both laboratory-based and clinical studies, many conflicting results have been published over the years, with initial data confirming both histopathological and neurodevelopmental deleterious effects after exposure to general anaesthetics. In more recent years, animal studies using non-human primates and new human cohorts have identified some specific deleterious effects on neurocognition. A clearer pattern of neurotoxicity seems connected to exposure to repeated general anaesthesia. The biochemistry involved in this neurotoxicity has been explored, showing differential effects of anaesthetic drugs between the developing and developed brains. In this narrative review, we start with a comprehensive description of the initial concerning results that led to recommend that any non-essential surgery should be postponed after the age of 3 yr and that research into this subject should be stepped up. We then focus on the neurophysiology of the developing brain under general anaesthesia, explore the biochemistry of the observed neurotoxicity, before summarising the main scientific and clinical reports investigating this issue. We finally discuss the GAS trial, the importance of its results, and some potential limitations that should not undermine their clinical relevance. We finally suggest some key points that could be shared with parents, and a potential research path to investigate the biochemical effects of general anaesthesia, opening up perspectives to understand the neurocognitive effects of repetitive exposures, especially in at-risk children.

Keywords: anaesthetic drugs; apoptosis; biochemical mechanisms; duration of exposure; neuro-developmental risks; paediatric anaesthesia; single and multiple exposures

One in 10, 100, 1000, 10 000, or 100 000 … anaesthetic risks are at the centre of the preassessment, informing children and their parents of the inherent risks associated with general anaesthesia. Some are immediate: sore throat, gum or lip or dental damage, airway reactivity, anaphylaxis, anoxic injury, or death. However, there is a risk that is much harder to assess: the neurotoxicity of general anaesthetics on the developing brain. Interestingly, rarely discussed by anaesthetists during preassessment, parents increasingly enquire about the neurocognitive outcome for their children after exposure to general anaesthesia. Awareness of this issue was initially brought to the attention of scientific and clinical communities after studies on rat puppies exposed to anaesthetic drugs. The widespread neurodegeneration witnessed raised the alarm, resulting in a recommendation by the Food and Drug Administration (FDA) in the USA, and other organisations, to postpone non-vital surgery in children until the age of 3 yr and to intensify research in this area. Since then, many animal and clinical studies have focused on this problem, which is listed as a top 10 UK research priority for perioperative medicine by the James Lind Alliance and the National Institute for Academic Anaesthesia. Here, we discuss the

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initial concerning animal data, explore the biochemical basis for this neurotoxicity, scrutinise further animal and early human investigations on the subject, before evaluating the results from the most recent human cohort studies and trials. From there, we outline a new framework for future investigations with the aim of providing reassurance as to the effect of general anaesthesia on the developing brain and a risk evaluation of its neurotoxicity in specific circumstances.

Initial concerns about the neurotoxicity of general anaesthetic agents on the developing brain

In the late 1990s, a specific blockade of glutamate *N*-methyl-D-aspartate (NMDA) receptor in the late fetal or early neonatal lives of rats led to widespread neurodegeneration via apoptosis.[1,2] Similar patterns were observed with other NMDA antagonists[3,4] and γ-amino-butyric acid (GABA) agonists.[5] The combination of midazolam, isoflurane, and nitrous oxide was also associated with long-term potentiation suppression, and impairment in spatial learning and memory.[6] In humans, brain development is different.[7] The FDA issued, in 2007, a recommendation to increase research into this specific area and to postpone all non-vital procedures under general anaesthetic until after the age of 3 yr.[8–10]

Many approaches have been used.[11] One of the key findings in rodents is the central role of the apoptotic pathway shown by the identification of structural damage and enzymatic activation.[12] Other findings include electrophysiological modifications and behavioural changes that can have a delayed onset.[13,14] Rodents have also been used to track morphological alterations and metabolic disruptions induced by general anaesthesia.[15–17]

In mammals, the majority of work focused on non-human primates, whereas some studies introduced other models.[18–20] Cellular and structural damage, and adverse cognitive outcomes, were reported with propofol,[21] ketamine,[22,23] isoflurane,[24–27] and sevoflurane,[28] although this was not confirmed in recent studies using inhaled anaesthetics.[29,30] Multiple long exposures to sevoflurane resulted in altered visual recognition memory in neonate rhesus monkeys.[31] Similar regimens using sevoflurane led to an increase in anxiety-related behaviours.[28,32] General anaesthesia also triggers a long-lasting change in glial fibrillary acidic protein concentration. These changes were identified in areas of the brain involved in visual recognition memory, anxiety and sociability.[33] Dexmedetomidine emerges as a non-neurotoxic option, both in histopathology and behavioural outcomes.[34,35]

The results observed in non-human primates are therefore concerning, with deleterious effects identified at biochemical, morphological, histopathological, and behavioural levels. The risk of general anaesthetic-induced neurotoxicity on the developing brain has species-, dose-, duration-, and repetition-dependent thresholds rarely used in paediatric anaesthesia. The interspecies extrapolation is not straightforward.[36,37]

General anaesthetics disrupt brain physiology

Brain physiology under anaesthesia

The CNS cellular pool is a complex assembly of cells (see Fig. 1). The main neurotransmitters affected by general anaesthesia are glutamate (major excitatory neurotransmitter), glycine, serotonin, norepinephrine, dopamine, acetylcholine, and GABA (major inhibitory neurotransmitter).[38] A list is proposed in Table 1.

Glutamate facilitates the transmission of the electrical impulses. It binds with the three ionotropic receptors: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate (controlling Na^+ ion channels), and NMDA receptors, which with the metabotropic glutamate receptors are driving Ca^{2+} ion channels. Once activated, they promote a postsynaptic increase of intracellular Ca^{2+} and could result in a depolarisation progressing along the postsynaptic neuron.

GABA has a more complex and versatile role, also regulating the opening of some ion channels when linked to its receptors. Two transporters are critical to understand this versatility: the NKCC1 (Na–K–2Cl cotransporter isoform 1) brings chloride inwardly increasing the intracellular concentration, whereas the KCC2 (K–Cl cotransporter isoform 2) releases chloride outwardly decreasing the intracellular concentration. GABA has an excitatory role in the early stages of brain development (predominance of NKCC1 over KCC2) before becoming an inhibitory amino acid in later stages (predominance of KCC2 over NKCC1).

The glutamate and GABA synaptic transmissions in the mammalian spinal cord have been comprehensively investigated.[39–41] In the brain, glutamate metabolism was explored,[42,43] especially its excitatory role in neurotransmission.[44,45] Glutamate was shown to be predominant and ubiquitous[46] and its receptors identified.[47,48] Similar observations were made for GABA,[49,50] with the ligand gated ion channel $GABA_A$ receptors and the $GABA_B$ receptors coupled to G proteins.[51,52] Studies of anaesthetic effects on neurotransmitter release and reuptake have produced a variety of results.[53–55]

There is strong metabolic coupling between presynaptic and postsynaptic neurons, and astrocytes,[43] with specific cycles for glutamate,[56] glycine, serine, and GABA.[57] General anaesthesia will usually induce an enhancement of inhibitory synaptic transmission and an inhibition of excitatory synaptic transmission.[58,59] NMDA, $GABA_A$, serotonin, and nicotinic receptors can come in different subunit compositions, supporting the differing clinical effects observed with anaesthetics: sedation, antiepileptic, anterograde amnesia, anxiolysis, and myorelaxation, among others.[60] Effects of opioids and neuromuscular blocking agents were also studied.[61,62]

Excitotoxicity

When neurotransmitter concentrations increase and overload the receptors, excitotoxicity can occur. If compensatory mechanisms are overwhelmed, the activation of proteases will damage cytoskeletal proteins, membrane receptors, and enzymes. Glutamate concentration in the synaptic cleft will sharply increase after an action potential, before normalising rapidly.[63,64] GABA concentration is more stable inside and outside the synaptic cleft.[65,66] These changes can potentially trigger apoptosis and necrosis.[67,68] Excitotoxic lesions have been isolated in many pathological processes of the CNS with apoptotic and necrotic mechanisms involved.[69,70] Many mechanisms of neuronal cell death have been described.[71] Only apoptotic features have been identified after exposure to general anaesthetics.

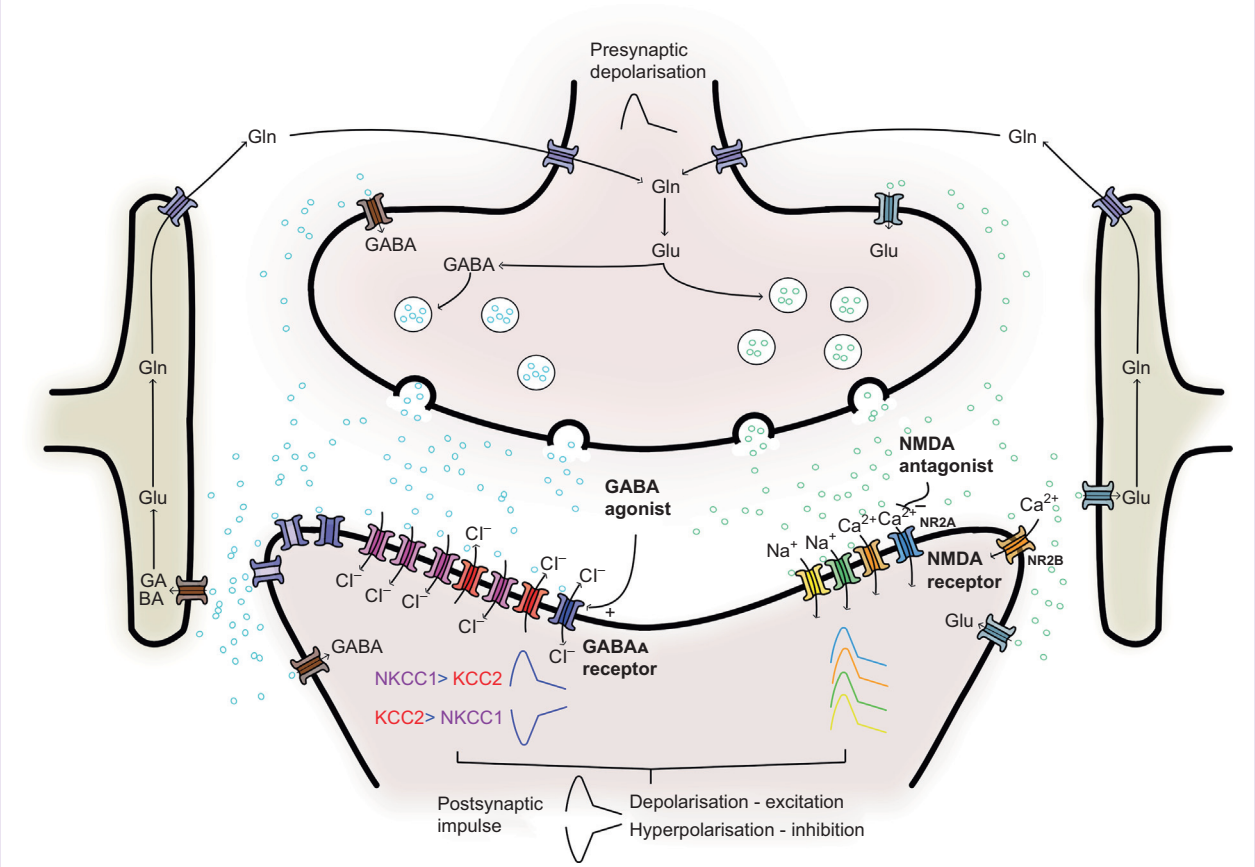

Fig 1. Effect of an action potential on a glutamatergic synapse with principal targets of main anaesthetic drugs (NMDA and $GABA_A$ receptors). A presynaptic depolarisation leads to an influx of calcium into the presynaptic neuron and the release of neurotransmitters into the synaptic cleft. Glutamate targets its postsynaptic receptors, leading to an influx of cations in the postsynaptic neuron and facilitates further depolarisation. NR2A subunit-containing receptors are synaptic receptors whereas NR2B subunit-containing receptors are extrasynaptic. The blockade of NR2B receptors has neuroprotective effects; however, in the developing brain, the associated blockade of NR2A receptors is deleterious. GABA targets its postsynaptic receptors leading to a chloride exchange. In the immature brain, the NKCC1 transporters are predominant, leading to a high intracellular concentration of chloride. The activation of the GABA receptor leads to the release of chloride ions into the synaptic cleft, favouring a further depolarisation. When the brain matures, the KCC2 transporters become more predominant than the NKCC1 transporters, leading to a lower intracellular concentration of chloride in the neuron. When the $GABA_A$ receptor is activated, chloride is driven inwardly into the postsynaptic neuron. This favours a hyperpolarisation of the postsynaptic membrane; the activation of the GABA receptor inhibits the transmission of the influx. This phenomenon, when the $GABA_A$ receptor changes from an excitatory to an inhibitory role, is called the GABA shift. NMDA antagonist anaesthetic drugs will block the NMDA receptor and reduce the depolarisation and thus excitation of the postsynaptic neuron after the arrival of the presynaptic depolarisation. GABA agonist drugs will favour depolarisation and excitation in the immature brain, but hyperpolarisation and inhibition in the more developed brain after the GABA shift. If the summation of the postsynaptic depolarisations and hyperpolarisations are sufficient, a new action potential will progress along the postsynaptic neuron. Specific biochemical cycles allow the recycling of glutamate and GABA by specific reuptake mechanisms. Glutamate can be transformed into the non-active glutamine in the astrocytes via the glutamine synthetase. Once sent back to the neurons, the glutamine can be transformed back to glutamate via the glutaminase. Glutamate can then be used directly or transformed further into GABA via the glutamate decarboxylase. GABA recaptured by the astrocytes can be changed into glutamate via the mitochondria. GABA, γ-amino-butyric acid; Gln, glutamine; Glu, glutamate; KCC2, K–Cl cotransporter isoform 2; NKCC1, Na–K–2Cl cotransporter isoform 1; NMDA, *N*-methyl-D-aspartate

Biochemical mechanisms that could cause neurotoxicity of general anaesthetic agents on the developing brain

Multiple factors could be involved. The modulation of the $GABA_A$ receptor activity, coined the GABA shift,[72] could explain the increased susceptibility of the developing brain to anaesthetic drugs. This cotransporter imbalance has also been identified in epilepsy, post-traumatic brain injury seizures, and ischaemic brain injury.[73–75]

The blood–brain barrier is leaky in the developing brain and more permeable to certain substances such as amino

Table 1 Principal neurotransmitters, receptors and type, effects, and hypnotic drugs used in clinical paediatric anaesthetic practice. GABA, γ-amino-butyric acid; NMDA, N-methyl-D-aspartate.

Neurotransmitter	Receptor	Type	Effects	Anaesthetic drugs
Acetylcholine	Nicotinic receptor Muscarinic receptor	Ligand-gated G protein-coupled	Inhibition	Agonists: halogenated volatiles, ketamine, propofol, barbiturates, etomidate, neuromuscular blocking agents (neuromuscular junction receptors), nitrous oxide
GABA	$GABA_A$ receptor $GABA_B$ receptor	Ligand-gated G protein-coupled	Inhibition	Agonists: halogenated volatiles, propofol, barbiturates, benzodiazepines, etomidate, nitrous oxide
Glycine	Glycine receptor	Ligand-gated	Inhibition	Agonists: halogenated volatiles, propofol, barbiturates, benzodiazepines, etomidate, nitrous oxide
Glutamate	NMDA receptor	G protein-coupled	Excitation	Antagonists: ketamine, nitrous oxide, halogenated volatiles
Serotonin	5-HT receptor	Ligand-gated (3) G protein-coupled (1,2,4,7)	Inhibition/excitation	
Dopamine	D1 and D2 receptors	G protein-coupled	Inhibition	Antagonists: halogenated volatiles
Norepinephrine	α and β receptors	G protein-coupled	Inhibition	Agonists: dexmedetomidine, clonidine

acids and drugs that could be harmful.[76–78] Brain glutamate concentration increases over the first 90 days of life.[16,17] Repeated anaesthesia also led to an increase in the posterior cortical concentration of glutamate and taurine.[16] Finally, excessive activation of glutamate receptors could contribute to neuronal dysfunction, damage, and absence of repair in the developing brain.[79] Mechanisms by which widespread neurodegeneration and dendritic arborisation alteration occur[1,80–82] are still not fully understood, but better descriptions of the effects of general anaesthesia on the developing brain have been reported.[83] A summary is given in Table 2.

Table 2 Potential biochemical mechanisms involved in general anaesthesia toxicity for commonly used hypnotics (sevoflurane, propofol, and ketamine). Effects are listed in the mature and developing brains, with neuroprotective in italic font and deleterious in bold font. GABA, γ-amino-butyric acid; NMDA, N-methyl-D-aspartate; TGF-β, transforming growth factor-β.

Drug	Effects on the mature brain	Effects on the developing brain
Sevoflurane	• *Decreases metabolic rate* • **Increases glutamate** • **Increases oxidative stress** • **Increases DNA damage** • *Reduces pro-apoptotic proteins* • *Increases anti-apoptotic proteins* • *Decreases inflammatory cytokine activity* • *Interaction with TGF-β superfamily and activin A (cellular survival)*	• *Decreases metabolic rate* • **Increases glutamate** • **Increases oxidative stress** • **Increases DNA damage** • **Increases pro-apoptotic proteins** • **Decreases anti-apoptotic proteins** • **Increases inflammatory cytokine activity** • **Interacts with the neutrophin receptor pathways (neuronal growth and plasticity)**
Propofol	• *Decreases metabolic rate* • *Decreases glutamate* • *Decreases oxidative stress* • *Decreases DNA damage* • *Reduces pro-apoptotic proteins* • *Increases anti-apoptotic proteins*	• *Decreases metabolic rate* • *Decreases glutamate* • *Decreases oxidative stress* • *Decreases DNA damage* • *Reduces pro-apoptotic proteins* • *Increases anti-apoptotic proteins* • **Excitatory effects on the GABA receptors before the GABA shift**
Ketamine	• **Increases metabolic rate** • *Reduces glutamate* • **Increases oxidative stress** • **Increases DNA damage** • *Reduces pro-apoptotic proteins and upregulates dendritic spine density (interaction with NR2B subunit of the NMDA receptor)* • *Decreases inflammatory cytokine activity*	• **Increases metabolic rate** • *Reduces glutamate* • **Increases oxidative stress** • **Increases DNA damage** • **Interacts with NR2A subunit of the NMDA receptor (disruption of key effectors of neurodevelopment and plasticity)**

Sevoflurane decreases cerebral metabolic rate,[84,85] and an increase in glutamate,[86–88] oxidative stress,[89] and DNA damage.[90] Sevoflurane reduces levels of pro-apoptotic proteins, whilst increasing the amount of anti-apoptotic proteins, regulated by an activation and increase in protein kinase B. Sevoflurane also interacts with the transforming growth factor-β superfamily and activin A. This leads to the activation of cellular survival mechanisms and the reduction of inflammatory cytokine production.[91–94] However, in the developing brain, sevoflurane has an opposite effect, consisting of an increase in caspase-3 activity. It also increases inflammatory cytokine activity and seems to damage physiological neuronal growth and plasticity by interacting with the neutrophin receptor pathways. Finally, sevoflurane could have a neuroprotective effect on a brain already sustaining an injury, whilst being neurotoxic on brains without any ongoing pathological process.[94] Considering the different known apoptotic mechanisms, ferroptosis, lysosomal, MitoPore, and parthanatos mechanisms could be triggered by sevoflurane.[71] Sevoflurane also interferes with dendritic arborisation, which leads to impaired physiological patterns and circuit assembly. This could be species- and localisation-dependent, but would induce cognitive damage such as memory loss.[95–99]

Propofol has the opposite effect on glutamate concentration, oxidative stress, and DNA damages.[89,100–104] It reduces the release of glutamate by interacting with the presynaptic cannabinoid receptor 1 receptors. It also inhibits the limiting effects of reactive oxygen species on the EAAT and Na+/H+ exchanger NHE1 exchangers, and increasing the activity of antioxidant proteins. Propofol reduces mitochondrial swelling induced by Ca^{2+} overload in case of brain injury,[105] promotes the expression of anti-apoptotic factor Bcl2, and limits the expression of pro-apoptotic factor Bax. Finally, it interacts with reactive oxygen species as a scavenger and restricts lipid peroxidation. In young children, the activation of $GABA_A$ receptors leads to an excitatory circuit, reducing the neuroprotective effects of propofol.[100]

Ketamine shares biochemical patterns with sevoflurane but increases cerebral metabolic rates.[82,85,106–109] It protects against excitotoxicity by interacting with the neurotoxic NR2B subunit containing NMDA receptors. This activity also upregulates the dendritic spine density that could show the tentative cerebral repair promoted by the brain. Additionally, ketamine reduces glutamate release by inhibiting the fusion of intraneuronal glutamate vesicles with the presynaptic membrane. Ketamine also leads to the synthesis of anti-apoptotic proteins and inhibits inflammatory cytokine production. These neuroprotective effects are, however, unlikely to be extendable to children. NMDA receptors on the synapse carry an NR2A subunit, which is associated with many key protein effectors of neurodevelopment and neuroplasticity. Interactions with these receptors are believed to cause neurodevelopmental harm.[110] Finally, opioids and neuromuscular blocking agents are also thought to promote apoptosis in the developing brain.[111–114]

Clinical evidence of neurotoxicity of general anaesthetic agents on the developing brain

Several cohort studies have explored neurocognitive outcomes in children exposed to general anaesthesia in their early years (see Fig. 2). They 'have yielded conflicting and inconclusive results'.[115] There is important literature about cognitive outcomes after exposure to anaesthesia in neonates undergoing laparotomies or infants requiring cardiac surgery.[116] In these specific situations, many confounding factors make it difficult to interpret neurocognitive outcomes. In this review, we focus on infants referred for elective noncardiac surgeries.

Most of the evidence is derived from cohort studies that explore a possible association between neurocognitive outcomes and exposure to one or multiple general anaesthetics. Neurocognitive outcomes have been explored/assessed in different ways, mainly looking at children's learning disabilities, academic achievements, behavioural issues, IQ, and some more specific evaluations in smaller studies.[116,117]

Single general anaesthesia

Effects of exposure to general anaesthesia have been explored mainly through retrospective cohort studies. Various methodologies were used in the recruitment or analyses, with country-wide surveillance in Denmark, Canada, Taiwan, the Netherlands, or involving large regions such as the Western Australian Pregnancy Cohort, as part of large-scale birth cohort studies.[118–127] Some used registers, such as the Texas or New York State Medicaid databases, to identify potential cases.[128,129] Others targeted more specific factors in their designs, such as the presence or absence of surgery, the timing of general anaesthesia in the first year of life, exposure to propofol or sevoflurane, evaluation of a child's cognitive score before and after exposure, or the use of other markers such as functional MRI data.[128,130–134] Others evaluated the use of psychoactive medicine years after exposure to general anaesthesia[135] or tried to link anaesthetic exposure to certain morphological changes.[136]

Although these studies were aimed at measuring cognitive and behavioural effects in children exposed to general anaesthetics, there was also a large variety of outcomes chosen to evaluate these effects. Some looked at specific diagnoses such as attention deficit hyperactivity disorder, autistic disorder, or developmental delays. Others considered less specific clinical markers such as intellectual coefficients, learning difficulties, language and cognitive disorders, early developmental vulnerability, academic achievements, recollection score, whereas another group of studies focused on a comprehensive range of cognitive tests. The age threshold for exposure fluctuates from one study to another, making it harder to draw clear conclusions. Some looked at exposures to general anaesthetics before the age of 1, 2, or 3 yr, and others looked at exposure in children up to 6 yr of age.

In the Australian cohort study, language and cognitive skills were impacted by general anaesthesia with an adjusted odds ratio >2. These results were confirmed in a wider analysis.[119,120] The exposure duration–cognitive risk link was also investigated showing that young children exposed for >35 min to volatile anaesthetics had lower total and expressive language scores.[120] In the Medicaid cohort studies, it was found that the hazard ratios for developmental delay and attention deficit hyperactivity disorder were around 1.3 after exposure to anaesthesia and the adjusted hazard ratio of developmental or behavioural disorders was 2.3.[128,129] The Swedish, Taiwanese, and Danish cohort studies, however, did not identify statistically significant differences.[121,122,125–127] A concordant monozygotic twin cohort study found that general

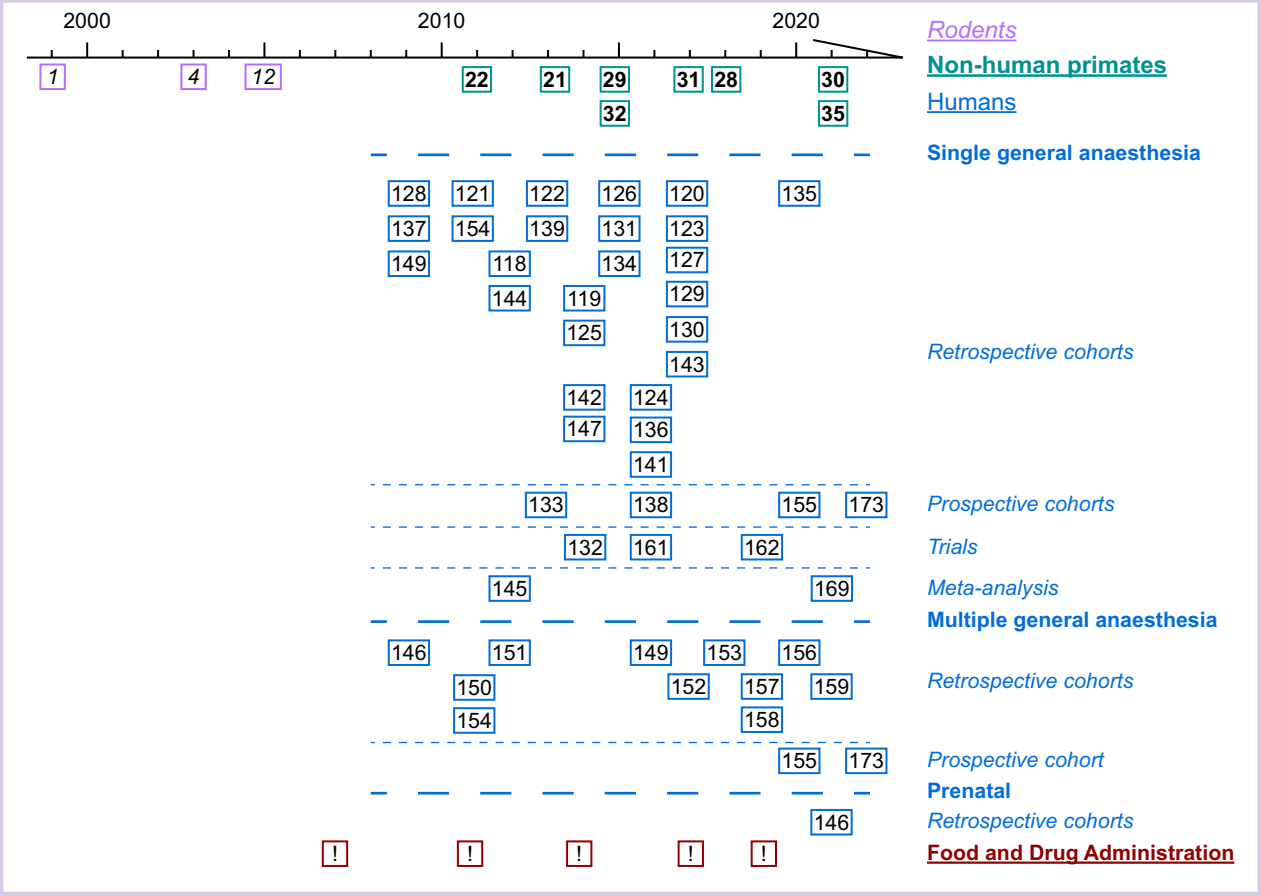

Fig 2. Timeline of a selection of relevant publications and the Food and Drug Administration (FDA) recommendations (concerning the toxicity of general anaesthetic drugs on the developing brain). Studies involving rodents are in italic fonts in purple boxes, non-human primates in bold fonts in green boxes, and human studies in blue boxes. Numbers refer to the reference list of this review. Exclamation marks in red boxes correspond to the FDA's Anesthetic and Life Support Drugs Advisory Committee meetings with deliberations and recommendations on the issue of neurotoxicity of general anaesthesia on the developing brain.

anaesthesia exposure led to significant cognitive problems and lower educational achievements.[137]

A sibling cohort study prospectively monitored children for neurocognitive development, and did not identify any significant difference between the exposed and non-exposed groups (PANDA Study) in their primary outcomes. However, worse scores were observed for emotional and behavioural problems in the group exposed to general anaesthesia (higher Child Behavior Checklist [CBCL] internalising and total scores).[138]

Surprisingly, the Canadian cohort study identified a discrete risk of early developmental vulnerability, but in children aged between 2 and 5 yr and not in those under the age of 2 yr.[124] The cohort study from Singapore (surgery before the age of 1 yr and academic achievements at the age of 12 yr), reported an odds ratio of 4.5 to develop learning disability,[139] whereas the Danish cohort study (surgery before the age of 1 yr and academic achievements at the age of 15 yr) did not identify any significant difference.[121,122] Some studies reported deleterious effects whereas others did not identify any.[116,134,140–144] It is, however, interesting to note that there was no difference between sevoflurane and propofol,[132] or between an early or late anaesthesia in the first year of life.[131]

Fan and colleagues[133] proposed a preoperative cognitive evaluation rarely reported in other studies, but in children aged 4–7 yr, after the risk period for anaesthetic exposure. There was no difference between the preoperative evaluation and the postoperative evaluation, conducted at 1 and 6 months after surgery.

Nestor and colleagues[130] also investigated the effects of general anaesthesia when there is no associated surgery (i.e. imaging). They found that the group undergoing general anaesthesia for imaging had poorer outcomes, but this was probably as a result of underlying health conditions. Finally, a meta-analysis identified a moderately elevated risk of adverse behavioural or developmental outcomes with early exposure to general anaesthesia, with an adjusted odds ratio of 1.4.[145]

The development of prenatal surgery also raises the question of the toxicity of general anaesthesia on an even more immature brain. Early findings show an association with increased externalising behavioural problems in childhood.[146] Further studies will be needed to explore this specific domain. Finally, a study specifically focused on exposure to general anaesthesia at older ages (3–16 yr of age) did not identify any deleterious effects on language or cognitive function.[147]

Multiple general anaesthesia

There is stronger consensus around the deleterious effects of repeated general anaesthesia. The association with an increased risk was not confirmed in a Canadian retrospective study,[148] but in other studies, retrospective or prospective, from Minnesota,[149–153] New York State,[154] the UK,[155] and Japan[156] increased risk of adverse outcomes was associated with the number of episodes of general anaesthesia, more so than with the duration of anaesthesia.[120]

Looking at this in more detail, one general anaesthetic was not associated with an increased risk of learning disabilities (hazard ratio=1.00), but two or three general anaesthetics led to a risk of learning disabilities (hazard ratios=1.59 and 2.60, respectively).[149] Including the development of attention deficit/hyperactivity disorder, this hazard ratio for multiple exposures increases to 2.17.[152] Using the Wechsler Abbreviated Scale of Intelligence, multiply and singly exposed did not score lower than unexposed children for the intelligence quotient, but processing speed and fine motor abilities were altered in the multiply exposed group. It is also of note that, in this study, single exposure to general anaesthesia led to worse scores for emotional and behavioural problems (higher CBCL scores), whereas multiple exposures had deleterious effects on the aforementioned aspects but also on executive functions (higher Behavior Rating Inventory of Executive Function [BRIEF] scores).[153] The risk of developmental and behavioural disorders increased from 1.10 with a single exposure to 2.90 for two exposures and 4.00 for three or more exposures.[154] In a prospective cohort study, dynamic balance (0.30 standard deviation lower), manual dexterity performance (0.10 standard deviation lower), and social communication scores (0.10 standard deviation lower) were lower in the children exposed to general anaesthetics multiple times *vs* once. All other aspects of neurocognitive development were similar in both groups, but also in comparison with unexposed children.[155]

In Japan, three or more exposures significantly increased the risk of developmental delay in the five domains of the Japanese translation of the Ages and Stages Questionnaires—3rd edition. Adjusted odds ratios of developmental delay were 3.32 for communication, 4.69 for gross motor skills, 2.99 for fine motor skills, 2.47 for problem solving, and 2.55 for personal-social skills).[156] Similar results were confirmed by a secondary analysis of the MASK study.[157,158]

Recently, a higher hazard ratio was associated with the diagnosis of attention deficit hyperactivity disorder after multiple exposures to general anaesthesia compared with a single exposure in a retrospective study.[159] A more detailed description of most of these studies is out of the scope of this review, and can be found elsewhere.[160]

The GAS trial

Methodology and primary outcome

The GAS trial is an international assessor-masked randomised controlled equivalence trial, comparing cognitive outcomes in infants <60 weeks of postmenstrual age and without underlying medical conditions, undergoing herniorrhaphy under general anaesthesia or spinal anaesthesia.

One of the gold standard tests for neurocognitive assessment of young children, the Wechsler Preschool and Primary Scale of Intelligence, 3rd edition (WPPSI-3), was administered to evaluate 'attention, memory, sensorimotor development, exploration, concept formation, and simple problem solving'. A composite cognitive score was used to summarise these tests. The standardised scores are centred around 100, with a standard deviation of 15. A margin of 5 points was used to establish equivalence on both sides. The trial was designed to observe a difference of 1 point in the standardised score, with a type 1 error risk of 5% and a type 2 error risk of 10%. This corresponds to a sample size of 598 infants.

The GAS trial recruited 722 children across 28 hospitals around the world. It reported the absence of a neurocognitive effect from general anaesthesia compared with regional anaesthesia for single and short exposures (<1 h of sevoflurane) in children undergoing herniorrhaphy. The WPPSI-3 full-scale IQ scores at 5 yr were equivalent in the regional anaesthesia (mean 98.9, standard deviation 18.0) and general anaesthesia groups (mean 98.8, standard deviation 19.2) at 2 and 5 yr after general anaesthesia. Anaesthesia lasted on average just under 60 min.[161,162] These values are similar to the population mean and standard deviation observed in children who had never been anaesthetised.[163]

Secondary outcomes

In the per-protocol analysis, executive functions seemed to be affected by general anaesthesia (higher BRIEF-P scores), but it is not clear how the authors adjusted results for multiple hypothesis testing. This result was not observed in the intention-to-treat analysis.[162] As rightly pointed out in the statistical analysis section of the GAS paper, both intention-to-treat and per-protocol analyses are important in equivalence trials, with a risk of claiming non-inferiority with the former with insufficient evidence when non-adherence to the allocated treatment is observed.[164]

In the GAS trial, 'there were unavoidable protocol violations in this study (the majority of which were in babies allocated to receive regional anaesthesia who had some exposure to general anaesthesia, particularly if the awake-regional anaesthesia failed)'.[162] An association between young age and non-adherence was a potential confounding factor when comparing regional and general anaesthesia groups. The information shown in Table 1 of the paper evaluating 5 yr outcomes of the GAS trial[162] was obtained after imputation of missing data. Table S3 of the same paper shows a difference in age at surgery between groups for those who did attend the 5-year visit (67.1 and 71.9 days for regional and general anaesthesia groups).[162] There is also a difference amongst those who did not attend the 5-year visit (79.1 and 68.7, respectively). Data were not missing at random and multiple imputation was required to produce valid inferences, including variables associated with loss to follow-up data in the imputation models. In the per-protocol analysis populations, the difference of the BRIEF-P scores between the two groups is at the limit of significance. Focusing on complete cases only, the difference appears stronger, but with groups that are potentially less comparable for both birth weight and age at surgery. The per-protocol analysis focusing on complete cases represents a subgroup of 205 patients from the regional anaesthesia group and 242 patients from the general anaesthesia group. The characteristics of these two subgroups are not provided. A risk could be that the age is unbalanced between the two groups and could potentially favour one group over the other in the identification of deleterious neurocognitive outcomes. Finally, the GAS trial is an equivalence study. The identification of a difference would mean lack of equivalence but would not establish a difference between the two groups. The results

of these complete case analyses should thus be interpreted with caution.

The GAS trial demonstrated that a short and single exposure to sevoflurane is not associated with deleterious neurocognitive outcomes and further dedicated studies should focus on the BRIEF score evaluation to evaluate a potential effect on executive functions.

Statistical limitations and clinical relevance

The GAS trial provides the highest level of scientific evidence in the evaluation of deleterious effects of general anaesthesia, but there are some statistical limitations to consider. The attrition was higher than anticipated, reaching almost 14% for the interim analysis 2 yr after exposure,[161] and nearly 38% for the 5-yr outcome (447 infants in the final analysis).[162] The control of type 2 errors is crucial in equivalence trials to limit the risk of not identifying a difference that truly exists (i.e. to mistakenly conclude an equivalence because of a lack of power).[165,166] In Fig. 3a, we illustrate the effect of attrition, the unplanned interim analysis at 2 yr, and testing of multiple hypotheses on sample size requirements for the GAS trial. Although imputation methods reduced bias and allowed data analysis, they do not compensate for the loss of statistical power.[162] Furthermore, when studying rare/single events, some would recommend to include three times as many patients as the likelihood of the event.[167]

Based on the experience of the GAS trial, one could argue that the quest for statistical power risks trial feasibility with unreachable sample sizes. Alternatively, it should focus attention on the clinical relevance of effect size, as the sample size fluctuates importantly with the equivalence margin.[168] A change of 1 point in these cognitive score margins is clinically irrelevant, but has a large impact on statistical power (see Fig 3b).

These statistical limitations should not diminish the scientific and clinical impact of the GAS trial. Despite the difficulties to recruit children and to follow them up for 5 yr, the GAS trial established that anaesthetised children (short and single exposure to sevoflurane of <1 h) exhibit similar WPPSI-3 scores to children in the regional anaesthesia group and non-exposed children. It provides the necessary reassurance that single and short exposures to sevoflurane do not harm our patients, opening perspectives for future human studies aimed at understanding the mechanisms of general anaesthetics and their neurocognitive effects after long or repetitive exposures.

Meta-analysis of prospective studies

A recent meta-analysis,[169] including data from the PANDA, MASK, and GAS studies showed that children exposed to general anaesthesia had worse CBCL scores, whereas the differences in BRIEF scores were not significant and the Full-Scale Intelligence Quotients (FSIQ) were similar in the exposed and non-exposed children. This meta-analysis merges an RCT and prospective cohort studies, potentially increasing precision by following a strict methodology.[170,171] Unfortunately, the changes in the BRIEF-P scores, seen in the GAS trial, are not confirmed in this analysis. Additionally, the worsening in CBCL scores seen here was not observed in the GAS trial.

In a secondary analysis considering scores after dichotomisation, the authors reported an increased risk of CBCL internalising behavioural deficit (47% increase) and impaired BRIEF executive functions (68% increase), but no multiple hypothesis testing correction is mentioned, whilst the Hohm-Bonferroni procedure was applied for the primary analysis. These discrepancies and statistical elements invite, once again, the reader to interpret these results with caution.

Neurotoxicity induced anatomical damages and deleterious neurocognitive effects

It is difficult to establish, in humans, a clear link between structural damage in the brain and the observed neurocognitive effects. In animal models, especially rodents and non-human primates (NHPs), damage to neurons and astrocytes has been associated with specific effects observed after exposure to general anaesthetics: neuroapoptosis-impaired dendritic arborisation and long-term impairments in spatial learning and memory,[6] modifications of oligodendrocytes, and decreased myelination associated with cognitive deficits.[21,24,25] More recently, an alteration of astrocytes activity, called astrogliosis, was identified 2 yr after exposure, in areas of intense apoptosis of the NHP developing brain.[33] Animal data are numerous[14,22,23,26,29–32,98,142,172] and could be compatible with results observed in humans. Deleterious neurocognitive outcomes affecting executive functions and behaviours seems to have a delayed onset, as recently shown in humans.[173] Further monitoring of these children is necessary to establish if they later develop the neurocognitive deficits identified in recent studies.

Specificity of the evaluation of the neurotoxic risk of general anaesthesia

The neurocognitive risk is different from the usual anaesthetic and surgical risks, discussed during preoperative preassessment and consent, for multiple reasons. Interestingly, even recent European studies evaluated the risks of adverse outcomes associated with neonatal anaesthesia and surgery, but did not include the neurocognitive risks associated with general anaesthesia.[174,175]

First, a fine neurocognitive developmental assessment is difficult to obtain at a young preoperative age and outside the scope of a preoperative anaesthetic assessment. Second, many confounding factors occur when patients are anaesthetised that can have an impact on their brains:

- Multimodal anaesthesia implies polypharmacy preventing the discriminant analysis of each anaesthetic drug.[176]
- Protocols are left to the discretion of the anaesthetist to achieve similar general anaesthetic outcomes, so preventing standardisation of the factors under scrutiny.[177]
- Systemic inflammatory response induced by anaesthetics and surgery releases cytokines that can cross the blood–brain barrier and interact with anaesthetic targets.[178]
- Haemodynamic and respiratory instability during general anaesthesia induces additional insults with potential impacts on the brain.[112]

Third, neurocognitive developmental insults can take time to come to light. Other medical conditions or socio-environmental factors can also have an impact on these evaluations, preventing a proper estimate of the neurotoxic risk of general anaesthesia itself. Paediatric anaesthetists are thus facing a triple challenge: absence of baseline neurocognitive assessment, multiple confounding factors during

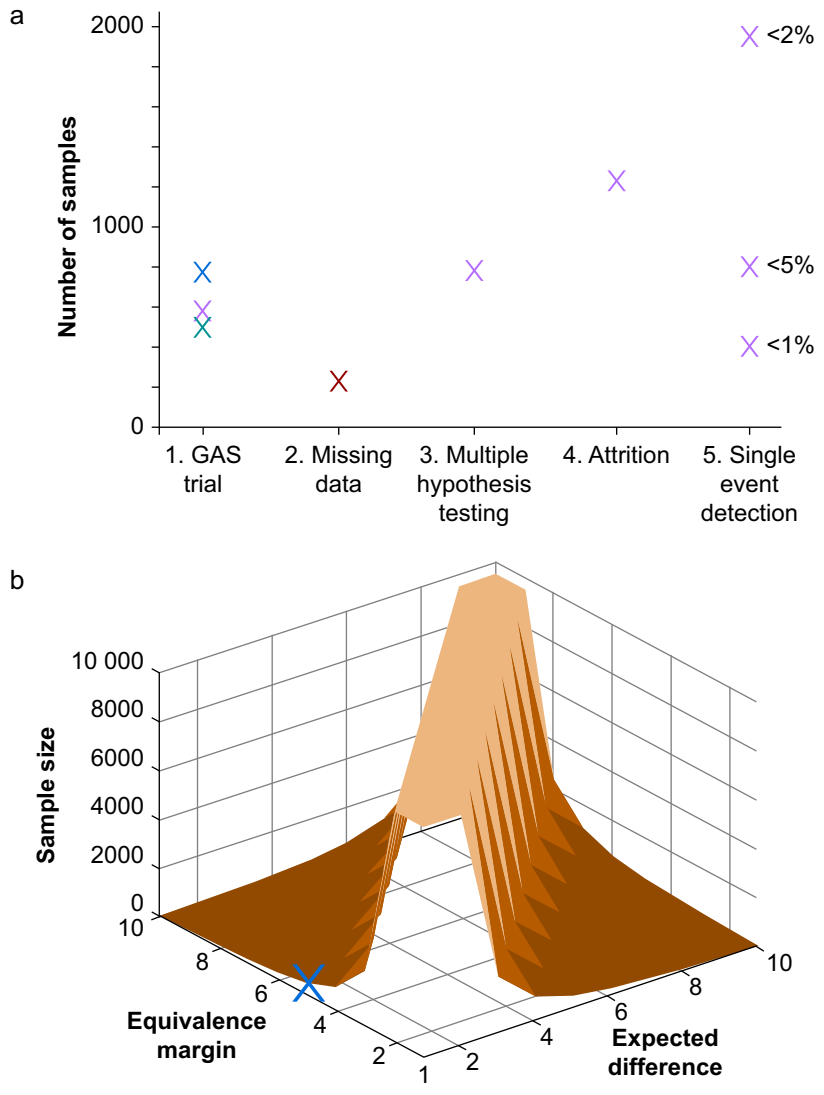

Fig 3. (a) Number of samples necessary to reach the statistical objectives of the GAS trial, from left to right: 1. Blue: targeted sample size to take into account attrition (722 children), purple: sample size obtained via sample size calculation (598 children), and green: observed sample size in the GAS trial (447 children). 2. Red: missing data treated with imputations in the GAS trial. 3. Sample size required to take into account the interim analysis at 2 yr of age (multiple hypothesis testing with the Bonferroni correction). 4. Sample size required to take into account the observed attrition. 5. Sample sizes required to control the single event rate below given thresholds in cohorts detecting no events (for one analysis only). The absence of detection of events in the GAS trial shows that the estimated proportion of the population that would experience the event has an upper 95% confidence interval limit around 1%. (b) Sample size as a function of the expected difference and equivalence margin in an equivalence trial with a power of 90% and a type 1 error risk of 5%. The sample size estimated for the GAS trial is represented in blue.

the general anaesthesia, and delayed long-term evaluation of the primary outcome.

Finally, what should paediatric anaesthetists say to the parents/carers of their patients. To the question 'Is there a neurocognitive risk?', the answer should probably be no for single exposure of <1 h to sevoflurane (primary outcome of the GAS trial) but uncertain for long exposure (effect not evaluated by the GAS trial) and highly probable for repetitive exposures (cohort studies).[155] It is obviously at the discretion of the anaesthetist to evaluate it, pondering the risk factors of each specific situation, and inform the parents if deemed necessary. The purpose is not to induce undue distress in parents, but to provide up-to-date information of the risks faced by children under general anaesthesia. It is important to nuance this risk, insisting that intellectual performances seem to be unaffected, whilst some deleterious effects may be seen, in some children, on internalised behaviours (mood disturbance, anxiety, depression, and social withdrawal) and executive

functions (inhibition, working memory, shifting, planning/organising, and emotional control) in specific circumstances. These last elements were only observed in secondary analyses and with some statistical limitations. They require further specific investigations. The GAS trial did not identify deleterious neurocognitive outcomes for a short exposure to sevoflurane (<1 h). Children having long or repetitive exposures should probably be flagged to their paediatricians or general practitioners to suggest a potential neurocognitive follow-up.

Surrogate biomarkers to evaluate neurotoxicity

There is still a grey area concerning the safety of paediatric anaesthetics. Further studies will be needed to understand which drugs are potentially less neurotoxic and under which conditions (dose, duration, repetition). New approaches are needed to tackle these issues.

One potential means of overcoming such difficulties would be to look at surrogate biomarkers, yet to be defined, that would identify instantaneous biochemical, cellular, or structural lesions induced by general anaesthesia on the developing brain. Many drugs show neuroprotective effects in animal models exposed to general anaesthesia.[179] The establishment of a phenotype of general anaesthesia could be a promising approach to understand general anaesthetics mechanisms and neuroprotective strategies.[87,115] Using magnetic resonance spectroscopy, a modality of MRI, one could measure metabolic variations induced by general anaesthesia and identify neurotoxic mechanisms, based on the expected cellular effects of anaesthetics. MRI scans also have the advantages of being considered harmless and not including other confounding effects, such as surgery.

Results so far can help us define a research framework with specific and restrictive conditions to prevent children from being exposed to harm while collecting valuable data. Based on the strongest evidence available brought by the GAS trial, it appears that, in young children with a clinical referral for a short procedure under general anaesthesia, an extension of the duration of a general anaesthesia up to 1 h in total, to complete clinical requirements and acquire research data, should not result in additional harm. A mechanistic approach would be key to identifying, explaining deleterious neurocognitive outcomes, and developing neuroprotective strategies. It would provide valuable insights for long or repetitive exposures, particularly in vulnerable children, and a platform to test the differential effects of anaesthetic drugs on the developing brain.

Conclusions

Thirty years after the initial concerning results, a large body of evidence has been gathered at the cellular, histopathological, animal model, and human levels regarding the neurotoxic effect of general anaesthetics on the developing brain. They can trigger apoptosis via different mechanisms, associated with specific biochemical patterns. Some drugs, known to be neuroprotective in the adult brain, exhibit neurotoxic effects in the developing brain. The GAS trial provides some necessary reassurance for parents and clinicians.

Are we there yet? The amount of evidence describing a neurocognitive risk after specific exposures to general anaesthesia is clear: no impact after short and single exposure, uncertainty for duration >1 h and probable deleterious neurcognitive effects for repetitive exposures. More research needs to be supported and carried out to refine this risk: understand the conditions that can trigger it (drug, dose, duration, repetition), decipher biochemical mechanisms sustaining this toxicity, identify the link between anatomical damage and neurocognitive deleterious outcomes, and design neuroprotective strategies. In daily clinical practice, these results should be considered carefully, especially outside the ASA score 1 or 2 in patients referred for elective procedures under general anaesthesia. When dealing with complex children or in emergency situations, drugs with suspected deleterious neurocognitive effects can provide the required haemodynamic or respiratory stability. Anaesthetists should keep using them until further evidence and recommendations are established.

Author's contributions

Review conception: BJB.
Selection/analysis of included articles: EJR, BJB.
Drafting of paper: all authors.
Critical review of paper: all authors.
Writing of final version of paper: BJB.
Approval of final version of paper: all authors.

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Declarations of interest

The authors declare that they have no conflicts of interest.

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