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REVIEW ARTICLES

Balancing paediatric anaesthesia: preclinical insights into analgesia, hypnosis, neuroprotection, and neurotoxicity

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Logistical and ethical reasons make conducting clinical research in paediatric practice difficult, and therefore safe and efficacious advances are dependent on good preclinical research. For example, notable advances have been made in preclinical studies of pain processing that correlate well with patient data. Other areas of paediatric anaesthetic research remain in their infancy including mechanisms of anaesthesia and anaesthetic neuroprotection and neurotoxicity. Animal data have identified the potential 'double-edged' sword of administering anaesthetic agents in the young; although these agents can be neuroprotective in certain circumstances, they can be neurotoxic in others. The potential for this toxicity must be balanced against the importance of providing adequate anaesthesia for which there can be no compromise. We review the current state of preclinical research in paediatric anaesthesia and identify areas which require further exploration in order to provide the foundations for well-conducted clinical trials.

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The deleterious effects of insufficient anaesthesia and analgesia in children were highlighted 20 yr ago.[3 4] Since then clinical and preclinical studies have demonstrated the importance of providing adequate analgesia to the young. However, we are now faced with a potentially even more vexing problem; animal research suggests anaesthetic agents may be neurotoxic to the developing nervous system[44] leaving the clinician with a dilemma of how much anaesthesia or analgesia to provide. Unfortunately, for commonly used agents, such as isoflurane, the neurotoxic burden correlates with the depth of anaesthesia.[44 46 57] A further apparent paradox is the ability for anaesthetic agents to protect the brain in pathological situations such as hypoxia–ischaemia yet also be inherently toxic.[87] It is clear that these factors, although intertwined, may be difficult to balance. We have sought to review the current literature analysing the importance of providing adequate analgesia, hypnosis, and anaesthesia in children balanced against the potential to induce neurotoxicity in the brain. A second conundrum existing between the neuroprotective and neurotoxic actions of anaesthetics is also explored.

An important, early caveat to introduce is the difficulty in extrapolating findings from the developing nervous systems of animals to humans. It is clear that direct extrapolation of rodent developmental data (an altrical species) to humans (a precocial species) may be confounded.[2 16] The understanding of neurodevelopmentally equivalent ages across species is similarly controversial. Much of the work described here has utilized 7-day-old neonatal rat pups, but the exact equivalent age in the human remains a source of discussion with estimates ranging from preterm to 1–2 yr post-term in humans[2 16 44 72] based on neuro-anatomical and neuro-physiological research. This is consistent with resistance to minimum alveolar concentration (MAC) of anaesthetic which peaks in the first year of human life and at 9 days in rats[73] (7-day-old data are not available); thus we believe the rat data could model anaesthetic effects in the first year of human life. Unfortunately, we cannot be more precise than this estimate at present. A conservative approach is therefore recommended. Hasty extrapolation of any findings to

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humans would be inadvisable, but inappropriate dismissal of the preclinical findings could prove more detrimental. Rodent data should form the foundation for future studies involving anaesthetic regimens in non-human primates or clinical studies (where appropriate) to inform us about the potential vulnerability of the developing human nervous system. Until we understand the consequences of these findings, in these latter models, we should remain cognizant of the potential effects of our pharmacopoeia on development.

Notwithstanding these concerns, preclinical data have explained much about the developing human and will continue to be a test-ground for future clinical exploitation. Rather than be daunted by the difficulties in applying preclinical research, we should be buoyed by the advances made to date and continue to explore possible preclinical solutions to clinical problems.

Preclinical advances in analgesia

Impact of nociception on neurodevelopment

Data from animal studies suggest that painful experiences in early life have long-term consequences. During growth, neuroplasticity keys the development of appropriate sensory and motor processing; experience of severe pain in the very young provokes neuroplastic changes in the central nervous system (CNS) that causes hyperalgesic responses to noxious stimuli later in life[83 97] with associated neuroendocrine disturbance.[33] Animal data demonstrate that skin injury in newborn rats causes an acute expansion of the C fibre termination field with denser CGRP-positive terminals in lamina II of the dorsal horn[100] and neonatal skin wounds result in prolonged hyperinnervation of the wound site[80] with permanent expansion of dorsal horn receptive fields.[101] Importantly, unattenuated pain can provoke cell death in cortical and thalamic, hypothalamic, amygdaloid, and hippocampal areas of the neonatal rat brain with subsequent neurocognitive impairment such as the impaired formation of memory.[5] Thus, the importance of combating pain in the young to avoid adverse neurodevelopmental changes is clear.

The clinical correlate of these findings was demonstrated by Taddio and colleagues,[97] who followed up 87 male infants in three groups (uncircumcised or circumcised with the intervention of Emla cream or placebo) and analysed their behavioural responses to vaccination at 4–6 months of age. The uncircumcised group exhibited the lower pain scores whereas those circumcised with placebo demonstrated the highest pain scores. Surgery in the first 3 months of life leads to higher analgesic requirements with subsequent surgery (even up to 2 yr later) when compared with children without previous operations.[75] Changes in pain sensitivity of children, who had previously been treated in the neonatal intensive care and undergone repeated painful procedures, have been recorded up to 14 yr later.[35] Exactly how long these developmental changes persist is unknown; however, in aggregate, the preclinical and clinical evidence suggests that unopposed painful states predispose to persistent hyperalgesia. Experience of pain in the young can also result in hypoalgesia.[1 13] Whether hypo- or hyperalgesia is provoked is likely to be related to the age at which pain is experienced. Hypoalgesia appears to follow pain experienced by preterm babies and hyperalgesia follows pain experienced by post-term babies. Interestingly, while hyperalgesia readily develops in the paediatric population, neuropathic pain does not follow neonatal nerve damage as it does in the adult,[37] paralleling the clinical finding of a lack of chronic pain from brachial plexus avulsions at birth.[6]

Development of the pain response

Two recent studies have shown that activation of the somatosensory cortex occurs after the painful stimulation of venipuncture or heel-lancing in preterm human neonates[10 94] providing further evidence of higher level processing of painful stimuli in the very young (i.e. at 25 weeks gestation). Neuro-anatomical arguments concerning the onset of pain perception suggest that 7 weeks post-conception (the onset of free nerve endings)[25] is the earliest that pain sensation could be recognized. Thalamo-cortical connectivity occurs by 12–16 weeks with more mature-type connectivity occurring by 23–25 weeks.[26] Therefore, the anatomical and the physiological pathways required to 'feel' pain are present by 25 weeks, possibly earlier. These data provide the basis for the clinical practice of providing adequate analgesia during painful procedures early in life.

Development of the antinociceptive system

In the very young, descending inhibitory neurones (DINs) that connect supraspinal centres to the dorsal horn of the spinal cord and provide potent antinociception are absent.[26] This, in part, explains why the young are sensitive to acute pain; for example, preterm infants have a dorsal cutaneous flexor reflex reaction to pain that is twice as sensitive as that of term infants.[8 26] Likewise, term infants are more sensitive to pain compared with older children and adults.[1]

Fortunately, an endogenous antinociceptive system at the level of the spinal cord also exists[26 48] that may compensate partly for the lack of a functional DIN early in life. In the young, both GABAergic synapses and novel co-synapses for both GABA and glycine provide a functional inhibitory system to reduce neuronal excitability.[26 48] Further preclinical research is required to determine whether neuraxial targeting of GABAergic signalling will provide effective analgesia in the young; this mechanism may also underlie isoflurane's antinociceptive efficacy in young animals.[88]

Opioid analgesia

Along with increased pain sensitivity in the young, increased sensitivity to the analgesic effects of opioids is also noted,[67] although this appears related to both gender[13] and the type of painful stimulation.[1] In the young, opioid receptors are expressed on Aβ, and Aδ and C fibres. This may explain why morphine provides greater analgesia against mechanical stimulation (requiring all three nerve types in the young) than against thermal pain stimulation (requiring Aδ and C fibres only) early in life.[68] Furthermore, in the young, Aβ fibres play a greater role than C fibres in the development of central sensitization, explaining the sensitivity to opioid-induced analgesia. However, morphine is only able to prevent the long-lasting hyperalgesia associated with neonatal pain in male but not in female rat pups.[13] Morphine administration in the absence of painful stimulation also promotes hypoalgesia in males in later life, underpinning the importance of prudent analgesic therapy in the young.

N-*methyl-D-aspartate antagonist analgesia*

Ketamine, nitrous oxide, and xenon are antagonists at the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor that plays a prominent role in nociception. Clinical evidence suggests that ketamine is a potent analgesic drug in the paediatric population.[1] While ketamine has been shown to induce neurotoxicity in the young,[39 76] ketamine also inhibits pain-induced neurotoxicity in the neonatal rat brain (important to later discussions on the neurotoxicity of anaesthetics). However, ketamine administration prevents the long-term hyperalgesia after neonatal pain only in female not in male animals[5] (in keeping with female sensitivity to NMDA antagonist anaesthesia[31] and neurotoxicity in adult models).[43] Thus, it is possible that there is also a gender-based sensitivity to the analgesic properties of NMDA antagonists. Furthermore, in combination with the evidence presented above, it would appear that males are relatively more sensitive to morphine analgesia and females to ketamine analgesia. Clinical exploration of these findings is required.

Nitrous oxide induces antinociception by the supraspinal stimulation of opioid and adrenergic centres activating DINs. However, these DINs are not functional in the young and, therefore, we hypothesized that nitrous oxide may be an ineffective antinociceptive agent in the very young. Nitrous oxide did not produce antinociception in the tail flick test,[29] or the formalin test,[71] in young animals. In contrast, xenon was antinociceptive for the formalin test in young and adult animals[59] consistent with more potent effects in the spinal cord[105] and at the NMDA receptor.[27]

α_2 adrenoceptor agonist analgesia

α_2 adrenoceptors are present in the spinal cord from birth[92] and the highly selective α_2 adrenoceptor agonist, dexmedetomidine, is antinociceptive at all ages tested from infant rats (7 days old) to adults.[86] Walker and colleagues[102] established the efficacy of neuraxially administered dexmedetomidine at early stages of rat development supporting recommendations for the clinical use of α_2 adrenoceptor agonists.[12]

Local anaesthetics

Regional anaesthetic techniques to provide analgesia in the very young have a proven safety and efficacy record in clinical practice.[12 15 53] Indeed, preclinical evidence suggests that the local anaesthetic, bupivacaine, may be more potent at producing analgesia in the very young than in older ages[36] supporting the use of regional techniques in the young.[12]

Summary

Preclinical evidence suggests that unopposed pain states produce long-term effects including hypoalgesia, hyperalgesia, neuroendocrine, and cognitive changes and this is supported by the currently available but limited clinical data. Therefore, the importance of prudent analgesic therapy is clear. Animal data support the use of regional and local anaesthetic techniques with increased susceptibility to local anaesthetics and adjuvants such as α_2 adrenoceptor agonists. Clinical investigation into the potential sex differences of analgesia with opioids and NMDA antagonists is required to inform us how best to use these agents in the young. Finally, preclinical data suggest that nitrous oxide may be an ineffective analgesic in the young.

Preclinical advances in understanding sedation/hypnosis

Advances in understanding the neuronal circuitry underlying the hypnotic pathways of anaesthetic action has recently made great strides with the discovery that anaesthetics act on endogenous sleep pathways.[21 69 70] However, these studies have been conducted entirely in the adult phenotype and anaesthetics exhibit significant pharmacodynamic differences at younger ages. Furthermore, sleep and EEG patterns are different in the young indicating differences in arousal and sleep pathways. The balance of REM and NREM sleep and the day–night cycle shifts during development[7] as does the rapidity with which sleep–wake cycles occur.[47] These differences may be attributable to the relative inactivity of the newly discovered orexin system which is an excitatory (arousal promoting) peptide neurotransmitter that activates G protein-coupled receptors.[84] Early in development ($\leq$15 days in rats) prepro-orexin is only weakly expressed in the hypothalamus.[109] However, after this time orexin signalling increases significantly. Orexin is thought to stabilize the wakefulness/sleep 'flip-flop switch' in the adult[91] and its relative deficiency may underlie the relatively rapid sleep–wake cycles in the young.[14] As suppression of orexin signalling appears to have a role in the anaesthetic state,[50] this may in part explain the sensitivity of the young to the hypnotic

effects of anaesthetic agents.[86 88 102] Thus, the relative deficiency of orexin may sensitize the young to the hypnotic qualities of anaesthetic agents. In addition, developmental shifts in the expression and function of other critical receptors involved in the anaesthetic state such as the $GABA_A$ receptor[42 81] and two pore domain potassium channels[108] are also likely to play a role. Furthermore, over the first postnatal week in rats, the locus coeruleus increases control over sleep–wake cycling[47] and it is thought that in the rat, this control peaks at approximately 7 days after birth when rat pups are extremely sensitive to the hypnotic effects of the α_2 agonist dexmedetomidine,[86] showing 10-fold enhanced sensitivity compared with that seen in adults.[70 86] Therefore, there appear to be multiple factors that, in the aggregate, will at least in part explain the sensitivity to the hypnotic qualities of anaesthetic agents in the young.

Although the young are sensitive to the hypnotic effects of anaesthetics, they also appear relatively resistant to the anaesthetic effects when assessed by MAC that also depends on spinal cord responses. For example, young rats are more susceptible to the hypnotic effects of isoflurane than the adult (assessed by loss of righting reflex)[88] (Fig. 1) yet they are relatively more resistant to the anaesthetic level (as assessed by MAC).[73] The latter is well described in humans, but there is a paucity of data relating to the hypnotic sensitivity of children. From the animal data, the fraction of MAC required to induce hypnosis in the 7–9-day-old pup is 10% whereas in older animals the value is much higher; in 30-day-old rats (estimated to be in late childhood in human terms) this value is 33% and in the adult this value is 50%. Thus, in younger animals, there is greater separation between the amount of isoflurane to make the animal 'sleep' and anaesthetize the animal. In the 7-day-old rat, this difference is 10-fold, in the adult there is a <2-fold difference. If these data can be extrapolated to humans, they suggest that when anaesthetizing children, there may be a larger window in which a young patient appears 'asleep' but is not anesthetized and therefore is vulnerable to the effects of ascending arousal stimuli. Clinically, this may account for increased incidence of awareness in the paediatric population which is 0.8–1.2%.[18 55] There is some evidence that classical signs, for example, movement, do not precede awareness in anaesthetized children.[17] It is conceivable that vulnerable children externally appear 'asleep' but are insufficiently anaesthetized. If an increased incidence of awareness is confirmed, it follows that a depth of anaesthesia monitor may be even more useful in paediatric anaesthesia. However, the notable EEG differences between children and adults[20] suggest extrapolation of EEG studies from adults to children may be imprudent and therefore focused paediatric research will be required in this field.

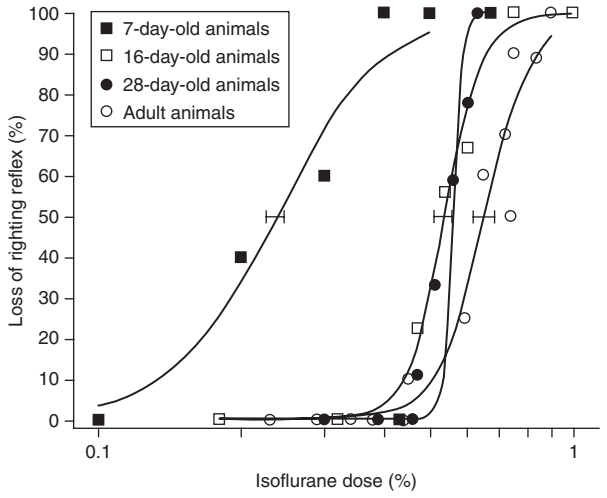

Fig 1 Log dose–response curves of the hypnotic effect of isoflurane at different ages in Fischer rats ($n = 8$–10). LORR (expressed as percentage of animals) was used to define the onset of hypnosis. Seven-day-old rats have lower ED_{50} (0.25%) than 16-day-old (0.54%), 28-day-old (0.56%), and adult (0.65%) rats ($P < 0.01$). Adult rats have significantly higher ED_{50} than younger rats ($P < 0.05$). Reproduced with permission from the *British Journal of Anaesthesia*.

Summary

Further research into the neural networks and molecular mechanisms mediating paediatric anaesthesia will hopefully allow tailoring of the drugs we use to provide even better balance to the delivery of anaesthetic care. Clinical research into the MAC fractions (such as MACawake) in children is also required.

Note added in proof

A recent publication has analysed the MAC fractions in children more closely and found the MAC-awake fraction to be lower in children aged 5–8 and 8–12 years old than in adults, which is consistent with the animal data described earlier. However, the margins are narrower than demonstrated in animals (Davidson AJ, Wong A, Knottenbelt G, *et al*., MAC-awake of sevoflurane in children. *Paediatr Anaesth* 2008; **18**: 702–7).

Preclincial advances in neuroprotection

Provision of neuroprotective strategies by anaesthetists may be required for perinatal hypoxic–ischaemic encephalopathy (HIE), traumatic head injury, and major cardiac and neurosurgery. Currently, only hypothermic neuroprotection has been shown to improve clinical outcome, albeit modestly; additional benefit will require adjunctive agents,[52 60] though further research is required on the use of hypothermia for perioperative neuroprotection.

Neuroprotection research in the paediatric population has centred on perinatal HIE which occurs in approximately 1–2 per 1000 full-term live births and until recently was bereft of interventions. For example, cardiotocography monitoring has reduced the incidence of neonatal seizures but not HIE.[98] The pathogenesis of perinatal

hypoxic–ischaemic injury differs from adult hypoxic–ischaemic injury with a greater degree of programmed (apoptotic) rather than necrotic cell death.[66] Therefore, anti-apoptotic strategies may have greater impact in neonatal and paediatric neuroprotection. Primary energy failure in the brain due to ischaemia-induced ATP depletion results in cells being unable to maintain ion gradients leading to cellular depolarization, glutamate release, and excitotoxicity, with necrotic injury occurring rapidly. Apoptosis is provoked either by death of an innervating cell leading to a trophic deprivation injury or by a toxic stimulus that is insufficient to cause necrosis. It is an energy-driven process that takes several hours to develop, providing an opportunity for post-injury treatment strategies.

Infection increases the brain's vulnerability to hypoxic–ischaemic injury by exacerbating inflammatory processes and increasing blood–brain barrier permeability. In the perinatal setting, the proinflammatory cytokine, IL-6, is associated with increased risk of cerebral palsy and periventricular leukomalacia.[111] This is corroborated by exacerbation of hypoxic–ischaemic injury in the neonatal rat after lipopolysaccharide (gram-negative endotoxin) administration.[24] In a similar manner, the systemic inflammatory response that accompanies cardiac or other surgery may also contribute to any consequent neurological injury.

Hypothermic neuroprotection

The clinical evidence for hypothermic neuroprotection in the paediatric population followed subgroup analysis from the *Cool Cap Study* that demonstrated significant benefit of mild hypothermia (34–35°C) initiated within 6 h and administered for 72 h in mild to moderate injury due to perinatal HIE but not in the most severely injured neonates (NNT=6).[30] A second large randomized control trial of neonates with HIE due to acute perinatal asphyxia demonstrated that systemic hypothermia (33.5°C) for 72 h reduces death and moderate or severe disability by 18% (NNT=6).[93] Hypothermia reduces cellular metabolism and targeting both excitotoxic and apoptotic cell death processes, including reduction of glutamate release, the apoptotic cascade, and neuroinflammation.

Importantly, hypothermia, in the absence of anaesthesia, is not neuroprotective in neonatal piglets.[99] These findings suggest that either the 'stress' response to the hypothermia is deleterious or that anaesthesia/sedation (or the agent) itself contributes to the protection. Indeed, there are significant preclinical data suggesting that anaesthetic agents themselves are neuroprotective, however the potencies for different agents vary. Some agents, such as xenon, provide protection at subanaesthetic doses and others, such as the volatile anaesthetics, require anaesthetic doses to induce neuroprotection.[87]

Xenon neuroprotection

Investigation of xenon's neuroprotective properties was stimulated by the finding that it inhibits the NMDA subtype of the glutamate receptor.[27] Studies have shown that xenon is an anti-apoptotic neuroprotective when given before, during, or after a hypoxic–ischaemic insult.[60 61 87] In combination, hypothermia and xenon are also synergistic providing potent neuroprotection in a model of perinatal HIE, even when xenon was administered in subanaesthetic doses (0.3 MAC; see Fig. 2)[60] or even asynchronously, indicating that xenon was not solely working via a sedative mechanism.[64] Asynchronous administration of the two interventions also provided post-insult protection even when the interval between the two interventions was 5 h.[64] Not only does this separate xenon's neuroprotective effects from its anaesthetic actions but also provides potential for asynchronous clinical application of the two therapies where hypothermia can be initiated early before transfer to a tertiary centre for xenon administration. Xenon also interacts synergistically with dexmedetomidine to provide neuroprotection in this model.[78] Importantly, xenon neuroprotection against neonatal HIE has also been confirmed in animals by another research group.[22] Before translating this to a clinical trial for HIE, it will be necessary to demonstrate the xenon–hypothermic interaction in a larger mammalian species in order to address issues related to a closed, recirculating delivery system.

Volatile anaesthetic neuroprotection

Desflurane (9%) has proven effective in newborn piglet models of deep hypothermic cardiac arrest[49] and low flow cardiopulmonary bypass[54] confirmed functionally and histopathologically; dose–response studies are required to characterize the interaction between volatile anaesthesia and hypothermia to assess its possible utility. A significant reduction in GABAergic neurone expression is noted after human perinatal brain injury;[82] therefore, therapeutic interventions that act through activation of GABA receptors may prove less effective for post-injury treatment.

α_2 adrenoceptor agonist neuroprotection

The neuroprotective effects of clonidine and dexmedetomidine have been demonstrated in mouse pups injected intracerebrally with the NMDA receptor agonist ibotenate, thus demonstrating efficacy against excitotoxic injury.[51 74] Recently, we have shown that dexmedetomidine concentration-dependently diminished neuronal injury provoked *in vitro* and infarct size *in vivo* in a perinatal HIE rat model[58] and this correlated with improved neurological function. Dexmedetomidine's effects appear to be mediated by the α_{2A} adrenoceptor[58 74] and thus there is scope for development of more specific agonists for this effect in the future. Importantly, anti-apoptotic effects also

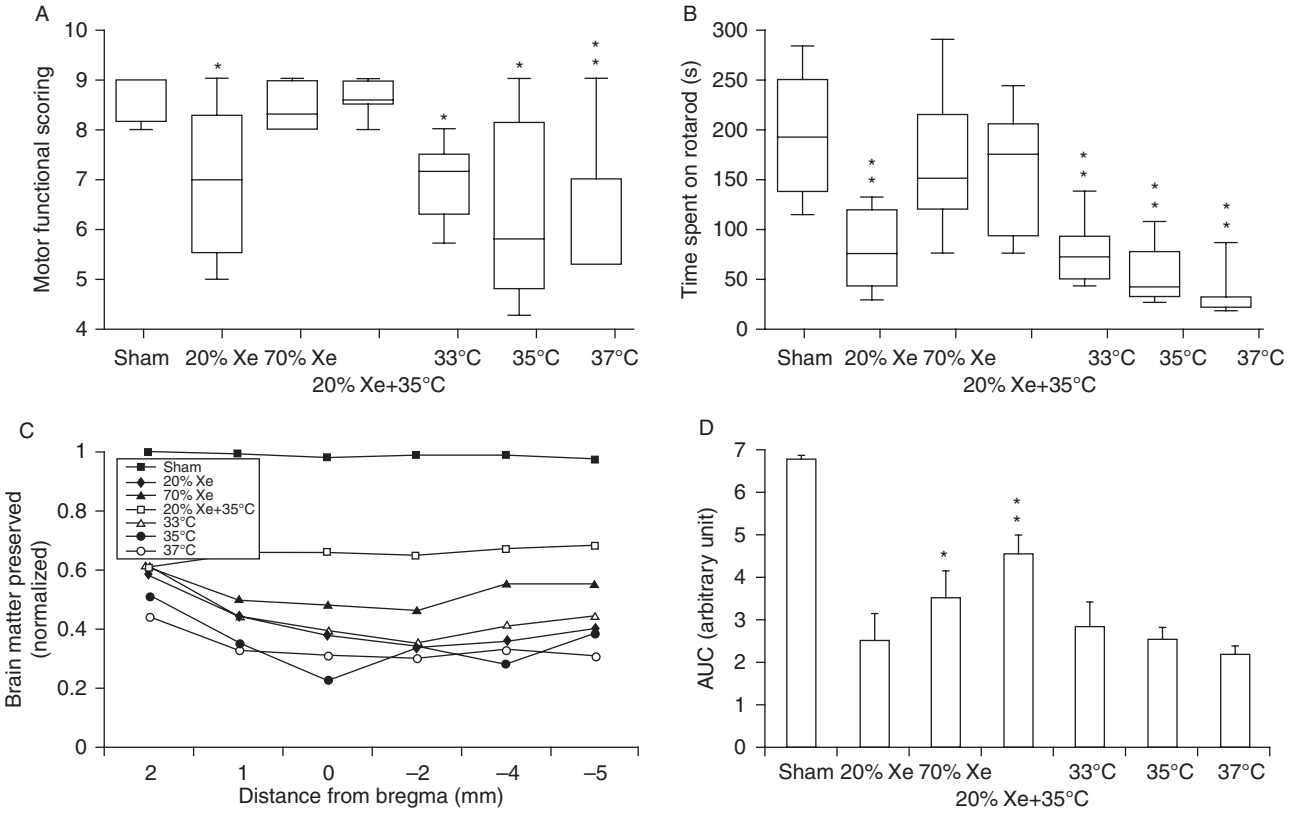

Fig 2 Xenon and hypothermia improve neurological function and attenuate brain matter loss 30 days after neonatal hypoxic–ischemic injury. Four hours after the injury, neonatal rats received either hypothermia (33°C or 35°C), xenon (20% or 70%), or a combination of these two. Effect of intervention on neuromotor function (A) and time spent on a rotarod (B) are shown. Higher scores indicate superior performance. $*P < 0.05$; $**P < 0.01$ *vs* control ($n = 6-8$). (C) The preservation of brain matter was assessed in slices obtained from six contiguous regions referred to as distance from the bregma. Data are presented as the ratio of brain matter on the lesioned hemisphere relative to the unlesioned hemisphere. Again higher scores indicate less hemispheric injury. (D) Area under the curve (AUC) was derived from (C) with variations. $*P < 0.05$; $**P < 0.01$ *vs* 37°C group ($n = 5$). Reproduced with permission from the *Annals of Neurology*.

appear to contribute to the neuroprotective profile[41 85 89] suggesting it may be effective for post-insult application. The interaction of dexmedetomidine and hypothermic neuroprotection would be of great interest, especially as dexmedetomidine is a sedative and anti-shivering agent used in critical care.[85]

Preconditioning

Preconditioning is a process whereby a sub-injurious stimulus can increase the cell, tissue, or organ's tolerance to withstand a subsequent injury-provoking stimulus. Much scientific interest has focused on the ability of transient hypoxia to prepare a fetus for a more severe neurological insult in the peripartum period.[34] Certain pharmacological agents can also precondition against ischaemic neurological injury including the NMDA antagonists.[79] Xenon, but not nitrous oxide, provides potent neuroprotection when given as a preconditioner in this setting[61] (Fig. 3) and thus may have potential to prepare a fetus against perinatal brain injury. Volatile anaesthetics can also precondition the neonatal brain.[113] For example, sevoflurane (1.5%) can precondition against HIE in rat pups but had no effect at the labour analgesia dose of 0.8% limiting its clinical application.[62] Interestingly, xenon (20%) and sevoflurane (0.75%) were synergistic in their ability to precondition in this model.[62] In the future, inhalation analgesia in obstetrics with this combination may provide neuroprotective benefits to the fetus.

Summary

There is a wealth of preclinical data showing that anaesthetics can provide neuroprotection against toxic insults to the brain. The neuroprotective potential of anaesthetic agents must also be balanced against the potential to do harm. Recently, the administration of anaesthetic agents in the young has also been associated with neurotoxicity.

Preclincial advances in understanding the neurotoxic potential of anaesthetics

The vulnerability of the neonatal brain to toxins such as alcohol has been well described and results in physical and neuropsychiatric problems referred to as fetal alcohol

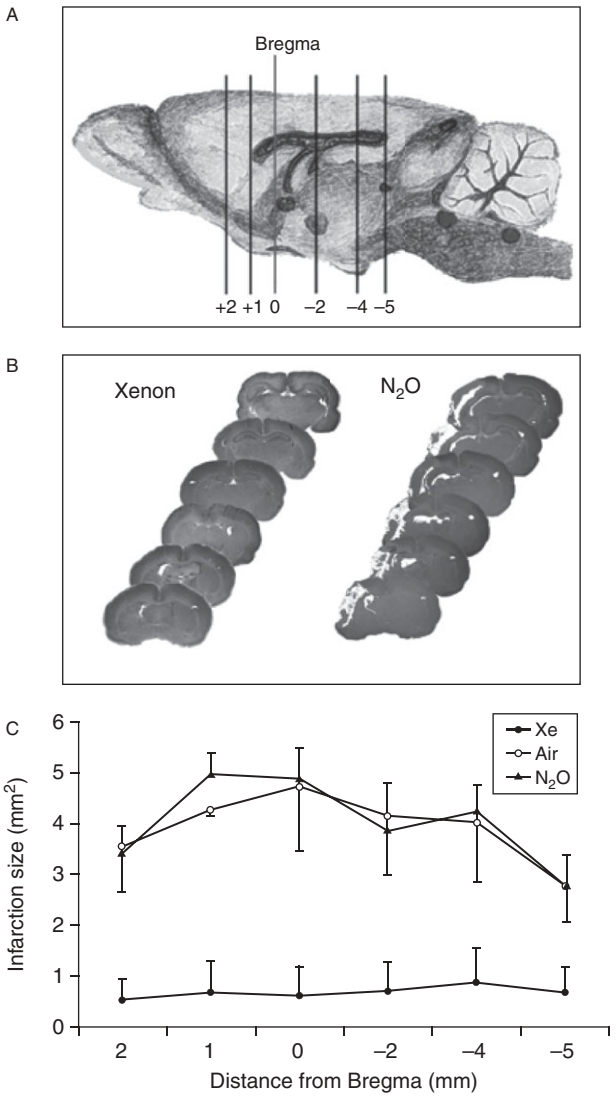

Fig 3 Effect of preconditioning against neonatal hypoxia–ischaemia on focal infarction size. After exposure to 2 h of xenon (70%), N_2O (70%), or air, 7-day-old rat pups underwent hypoxic–ischaemic injury 4 h later. Infarct size was assessed 4 days later. (A) Schematic graph of pup's brain and the location of representative sections harvested. (B) Representative sections from a pup preconditioned with either 70% xenon or 70% N_2O. (C) Mean infarction size at six adjacent slices relative to the bregma (+2, +1, 0, −2, −4, and −5 mm) after 70% xenon or 70% N_2O compared with air. Reproduced with permission from the *Journal of Cerebral Blood Flow and Metabolism*.

syndrome. Animal data suggest the neurotoxic effects are due to apoptotic neurodegeneration.[38] The mechanism underlying alcohol-induced neurodegeneration is thought to be due to the block of neuronal firing during a critical period of synaptogenesis with a reduction in trophic signalling causing the nerve to be eliminated.[72] Data from monkeys show that preventing synaptic transmission causes deleterious long-term cortical changes.[40] Ethanol interrupts synaptic transmission by a combination of activation of $GABA_A$ and antagonism of NMDA receptors, thereby triggering trophic deprivation. This has led to concern about the use of anaesthetic agents in the young as they also act at these receptors.

Inhalation anaesthetics

In 2003, Jevtovic-Todorovic and colleagues reported apoptotic neurotoxicity in 7-day-old rat pups exposed to 6 h of anaesthetic. The neurodegeneration was associated with learning and memory impairment up to 124 days later in adulthood[44] (Fig. 4). The neurotoxicity is also present after relatively short exposure periods and subanaesthetic isoflurane exposure for 1 h has recently been shown to also provoke apoptosis.[46] Furthermore, we and others have shown that the isoflurane injury occurs in *in vitro* hippocampal slice cultures, even at subanaesthetic doses.[63 107] These *in vitro* data indicate that physiological disruption of homeostasis during the anaesthetic state cannot account for the toxicity observed, rather the toxicity represents a direct insult to the brain.

I.V. anaesthetics

In a follow-up study by Olney's group, single doses of ketamine (20 mg kg^{-1} and above) or midazolam (9 mg kg^{-1}) induced apoptotic neurodegeneration in infant mice.[112] These apparently large doses are required to mimic the anaesthetic behavioural endpoint.[32] We consider this behavioural endpoint a more appropriate approach than measuring the pharmacokinetic endpoints (i.e. drug concentrations in the blood) to assess whether *anaesthesia* is toxic. This argument will hold as long as there is significant overlap between the mechanism of the toxicity of anaesthetics and the mechanism of their anaesthetic action (discussed below); if, subsequently, the toxicity appears to be related to specific drugs, then blood concentrations will become a more significant endpoint. Thus, rejection of drug toxicity purely based on overly 'high' blood concentration appears to be ill-considered at this early stage of research. Finally, as 1 h of isoflurane induces the injury at subanaesthetic concentrations in rat pups (assessed by pharmacokinetics or dynamics), dismissal of this toxicity would appear premature (Table 1).[46]

A further study has demonstrated that exposure to propofol or ketamine alone or in combination produced apoptosis in 10-day-old mice and the combination of propofol or thiopental and ketamine produced functional deficits in adulthood.[28] The affected groups showed altered spontaneous locomotor activity, deficits in spatial learning, and altered responses to diazepam in adulthood. Thus, this phenomenon affects both rats and mice using different anaesthetic agents and producing long-term functional effects.

A pivotal study has also demonstrated this injury in the monkey brain. This is critical as the monkey CNS is more likely to parallel the human CNS in terms of neurodevelopment. Ketamine was initially administered intramuscularly and then followed by an infusion with good

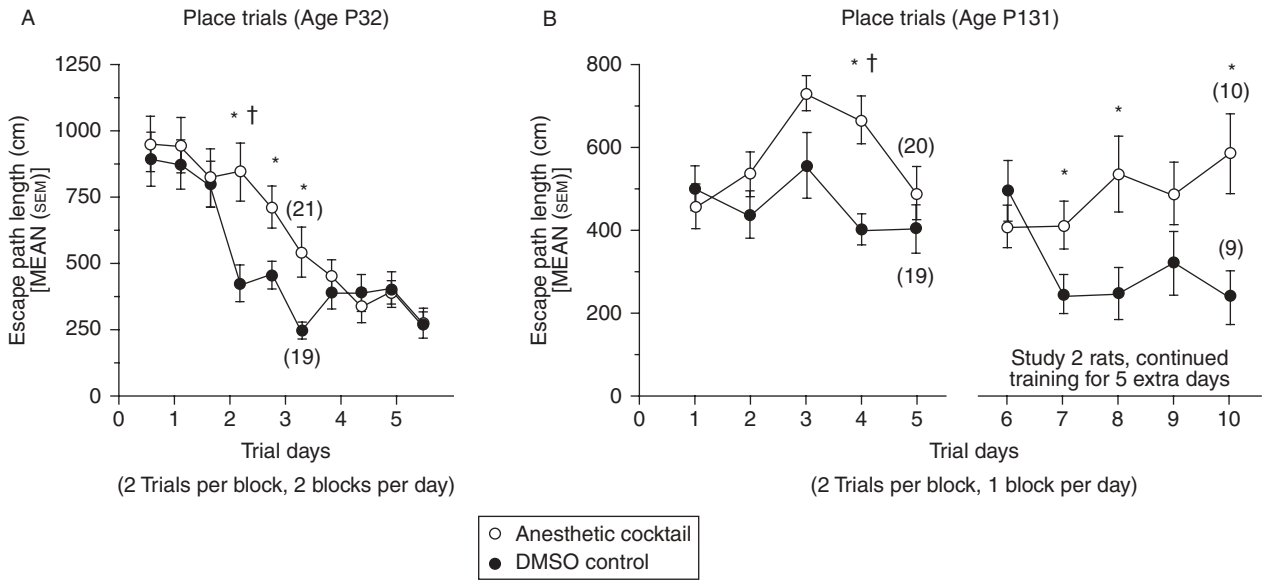

Fig 4 Effects of neonatal triple anaesthetic cocktail treatment (midazolam 9 mg kg^{-1}, isoflurane 0.75%, and nitrous oxide 75%) on spatial learning. (A) Rats were tested on post-natal day 32 (P32) for their ability to learn the location of a submerged (not visible) platform in a water bath (the Morris Water Maze). An ANOVA of the escape path length data yielded a significantly longer path length after treatment with midazolam, nitrous oxide, and isoflurane ($P = 0.032$) and a significant anaesthetic effect by blocks of trials interaction ($P = 0.024$), indicating that the cognitive performance of the rats that received the anaesthetic were significantly inferior to that of control rats during place training. Subsequent pairwise comparisons indicated that the differences were greatest during blocks 4, 5, and 6 ($P = 0.003$, 0.012, and 0.019, respectively). However, the rats receiving the anaesthetic cocktail improved their performance to control-like levels during the last four blocks of trials. (B) Rats were retested as adults (P131) for their ability to learn a different location of the submerged platform. The graph on the *left* represents the path length data from the first five place trials when all rats were tested. An ANOVA of these data yielded a significant main effect of treatment ($P = 0.013$), indicating that the control rats, in general, exhibited significantly shorter path lengths in swimming to the platform compared with anaesthetic cocktail rats. Subsequent pairwise comparisons showed that differences were greatest during block 4 ($P = 0.001$). The graph on the *right* shows the data from study 2 rats that received 5 additional training days as adults. During these additional trials, the control group improved their performance and appeared to reach asymptotic levels, whereas the anaesthetic cocktail rats showed no improvement. An ANOVA of these data yielded a significant main effect of treatment ($P = 0.045$) and a significant treatment by blocks of trials interaction ($P = 0.001$). Additional pairwise comparisons showed that group differences were greatest during blocks 7, 8, and 10 ($P = 0.032$, 0.013, and 0.017, respectively). Reproduced with permission from the *Journal of Neuroscience*.

Table 1 Controversies in neonatal anaesthetic neurotoxicity research

Criticisms that the doses of i.v. agents used are clinically irrelevant are confounded by (i) pharmacodynamic argument (animals require higher doses to induce anaesthesia) and (ii) isoflurane induces neuroapoptosis at subanaesthetic doses. Further studies dissociating anaesthesia and the neurotoxicity may lead to safer drugs
Duration of anaesthetic exposure is similarly controversial as 4–6 h may equate to a proportionally longer period of brain development in the rat than in the human neonate as the rat brain develops over weeks and human brain over years. However, as anaesthetic injury has recently been shown to occur with subanaesthetic isoflurane exposure for 1 h, this argument appears to have been weakened
Species differences likely contribute
Systemic effects (hypotension, hypoglycaemia, and hypoxia) have been implicated, though this can largely be discounted because (i) blood gases appear to be unaffected and (ii) the injury occurs *in vitro* where oxygen and glucose are readily controlled
The difference in findings between different research groups provokes concern, though most differences are likely species dependent or related to experimental protocols

control of physiological parameters. Significant cortical neuroapoptosis occurred when 24 h ketamine (to maintain a surgical plane of anaesthesia) was given to the fetus or in 5-day-old neonates but not in 35-day-old monkeys,[95] complementing monkey cortical neurone *in vitro* data showing ketamine is directly neurotoxic.[104] Plasma concentrations of ketamine in the 5-day-old monkey were three to five times greater than equivalent doses in humans, although this was the dose required to produce a surgical plane of anaesthesia. Crucially, injury did not occur when ketamine was infused for only 3 h. Thus, the monkey brain is vulnerable to ketamine injury, but the time course of the injury suggests that its use in general anaesthesia for short operations may not be toxic, although it must be noted that this is a small study and that cognitive function was not analysed. The doses given in this study were still in excess of those used in clinical practice, but a good functional/pharmacodynamic endpoint was used. This study demonstrates that drugs used in anaesthetic practice have the capability to induce neurodegeneration in an age- and duration of exposure-dependent manner. These findings are in the main reassuring for anaesthetic practice. However, the neurotoxicity associated with longer episodes of sedation/anaesthesia suggests that neonates sedated in the critical care environment may be vulnerable.

Is surgery important?

As noted earlier, unopposed pain states also lead to the long-lasting neuronal dysfunction including cognitive impairment[5] and, in this setting, ketamine administration prevented the pain-induced cognitive impairment. This is important as all the sedative regimes tested so far have been done in the absence of surgical or painful stimulation. Therefore, the experiments demonstrating harm from sedative agents may be more relevant to the critical care setting or anaesthesia in the absence of surgery such as for imaging studies. Surgical stimulation could in theory balance the anaesthetic injury, by increasing neuronal activity, thus mimicking the described interaction with ketamine and pain.[5] It is also conceivable that surgery may exacerbate any injury, as we have observed in an adult rat model of postoperative cognitive dysfunction.[103] Further preclinical studies with concurrent surgery are urgently required to explore this potential protective or deleterious effect of surgical stimulation.

Studies dissociating anaesthesia and neurotoxicity

As NMDA antagonists, such as ketamine, are broadly implicated in the provocation of this neuro-apoptotic phenomenon, we have investigated whether xenon itself is neurotoxic or exacerbates isoflurane induced neurodegeneration. Xenon itself lacked neurotoxicity and protected against isoflurane neurodegeneration in neonatal rats *in vivo* and *in vitro*.[63] Thus, unlike other NMDA antagonists, xenon lacks neurotoxicity and can prevent injury from other anaesthetic agents in both young and adult animals.[65] As xenon exhibits cardiostability, it may have a role in neonatal anaesthesia as the neonatal myocardium is particularly sensitive to the depressant effects of the volatile anaesthetics.[11]

We have also investigated whether the anti-apoptotic effects of dexmedetomidine could prevent isoflurane-induced neurodegeneration. Dexmedetomidine reduced the number of apoptotic neurones in the neonatal rat cortex, thalamus, and hippocampus induced by isoflurane (0.75%) administered for 6 h.[41 89] In addition, dexmedetomidine did not induce neurotoxicity even when given at 75 times the ED_{50} for hypnosis.[41 89] Dexmedetomidine may have utility to prevent anaesthetic-induced injury in the perioperative period and use as a sedative in critical care involving long-term sedation of neonates or children.[85]

As the anaesthetic state is not necessarily associated with neurodegeneration when isoflurane is combined with dexmedetomidine or xenon (i.e. there is no obligate association between anaesthesia and toxicity as both dexmedetomidine and xenon would deepen the anaesthetic state), we have sought to clarify the mechanism of isoflurane-induced neurotoxicity. Using gabazine, a $GABA_A$ antagonist, we attempted to clarify the role of the $GABA_A$ receptor in this injury as it has been implicated as mediating the injury of anaesthetics[44] and likely plays a role in the isoflurane anaesthetic state.[44 69] Gabazine did not attenuate the isoflurane injury as predicted indicating there may be differences in the mechanisms of the anaesthetic state and the toxicity observed.[41]

Isoflurane also inhibits the NMDA receptor which is an alternative mechanism for the observed injury.[46] This mechanism is consistent with data demonstrating that xenon is protective against isoflurane injury in the young and can inhibit ketamine neurotoxicity in the adult (thus potentially protecting against NMDA antagonist injury at different ages).[65] However, despite stereospecific potencies at inhibiting the NMDA receptor, ketamine does not induce apoptosis in a stereospecific manner in the neonatal rat suggesting NMDA antagonism may not be responsible.[96] Nonetheless, these data do further dissociate the mechanisms for anaesthesia and neurotoxicity in the neonatal rat as ketamine anaesthesia is stereoselective but the toxicity is not.[96]

Interestingly, exogenous administration of 17β-estradiol attenuates the neurotoxicity induced by phenobarbital, phenytoin, and, the NMDA antagonist, MK-801, in the neonatal rat brain.[9] However, recent evidence suggests that long-term treatment with oestradiol may alter neuronal development and therefore further safety data are required before extrapolation to clinical settings.[77] Erythropoietin (EPO) also protects against NMDA antagonist-induced injury, likely compensating for reduced EPO signalling in the treated brain and thus improving the local neurotrophic milieu.[23] Likewise, melatonin provides dose-dependent neuroprotection against anaesthetic-induced apoptosis in the anterior thalamus and cortex which are particularly vulnerable brain regions.[110] Melatonin has already been used clinically for premedication in the paediatric population as it possesses both hypnotic and analgesic qualities; further preclinical and clinical investigation of this drug in the perioperative phase is warranted.

Anaesthetic agents also provoke apoptosis in other cells such as lymphocytes, an action unrelated to the anaesthesia, suggesting that the toxicity may be related to the drugs themselves.[56] In aggregate, these data suggest that the state of anaesthesia itself may not be toxic, but the drugs we use currently to induce and maintain anaesthesia are toxic in animal models. If the toxic and desired effects of anaesthetics can be separated, by research into the mechanism of anaesthesia and toxicity, then we may be able to design drugs with enhanced safety. We should also be cautious about extrapolating current findings with one anaesthetic to another; sevoflurane does not induce apoptosis in cortical neurones *in vitro* unlike isoflurane,[106] thus further analysis of sevoflurane's safety profile is urgently required.

Summary

We firmly believe withholding adequate anaesthesia and analgesia in the perioperative period is not an option. We have outlined why adequate anaesthesia and analgesia is

critical in the young. However, because anaesthetic-induced neurodegeneration does occur in the neonatal rodents, we advocate further preclinical investigation to elucidate the factors that influence species vulnerability, the effects of different anaesthetics, their mechanism, and their duration of administration, and that of a concomitant surgical stimulus.

An important approach to this problem is the recently begun GAS clinical trial that compares the long-term cognitive and neurobehavioural effects of regional and general anaesthesia in the neonatal period.[19] Interpretation of a trial such as this will be facilitated by further preclinical studies addressing the mechanism of injury and the effects of different drugs and surgery. However, we have recently found that the combination of nitrous oxide (75%) and isoflurane (0.75%) administered for 6 h to 7-day-old rats provoked apoptosis in the rat spinal cord.[90] We should remain circumspect about the potential for regional anaesthesia to induce apoptosis in the spinal cord. As inhibition of neuronal transmission can occur for many hours in the perioperative period with spinal anaesthesia and local anaesthetics have been associated with neuroapoptosis *in vitro,*[45] it is plausible that neuraxial blocks may also induce neuroapoptosis *in vivo*. In the future, it is conceivable that if local anaesthesia-induced toxicity is observed, routine addition of an α_2 adrenoceptor agonist may not only extend analgesia but also provide neuroprotection. A combination of preclinical and clinical research is urgently needed in this area that remains so controversial in paediatric anaesthesia so that we may provide safe, non-toxic, tailored anaesthetic care in both perioperative and critical care environments.

Conclusions

Significant advances have occurred across the spectrum of paediatric anaesthesia that will continue to inform current and future clinical practice. However, it is clear that all four subjects addressed in this review of anaesthetic actions, namely analgesia, hypnosis, neuroprotection, and neurotoxicity, are interrelated and must be carefully balanced. Increased understanding in one area will inform research in another (Table 2) leading to changes in clinical practice. For example, understanding the mechanisms of hypnotic and analgesic actions in the young will drive development of more efficacious and refined anaesthetic agents and techniques that do not induce neurotoxicity and may reduce the risk of awareness in the paediatric population. Further understanding the targets mediating the neurotoxicity of anaesthetics may allow the development of 'cleaner', safer drugs. Investigation of the effects of agents, such as xenon, which are anaesthetic and neuroprotective but lack neurotoxicity will further aid development of safer anaesthetic drugs and neuroprotective agents. Thus, the rationale for translational research, where questions are asked in the preclinical setting before progression to clinical trials, is clear in paediatric anaesthesia. We hope that in the future dissection of the mechanisms of anaesthesia (encompassing analgesia and hypnosis), neuroprotection and neurotoxicity will aid design of safer anaesthetic agents to facilitate the development of truly tailored and balanced paediatric anaesthesia.

Table 2 Recommendations for further clinical and preclinical studies in paediatric anaesthesia

Analgesia	Sex differences in responses to analgesic agents in humans and animals Neurodevelopmental cognitive effects of unattenuated pain in animals Resistance to neuropathic pain in the young
Hypnosis/anaesthesia	Mechanisms of hypnosis in young animals MAC fractions and EEG studies in humans Further cohort studies to describe the problem of awareness in children
Neuroprotection	Animal studies to define the interaction of anaesthetics and hypothermia better The effect of hypothermia on perioperative neuronal injury in animals Further preclinical research to define the use of anaesthetic preconditioning in obstetric anaesthetic practice to improve neonatal outcome
Neurotoxicity	The safety profile of commonly used anaesthetics including sevoflurane in animals (especially non-human primates) The neurotoxic potential of regional anaesthesia in animals The potential for adjuncts (e.g. dexmedetomidine, xenon, or melatonin) to provide neurocognitive protection in animals The influence of surgery on anaesthetic neurotoxicity in animals

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