

# Impact of anaesthetics and surgery on neurodevelopment: an update

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## Editor's key points

- Robust evidence from neonatal non-primate animal models indicates increased programmed brain cell death after exposure to general anaesthesia.
- Emerging evidence from primate models confirms developmentally defined anaesthetic-induced neurotoxicity with associated neurocognitive deficits.
- Currently available retrospective clinical data are inconclusive, and the results of prospective studies are pending, making drastic changes to current paediatric anaesthesia practice premature.

**Summary.** Accumulating preclinical and clinical evidence suggests the possibility of neurotoxicity from neonatal exposure to general anaesthetics. Here, we review the weight of the evidence from both human and animal studies and discuss the putative mechanisms of injury and options for protective strategies. Our review identified 55 rodent studies, seven primate studies, and nine clinical studies of interest. While the preclinical data consistently demonstrate robust apoptosis in the nervous system after anaesthetic exposure, only a few studies have performed cognitive follow-up. Nonetheless, the emerging evidence that the primate brain is vulnerable to anaesthetic-induced apoptosis is of concern. The impact of surgery on anaesthetic-induced brain injury has not been adequately addressed yet. The clinical data, comprising largely retrospective cohort database analyses, are inconclusive, in part due to confounding variables inherent in these observational epidemiological approaches. This places even greater emphasis on prospective approaches to this problem, such as the ongoing GAS trial and PANDA study.

**Keywords:** brain, anaesthesia, molecular effects; nerve, damage (postoperative); nerve, neurotransmitters; nerve, regeneration

In this systematic review, we will update previous reviews of this topic published in the *BJA*<sup>1–3</sup> by describing the current weight of preclinical evidence for anaesthetic-induced neurodegeneration in the neonatal brain, along with a discussion of putative mechanisms of injury and potential adverse effects of surgery on neurodevelopment. Then, we review the clinical literature and ongoing prospective investigations that should shed further light on the potential harm of anaesthesia, surgery, or both in the neonatal period.

Preclinical studies in rodents and primates have shown that anaesthesia is neurotoxic to the developing brain after exposure in the neonatal period.<sup>1 4–13</sup> This neurotoxicity manifests as a pathological increase in apoptosis (programmed cell death), although other effects such as impaired neurogenesis and neuroinflammation likely also contribute. These studies randomized healthy neonatal animals to anaesthetic exposure or not; hence, a causal relationship has been established between the anaesthetic and neonatal brain injury. Furthermore, such neurotoxicity has been observed with exposure to drugs with similar mechanisms of action such as anti-epileptic medications<sup>14</sup> and ethanol.<sup>15</sup> Significant concern has arisen that present medical therapies could expose neonates to neurotoxicity.

Furthermore, in some animal studies, the anaesthetic injury is associated with impaired cognition that persists into adulthood. In primates, 24 h of ketamine exposure leads to apoptosis<sup>8</sup> and cognitive impairment up to 3.5 yr after the insult.<sup>10</sup> Several rodent studies also support this assertion of prolonged cognitive compromise associated with apoptosis after anaesthetic exposure.<sup>1 4 7 11 16</sup> Thus, the brain injury adversely affects neurodevelopment and leads to long-term impairment in cognition, at least in animals.

The difficulties of replicating these studies in humans include the ethical obstacle of being unable to randomize neonates to having an anaesthetic or not; it is unacceptable to perform surgery without any anaesthetic and it is unacceptable to give a neonate anaesthesia for no reason.<sup>1 11</sup> Both these options would likely provoke greater harm than current practice.<sup>1 11</sup> Therefore, current clinical studies have been limited to an observational cohort design, with the incumbent issues of confounding factors. These accumulating clinical data cannot exclude a clinically important effect of neonatal and paediatric anaesthesia and surgery on cognition in later life.<sup>17–26</sup> While these studies have used heterogeneous retrospective approaches (with varying degrees of

confounding effects), they provide sufficient preliminary evidence to cause concern in clinicians and parents alike.

## Search strategy methods

To provide an update on the weight of preclinical and clinical evidence of harm of anaesthetics and surgery on neurodevelopment, we performed literature searches for preclinical and clinical data. J.H. performed the search for preclinical data using the search terms: an(a)esth\*, neuroapoptosis, neuronal cell death, develop\* brain, and neurodevelop\*. The results included *in vivo* studies identified in Tables 1 (rodents) and 2 (primates). Two piglet studies were identified but were not tabulated. This search was designed to highlight the scope of the preclinical literature pertaining to anaesthetic-induced neurodegeneration in the developing brain. This search revealed an initial 4379 titles that were reviewed for relevance. Fifty-five rodent, two piglet, and seven primate studies were identified. The search for clinical data was conducted by R.D.S. using the search terms: cognitive or behavioral disorder and anaesth\* with the following limits: published in the last 10 yr, humans, English, infant: birth—23 months. To be included in the systematic review, some attempt to compare an anaesthetic exposed group with a non-exposed control group was required. However, we did include studies that used population controls, even if exposure status was not defined in this population. We only included studies of anaesthesia, not intensive care sedation, and only included studies from non-cardiac non-neurological surgery in term births. Our search revealed 3727 hits and the titles were then searched for relevant studies. The reference list of relevant studies and recent reviews were also hand searched for additional studies. Our formal search results are displayed in Table 3.

## Preclinical evidence for anaesthetic-induced neurodegeneration: rodent studies

After the seminal work of Jevtovic-Todorovic and colleagues<sup>4</sup> and Ikonomidou and colleagues,<sup>13</sup> numerous studies have now demonstrated that exposure of neonatal rodents to anaesthetics induces apoptosis in the developing brain (Table 1). This injury is most readily observed in the first 2 weeks of life in rodents when synaptogenesis peaks. The exact clinical correlate for this injury is unknown. Many factors influence the translation of these findings to humans, one being that synaptogenesis extends for many years post-partum in humans, suggesting that the window of vulnerability in humans may be longer.<sup>27</sup> Most rodent studies have also used relatively long exposures to anaesthetics, although isoflurane has been shown to induce apoptosis after exposure for just 1 h at concentrations of <1 MAC.<sup>28</sup> While ketamine and other *N*-methyl-D-aspartate (NMDA) antagonist drugs were initially implicated,<sup>13</sup> different combinations of agents have also been shown to induce apoptosis such as midazolam–nitrous oxide–isoflurane,<sup>4</sup>

nitrous oxide–isoflurane,<sup>29</sup> or combinations of ketamine and thiopental or propofol.<sup>30</sup> Similarly, benzodiazepine drugs, such as midazolam,<sup>31</sup> have been shown to be toxic.

Perhaps of most significance to modern neonatal clinical practice is that all volatile anaesthetics, and propofol,<sup>32</sup> have been shown to induce the injury.<sup>7 33–35</sup> The apoptosis is widespread among central nervous system loci, with cortical, thalamic, basal ganglia, and hippocampal injury prominent; the injury is also present in the spinal cord.<sup>4 6 7 36</sup> Significant effects on neurogenesis have also been noted, with suppressed proliferation of neural progenitors observed for 5 days after isoflurane exposure to 7-day-old rat pups.<sup>37</sup> This is paralleled by effects of isoflurane<sup>38</sup> (but not propofol)<sup>39</sup> on neuronal growth in *in vitro* culture, suggesting direct effects on these progenitor cells. Neurogenesis is important for both cognitive function and particularly brain repair; therefore, the inhibitory effects of isoflurane on neurogenesis likely play an important part in the development of impaired cognition in rodents.

When long-term follow-up of neonatal anaesthetic exposure have been performed, the majority of studies have identified some cognitive impairment persisting into adulthood. This relatively consistent finding is of particular interest, given the wide range of different tests used. Rodent studies suggest that general anaesthetics do not induce long-term motor impairment or altered nociceptive processing<sup>6</sup> but do impair memory function, likely through injury in both the hippocampus and frontal cortex.<sup>4 6 7</sup> Another study noted that anaesthetic exposure leads to behaviours that may be akin to autism;<sup>34</sup> this is particularly interesting as many clinical studies have focused on behavioural abnormalities rather than overt cognition. Of course, sophisticated cognitive function is difficult to test in rodents, limiting our knowledge about the exact effect on cognitive and other neurobehavioural domains. Rodent studies are also difficult to interpret due to the limited ability to control and monitor the physiology of the animal during anaesthesia. Indeed, in some of the studies, some of the newborn rodents die during anaesthesia implying that physiological control is not comparable with that in humans.

Spinal anaesthesia also exerts some neurotoxic effects (recently reviewed by Walker and Yaksh)<sup>40</sup> with ketamine again implicated. Intrathecal ketamine induced-apoptosis in the spinal cord was associated with abnormal gait and altered nociceptive processing.<sup>41</sup> Further information is needed on the effects of local anaesthetics, although recent data do not indicate harm.<sup>36</sup> Similarly, morphine and clonidine do not induce apoptosis when administered intrathecally to rats.<sup>42</sup>

*In utero* exposure to isoflurane also leads to apoptosis in the rat fetal hippocampus and retrosplenial cortex.<sup>43</sup> In a separate study, exposure to 1.4% isoflurane for 4 h during 1 day of pregnancy led to abnormal spatial memory acquisition and reduced anxiety in rats in adulthood.<sup>44</sup> Hence, there is evidence that the rodent fetus, like the neonates, is vulnerable to developmental neurotoxicity.

**Table 1** Rodent studies into the neurotoxic effects of anaesthetic agents. PND, post-natal day. Brain area codes: Am, amygdala; Th, thalamus (unspecified); ATH, thalamus anterior; VTh, thalamus ventral; ADTh, thalamus anterodorsal; LDTh, thalamus laterodorsal; AVTh, thalamus anteroventral; AMTh, thalamus anteromedial; Caud, caudate; Put, putamen; GP, globus pallidus; SN, substantia nigra; BF, basal forebrain; Cor, cerebral cortex (unspecified); RSC, retrosplenial cortex; CingC, cingulate cortex; PFC, prefrontal cortex; FroC, frontal cortex; ParC, parietal cortex; TemC, temporal cortex; OccC, occipital cortex; SomC, somatosensory cortex; PirC, piriform cortex; Hc, hippocampus; Sub, subiculum; Hyp, hypothalamus; StriaT, stria terminalis; Olf, olfactory bulb; SC, spinal cord. Studies identifying neuroprotective agents are identified in italics

Reference	Species	Age	Anaesthetic	Duration (h)	Histology outcome	Histology results	Brain area	Neurodevelopmental outcome	Neurodevelopmental result	Other outcomes/comments
Saito and colleagues, <i>Anesthesiology</i> 1993; 79: 1338–47	Rats	PND1	N <sub>2</sub> O 25–57% OR Halothane 0.5–1% in F <sub>10</sub> , 25%	6 h then killed PND 2–5	N/A	N/A	Whole	N/A	N/A	Whole brain homogenized, neuronal tip growth cone particles extracted by fractionation, polyacrylamide gel electrophoresis for 46 and 80 kDa proteins and protein kinase C activity assay—decreased protein-kinase C-mediated phosphorylation of 46 and 80 kDa proteins with increasing anaesthetic dose
Ikonomidou and colleagues, <i>Science</i> , 1999; 283: 70–4	Rats	PND 0, 3, 7, 14, 21	Dizocilpine 0.05–1 mg kg <sup>-1</sup> , single injection at 0, 8, or 16 h	Killed after 4, 8, 12, 16, 24, or 48 h	1. Apoptosis: TUNEL 2. Neuronal necrosis: DeOlmos silver	Dose-dependent increased apoptosis throughout maximal in LDTh, FroC, ParC, CingC. More apoptosis seen after longer time elapsed post-dose. Peak vulnerability on PND 7. Regional variation in degree of apoptosis at different ages	Hc, Sub, LDTh, MDTh, VTh, FroC, ParC, RSC, CingC	N/A	N/A	Dizocilpine (MK801)=NMDA-R antagonist. Maximally affected on PND 7=(ascending order) FroC, Cau, Sub, RSC, ParC, CingC, LDTh
Thompson and colleagues, <i>Brain Res Dev</i> 2001; 24: 167–71	Rats Sprague–Dawley	PND 6, 14, 21, 28, 56	Urethane 1.25 g kg <sup>-1</sup> single dose	Killed after 24, 24 or 96 h	Irreversible neuronal injury assessed by fluorescent LM after H&E staining	Age-dependent neuronal injury, max after urethane on PND 18–21	PirC	N/A	N/A	
Jevtovic-Todorovic and colleagues, <i>J Neurosci</i> 2003; 23: 876–82	Rats Sprague–Dawley	PND 7	Midazolam 3–9 mg kg <sup>-1</sup> , N <sub>2</sub> O 50–150%, isoflurane 0.75–1.5%, alone, or in combination	6 h	1. Neurodegen: DeOlmos silver 2. Apoptosis: caspase-3, electron microscopy	Dose-dependent apoptotic degeneration with triple cocktail, less marked with isoflurane alone. No significant increase in apoptosis with midazolam or N <sub>2</sub> O alone. Most vulnerable=thalamus	All areas—see paper for results	1. Ascent test at P10, P12, P14 2. Auditory/tactile startle P20 3. Locomotor activity test P21 4. Sensorimotor test P22 5. Morris water maze for spatial reference memory P28 6. Radial arm maze P53	Triple anaesthetic induced long-term impairment in spatial/learning memory	Anaesthesia did not significantly affect arterial blood gases
Dzieiko and colleagues, <i>Neurobiol Dis</i> 2004; 15: 177–87 <sup>†</sup>	Rats Wistar	PND 7	NMDA receptor antagonist MK801 0.5 µg kg <sup>-1</sup> at 0, 8, and 16 h ± rEPO 5–30 000 IU kg <sup>-1</sup> at 0 h. Subgroup pretreated—16 h	Killed at 24 h from first dose MK801	1. Apoptosis: TUNEL 2. Neuronal degeneration: DeOlmos silver	Increased apoptosis with MK801. Apoptosis reduced with rEPO 5000 IU kg <sup>-1</sup> co-treatment. No significant increase in protection with pre-treatment or increasing doses	Cor, Th, Sub	N/A	N/A	<sup>†</sup> Neuroprotection study. rEPO conferred 50% neuroprotection and partially restored MK801-enhanced neurotrophin-associated signalling pathways. RT-PCR for BDNF–MK801 reduced BDNF, partially restored by rEPO. Western blot for ERK1/2 and Akt–MK801 reduced phosphorylated ERK and Akt, reversed by rEPO
Scallet and colleagues, <i>J Chem Neuroanat</i> 2004; 29: 71–80	Rats Sprague–Dawley	PND 7	Ketamine 10–20 mg kg <sup>-1</sup> every 90 min, total 7 doses	Killed after seventh dose	1. Apoptosis: caspase-3, DAPI 2. Neuronal necrosis: Fluoro-Jade B staining, DeOlmos silver	Increased apoptosis and necrosis with high dose (20 mg kg <sup>-1</sup> )*	DLTh, Am	N/A	N/A	*20 mg kg <sup>-1</sup> produced blood levels 7 × greater than anaesthetic blood level in humans. No increased cell death seen at therapeutic levels

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Table 1 Continued

Reference	Species	Age	Anaesthetic	Duration (h)	Histology outcome	Histology results	Brain area	Neurodevelopmental outcome	Neurodevelopmental result	Other outcomes/comments	
Young and colleagues, <i>Br J Pharmacol</i> 2005; <b>146</b> : 189–97	Mice C57BL6	PND 7	Ketamine 10–40 mg kg <sup>-1</sup> or midazolam 9 mg kg <sup>-1</sup> , single dose, alone or combination	Killed after 5 h	1. Apoptosis: caspase-3 2. Neuronal necrosis: DeOlmos silver	Significant dose-dependent increase in apoptosis with ketamine or midazolam alone. Combined administration triggered significantly greater increase in neuroapoptosis	Cor, Cau, Put	N/A	N/A	Anaesthesia did not significantly affect arterial blood gases	
Yon and colleagues, <i>Neuroscience</i> 2005; <b>135</b> : 815–27	Rats Sprague-Dawley	PND 1, 3, 7, 10, 14	Midazolam 9 mg kg <sup>-1</sup> , N <sub>2</sub> O 75%, isoflurane 0.75% alone or combination	2, 4, or 6 h	1. Apoptosis: caspase-3, caspase-8 (extrinsic apoptotic pathway), caspase-9 (intrinsic apoptotic pathway) 2. Neuronal necrosis: DeOlmos silver	Age-dependent anaesthesia-induced apoptosis (max PND 7) with triple anaesthesia. Intrinsic pathway activated at 2 h, extrinsic pathway activated at 6 h, but both remain inactive at PND 14. Thalamus vulnerable earlier, cortex later	OccC, CingC, ParC, RSC, Sub, ADTh, LDTh, AVTh, AMTh, Am	N/A	N/A	Peak vulnerability=PND 7=peak synaptogenesis. Western blot—Cytochrome c (intrinsic apoptotic pathway), Bcl-X <sub>L</sub> (intrinsic), Fas (extrinsic)—all increased with triple anaesthesia. Cerebral perfusion—laser-Doppler—no decrease in CBF with anaesthesia	
Yon and colleagues, <i>Neurobiol Dis</i> 2006; <b>21</b> : 522–39 <sup>f</sup>	Rats	PND 7	Combination: midazolam 9 mg kg <sup>-1</sup> , N <sub>2</sub> O 75%, isoflurane 0.75% ± melatonin 1–20 mg kg <sup>-1</sup> pre-/3 h into anaesthesia	6 h	1. Apoptosis: caspase-3 2. Neuronal necrosis: DeOlmos silver	Increased apoptosis in the cortex and thalamus with anaesthesia, counteracted by melatonin in a dose-dependent manner (35–40% reduction at 1 mg kg <sup>-1</sup> , 75–90% at 20 mg kg <sup>-1</sup> )	OccC, CingC, ParC, RSC, ADTh, LDTh, AVTh, AMTh	N/A	N/A	<sup>f</sup> Neuroprotection study. Melatonin causes dose-dependent decrease in severity of anaesthesia-induced apoptosis/neurodegeneration and induces up-regulation of Bcl-X <sub>L</sub> . Western blot—Cytochrome c, Bcl-X <sub>L</sub> (intrinsic apoptotic pathway)—results as for histology and mel upregulated Bcl-X <sub>L</sub>	
Lu and colleagues, <i>Apoptosis</i> , 2006; <b>11</b> : 1603–15 <sup>f</sup>	Rats Sprague-Dawley	PND 7	Combination: midazolam 9 mg kg <sup>-1</sup> , N <sub>2</sub> O 75%, isoflurane 0.75% ± β-oestradiol 300 μg kg <sup>-1</sup> pre-/8 h post-anaesthesia	2, 4, or 6 h	Apoptosis: caspase-3, -9, activated ceramide, Trk-B activation	Increased apoptosis via Trk-dependent and independent pathways in the cerebral cortex	Th, CingC, OccC, ParC, RS, Sub	N/A	N/A	<sup>f</sup> Neuroprotection study. β-oestradiol (known to up-regulate Akt) protects against anaesthesia-induced neuroapoptosis. Western blot—BDNF, p75 <sup>NTR</sup> , Akt protein—results as for histology	
Fredriksson and colleagues, <i>Anesthesiology</i> 2007; <b>107</b> : 427–36	Mice NMRI	PND 10	1. Ketamine 25 mg kg <sup>-1</sup> 2. Thiopental 5 or 25 mg kg <sup>-1</sup> 3. Propofol 10 or 60 mg kg <sup>-1</sup> 4. Ketamine+ thiopental 5. Ketamine+ propofol	Killed 24 h post dose or tested PND 55–70	Apoptosis: Fluoro-Jade B	Increased apoptosis in all groups. Significant in ketamine+propofol/thiopental and high-dose propofol groups	Olf, StriaT	At PND 55–70	1. Decreased spontaneous activity in all groups 2. More errors in combination anaesthesia groups 3. No difference in behaviour across groups, but significantly less anxiolytic effect of diazepam in the propofol group	1. Spontaneous behaviour 2. Spatial learning: radial arm maze 3. Anxiety level: elevated plus maze ± anxiolytic diazepam	'Both GABA agonist (thiopental/propofol) and NMDA antagonist (ketamine) potentiated neonatal brain cell death and resulted in functional deficits in adulthood'

Ma and colleagues, <i>Anesthesiology</i> , 2007; <b>106</b> : 746–53 <sup>1</sup>	Rats Sprague-Dawley	PND 7	8 gas mixtures: 1. Air 2. N <sub>2</sub> O 75% 3. Isoflurane 0.75% 4. Isoflurane 0.75%+N <sub>2</sub> O 35% 5. Isoflurane 0.75%+N <sub>2</sub> O 70% 6. Xenon 75% 7. Isoflurane 0.75%+xenon 30% 8. Isoflurane 0.75%+xenon 60%	6 h	Apoptosis: caspase-3	Isoflurane enhanced neuroapoptosis. Combination with N <sub>2</sub> O significantly increased apoptosis. Combination with xenon reduced apoptosis to that of controls (statistically greater reduction with 60% than 30% xenon). Neither N <sub>2</sub> O or xenon alone increased apoptosis	Hc, Cor				<sup>†</sup> Neuroprotection study. Xenon reduced isoflurane-induced apoptosis to that of controls. Unblotting for caspase-3, -8, -9, and cytochrome c—no change in caspase-8 suggesting extrinsic apoptotic pathway not activated after 6 h. Caspase-3, -9, and cytochrome c (intrinsic apoptotic pathway) all increased with isoflurane ± N <sub>2</sub> O, ameliorated by xenon
Nikizad and colleagues, <i>Ann N Y Acad Sci</i> 2007; <b>1122</b> : 69–82	Rats Sprague-Dawley	PND 7	Combination: midazolam 9 mg kg <sup>-1</sup> , N <sub>2</sub> O 75%, isoflurane 0.75%	6 h then killed after 0, 18, 42 h, 3 or 23 days	Neuronal density: Nissl	Decreased cell counts in the thalamus and subiculum by day 3 (PND 10) and in the cortex by day 23 (PND 30) post-anaesthesia	CingC, OccC, ParC, RSC, ADTh, LDTh, AVTh, AMTh, Subi	N/A	N/A		Western blot—synaptophysin, synaptobrevin, amphiphysin, SNAP-25 (all presynaptic), CaM kinase II (postsynaptic)—decreased presynaptic proteins within 18 h, decreased SNAP and CaM at 42 h, recovered by day 23
Rizzi and colleagues, <i>Brain Pathol</i> 2007; <b>18</b> : 198–210	Guinea-pigs, Hartley, pregnant	Gest age (days) 20–25, 35–40, >50	Midazolam 1 mg kg <sup>-1</sup> , N <sub>2</sub> O 75%, isoflurane 0.55% alone or combination. Fentanyl 15 µg kg <sup>-1</sup> h <sup>-1</sup> , rocuronium	4 h	1. Apoptosis 2 h post-anaesthesia: caspase-3, -9 2. Neuronal density 35–40 days post-anaesthesia: NeuN, Nissl	1. Increased apoptosis with isoflurane alone and with triple, at GA 20–25 and 25–40 but not GA >50 2. 40–50% neuronal loss compared with controls	Am, ATTh, Subi, RSC, ParC, OccC, CingC, PirC	N/A	N/A		
Johnson and colleagues, <i>J Neurosurg Anaesthesiol</i> 2008; <b>20</b> : 21–28	Mice, infantile C57BL6	PND 5–7	Isoflurane 0.75%, 1.5%, 2%	4, 2, 1 h	Apoptosis: caspase-3	Increased apoptosis at all concentrations, min for 2% × 1 h, max for 75% × 4 h	Caud, Put	N/A	N/A		MAC for isoflurane in infant mice reported to be 2.26% (Loepke and colleagues 2006)
Sanders and colleagues, <i>Anesth Analg</i> 2008; <b>106</b> : 1708–11	Rats Sprague-Dawley	PND 7	N <sub>2</sub> O 75%+isoflurane 0.75% in F <sub>IO</sub> <sub>2</sub> 25%	6 h	Apoptosis: caspase-3	Increased apoptosis in the spinal cord	SC		1. Tail flick latency PND 8, 15, No difference 30 2. Time on rotarod PND 30—max latency 300 s		
Cattano and colleagues, <i>Anesth Analg</i> 2008; <b>106</b> : 1712–14	Mice infantile C57BL6	PND 5–7	Propofol 25–300 mg kg <sup>-1</sup> single dose	Killed after 6 h	Apoptosis: caspase-3	Increased apoptosis—linear dose–response curve	Cau, Put, Cor	N/A	N/A		
Cattano and colleagues, <i>Can J Anaesth</i> 2008; <b>55</b> : 403–7 <sup>†</sup>	Mice infantile C57BL6	PND 5–7	Isoflurane 0.75% ± xenon 70%	4 h then killed after 2 h	Apoptosis: caspase-3	Increased apoptosis with all: isoflurane > Xe+iso > xenon	Cau, Put, Cor	N/A	N/A		<sup>†</sup> Neuroprotection study. Xenon is apoptogenic used alone, but suppresses apoptogenic activity of isoflurane in combination
Xou and colleagues, <i>Neuroscience</i> 2008; <b>19</b> : 1053–65 <sup>†</sup>	Rats Sprague-Dawley	PND 7	N <sub>2</sub> O 75%, isoflurane 0.55%, alone or combination, in F <sub>IO</sub> <sub>2</sub> 25% ± L-carnitine 300–500 mg kg <sup>-1</sup>	2, 4, 6, or 8 h then killed after 6 h	1. Apoptosis: caspase-3 2. Neuronal necrosis: Fluoro-Jade B staining, DeOlmos silver	No increase in apoptosis with single agent. Significantly increased apoptosis after 6 h combination anaesthesia. L-carnitine blocked apoptosis	FroC, Th, Am, Hc	N/A	N/A		<sup>†</sup> Neuroprotection study. L-carnitine known to attenuate neurological injury associated with mitochondria-related degenerative disorders—neuroprotective in this study. Western blot for Bax, Bcl-XI, β-actin—down-regulated with combination anaesthesia, protected by L-carnitine
Zhang and colleagues, <i>Neurosci Lett</i> 2008; <b>12</b> : 109–14	Mice C57BL/6	PND 7	Sevoflurane 1.7% in F <sub>IO</sub> <sub>2</sub> 30%	2 h then killed after 0–12 h	Apoptosis: caspase-3, DAPI, electron microscopy	Significantly increased apoptosis at a subclinical dose of sevoflurane	Th, SomC, CingC	N/A	N/A		Sevoflurane 1.7% = MAC 0.75 in infants (subclinical dose). Blood glucose in the anaesthetized group was not different from the control group

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Table 1 Continued

Reference	Species	Age	Anaesthetic	Duration (h)	Histology outcome	Histology results	Brain area	Neurodevelopmental outcome	Neurodevelopmental result	Other outcomes/comments
Satomoto and colleagues, <i>Anesthesiology</i> 2009; <b>110</b> : 528–37	Mice C57BL/6	PND 6	Sevoflurane 3% in air	6 h	1. Apoptosis: caspase-3, TUNEL 2. Neuronal density: Nissl	Increased apoptosis immediately after anaesthesia, most severe in RSC and Th	Hc, Sub, Cau, Put, Th, FroC, TemC, RSC, Am	Assessed at age 14–17 weeks: 1. Cognitive function: Pavlovian conditioned fear test 2. Social behaviour: social recognition and interaction tests	General behaviour was normal in sevoflurane-exposed mice. 1. Sevoflurane caused persistent learning deficits 2. Sevoflurane-exposed mice did not develop social memory and showed decreased interactions with a social target. This could not be attributed to impaired olfaction or general interest	Exposure to sevoflurane did not cause significant changes in cerebral blood flow or arterial blood gas. Exposure of neonatal mice to inhaled sevoflurane could cause not only learning deficits but also abnormal social behaviours resembling autism spectrum disorder. Western blot for PARP (apoptotic biomarker)—increased with sevoflurane
Sanders and colleagues, <i>Acta Anaesthesiol Scand</i> 2009, <b>54</b> : 710–16 <sup>1</sup>	Rats Sprague-Dawley	PND 7	Isoflurane 0.75% in $F_{iO_2}$ 25% ± dexmedetomidine 25, 50, or 65 $\mu\text{g kg}^{-1}$ at 0, 2, and 4 h	6 h	Apoptosis: caspase-3	Isoflurane induced apoptosis, partially attenuated by dexmedetomidine 25 $\mu\text{g kg}^{-1}$ . Dose escalation of dex did not further increase neuroprotection	Hc, Thal, Cor	N/A	N/A	<sup>1</sup> Neuroprotection study. Dexmedetomidine protected against neuroapoptosis <i>in vitro</i> . Western blot for Bcl-2 and p-ERK1/2—expression decreased by isoﬂurane, effect reversed by dex
Sanders RD and colleagues, <i>Anesthesiology</i> 2009; <b>110</b> : 1077–85 <sup>1</sup>	Rat Sprague-Dawley	PND 7	Isoflurane 0.75% in $F_{iO_2}$ 25% ± dexmedetomidine 1, 10 or 25 $\mu\text{g kg}^{-1}$ at 0, 2, and 4 h	6 h	Apoptosis: caspase-3	Dexmedetomidine provided dose-dependent protection against anaesthesia-induced neuroapoptosis. In Hc and Th but not Cor, 25 $\mu\text{g kg}^{-1}$ reduced injury to baseline	Hc, Thal, Cor	Long-term memory: context fear conditioning test at P40	Dexmedetomidine ameliorated neurocognitive impairment induced by anaesthesia	<sup>1</sup> Neuroprotection study. Dexmedetomidine protected against neuroapoptosis and long-term memory impairment in this study
Straika and colleagues, <i>Anesthesiology</i> 2009; <b>110</b> : 862–8 <sup>1</sup>	Mice C57/B16	PND 5	Ketamine 40 $\text{mg kg}^{-1}$ or propofol 50–100 $\text{mg kg}^{-1}$ single dose ± lithium 3–6 $\text{mEq kg}^{-1}$	Killed after 2–5 h	Apoptosis: caspase-3	Increased apoptosis with ketamine or propofol, significantly reduced by 3 and 6 $\text{mEq kg}^{-1}$ lithium	Cau, Put	N/A	N/A	<sup>1</sup> Neuroprotection study. Lithium reduces neuroapoptosis-induced by ketamine or propofol. Western blot for phosphorylated ERK 1/2 and phosphorylated Akt—phosphorylation suppressed by ketamine or propofol; suppressant effect reversed by lithium for ERK1/2 only
Pesic and colleagues, <i>Int J Dev Neurosci</i> 2009; <b>27</b> : 279–87	Rats Wistar	PND 7	Propofol 25 $\text{mg kg}^{-1}$ single dose	Killed after 0, 1, 2, 4, 8, 16, or 24 h	Neuronal necrosis: Fluoro-Jade B staining	Increased apoptosis	Cor, Th	N/A	N/A	Western blot for TNF $\alpha$ , NGF, p-Akt1/2/3, cleaved caspase-3, $\beta$ -actin; RT-PCR for NGF $\beta$ , caspase-8—results show extrinsic apoptotic pathway induced
Head and colleagues, <i>Anesthesiology</i> 2009; <b>110</b> : 813–25	Mice	PND 1–21 mice and PND 1neurons	Isoflurane 1.4% in air	4 h	1. Apoptosis: caspase-3, NeuN, debris and doublecortin 2. Synapse characteristics: electron microscopy	Isoflurane increased apoptosis/reduced synapse number. Apoptosis mitigated <i>in vitro</i> by tPA, plasmin, or p75 <sup>NTR</sup> inhibitor (Fc-p75 <sup>NTR</sup> or TAT-Pep5) and <i>in vivo</i> by TAT-Pep5	Hc	N/A	N/A	Supports hypothesis that isoﬂurane neurotoxicity in the developing rodent brain is mediated by reduced synaptic tPA release and enhanced proBDNF/p75 <sup>NTR</sup> -mediated apoptosis
Bercker and colleagues, <i>Neurotox Res</i> 2009; <b>16</b> : 140–7	Rats Wistar	PND 6	Sevoflurane 3–5% for 6 h or propofol 30 $\text{mg kg}^{-1}$ every 90 min for 3 doses	6 h then killed 24 h after start	Neurodegen: DeOlmos silver	Significant difference in neurodegeneration between control and propofol, but not sevoflurane	FroC, ParC, CingC, RSC, Cau, Thal Hc, Sub, Hyp	Spatial memory learning: Morris water maze	Persistent learning deficits in the propofol group but not in the sevoflurane group	

De Roo and colleagues, <i>PLoS One</i> 2009; <b>16</b> : e7043	Mice H-line	PND 15–30	Midazolam 25 mg kg <sup>-1</sup> or propofol 50 mg kg <sup>-1</sup> or ketamine 40 mg kg <sup>-1</sup>	5 h then killed	Spine dynamics assessed on hippocampal slice cultures	All agents increased spine protrusion density on tufted apical dendrites, more marked on PND 15 than 30	Cor, Hc	N/A	N/A	Exposure to general anaesthetic agents rapidly induces dendritic spine growth (up to double) and the formation of functional synapses within a few hours
Wang and colleagues, <i>Pediatr Res</i> 2009; <b>66</b> : 435–40	Rats Sprague–Dawley pregnant	Gest age (days) 21	Isoflurane 1.3–3% plus F <sub>IO<sub>2</sub></sub> 100% Controls in F <sub>IO<sub>2</sub></sub> 100%	1 h then delivery after 6 h	Apoptosis: caspase-3	Increased apoptosis significant with 3% isoflurane, not with 1.3%	Hc, RSC	N/A	N/A	Plasma S100β levels significantly increased with 3% isoflurane, not with 1.3%. Blood S100β may be a useful predictor of fetal brain damage during pregnancy
Zhao and colleagues, <i>J Pharmacol Exp Ther</i> 2010; <b>333</b> : 14–22	Rats	PND 7 plus in vitro study	Isoflurane 1–1.5% ± xestospongine C 30 min prior	6 h	Apoptosis: caspase-3	Increased apoptosis with isoflurane, abolished by xestospongine C	Hc	Memory and learning ability evaluated through Morris water maze at P42–46	No significant learning impairments noted in either group	Xestospongine C=InsP <sub>3</sub> R inhibitor. Inhibition of InsP <sub>3</sub> R significantly inhibited isoflurane-induced neurodegeneration in this study. Western blot for C/Clcsp3, BACE, Bax/Bcl-X <sub>L</sub> , and Bax—isoflurane increased BACE, ameliorated by xestospongine. Isoflurane did not change expression of Bax/Bcl-X <sub>L</sub> or Bax
Shu and colleagues, <i>Anesthesiology</i> 2010; <b>113</b> : 360–81	Rats Sprague–Dawley	PND 7	70% N <sub>2</sub> O+0.75% isoflurane. Preconditioning: 70% xenon, 70% N <sub>2</sub> O or F <sub>IO<sub>2</sub></sub> 8% for 2 h	6 h, given 24 h after precondition	Apoptosis: caspase-3	Anaesthesia caused increased apoptosis. Pretreatment with hypoxia exacerbated anaesthesia-induced apoptosis. Pretreatment with N <sub>2</sub> O had no effect. Pretreatment with xenon prevented apoptosis	Hc, Cor	Trace fear conditioning at P40	Impaired cognitive function with anaesthesia ± hypoxic preconditioning. With xenon preconditioning, no significant difference in cognitive function of control	<sup>†</sup> Neuroprotection study. Xenon protected against anaesthesia-induced apoptosis and learning deficits in this study. Western blot for Bcl-2, C/Clcsp3, cytochrome c, p53, mitogen-activated protein kinases—results as for histology. Xenon pretreatment increased Bcl-2 expression and decreased cytochrome c release and p53 expression
Milanovic and colleagues, <i>Dev Neurosci</i> 2010; <b>32</b> : 288–301	Rats Wistar	PND 7	Propofol 20 mg kg <sup>-1</sup> hourly	2, 4, or 6 h then killed after 0, 4, 16, or 24 h	1. Apoptosis: caspase-3 2. Neuronal necrosis: Fluoro-Jade B staining	Increased apoptosis and necrosis 24 h after 6 h long exposure but not after 2 or 4 h long exposure	RSC, ParC, CingC, OccC, PirC, ATH	N/A	N/A	Western blot for proteolytic cleavage products of α-II-spectrin, protein kinase C and poly(ADP-ribose) polymerase 1—showed increased caspase-3 and calpain activity, in a time-dependent and brain structure-specific manner
Cattano and colleagues, <i>Minerva Anestesiol</i> 2010; <b>76</b> : 420–2	Rats Sprague–Dawley	PND 7	N <sub>2</sub> O 75%+F <sub>IO<sub>2</sub></sub> 25%	2 h	N/A	N/A	N/A	N/A	N/A	Total RNA isolated from each brain. Real-Time PCR showed up-regulation of nNOS (but not iNOS) and p53, providing evidence of pro-apoptotic action of N <sub>2</sub> O
Briner and colleagues, <i>Anesthesiology</i> 2010; <b>112</b> : 546–56	Rats Wistar	PND 16	Isoflurane 1.5% or sevoflurane 2.5% or desflurane 7%	30, 60, or 120 min	1. LM analysis of dendritic arbor architecture after inophoretic injection 2. Apoptosis: TUNEL 3. Neuronal necrosis: Fluoro-Jade B	1. Increased dendritic spine density with increasing duration of anaesthesia 2. No increase in apoptosis 3. No increase in neuronal necrosis	Cor	N/A	N/A	These data strongly suggest that exposure to volatile anaesthesia does not induce cell death in the developing cerebral cortex at PND 16
Liang and colleagues, <i>Anesthesiology</i> 2010; <b>112</b> : 1325–34	Mice C57BL/6	PND 7	Isoflurane 0.75% or sevoflurane 1.1% (to achieve MAC 0.5)+ F <sub>IO<sub>2</sub></sub> 30%	6 h	Apoptosis: caspase-3	Increased apoptosis isoflurane>sevoflurane	Hc, Cor	1. Spatial learning and memory: Morris water maze at P42 2. Non-cognitive impairment: Cued trials P31 3. Ability to learn spatial relations: Place trials P45 4. Memory retention: probe trials P45	No statistically significant difference in learning or memory with either agent	Plasma S100β significantly increased with isoflurane but not sevoflurane. Western blot for C/Clcsp3 and PARP (apoptosis biomarkers) and CDK4 and cyclin D1 (cell cycle regulatory proteins)—showed increased apoptosis results as for histology, but no change in cell cycle biomarkers

Continued

Table 1 Continued

Reference	Species	Age	Anaesthetic	Duration (h)	Histology outcome	Histology results	Brain area	Neurodevelopmental outcome	Neurodevelopmental result	Other outcomes/comments
Yeude and colleagues, <i>PLoS One</i> 2010; 5: e11374	Mice C57BL/6	PND 2 or 7	Phencyclidine 35 mg kg <sup>-1</sup> on P2, or 50 mg kg <sup>-1</sup> on P7, or both—single injections	Killed 10 h after last drug dose	Apoptosis: caspase-3	Increased apoptosis with phencyclidine P2+7>P7>P2. Dense staining of Th, Cau, and Put in all groups. More apoptosis in Hc and Amy in the P2 group, more apoptosis in the neocortex in the P7 group	CingC, RSC, Cau, Put, LDTh, Hc, Amy	1. Spatial learning and memory: Morris water maze at P28–30 and P170 2. Acoustic startle response test at P50 3. Conditioned fear test at P60	The P2 group showed mild retention impairments in water maze test. The P7 group showed impaired acoustic startle response. P2+7 mice showed significant impairment in all tests, deficits persistent at P170	Phencyclidine=NMDA antagonist. Phencyclidine induced different patterns of neuroapoptosis depending on developmental age at the time of exposure. Two separate exposures caused more severe neuropathological and neurobehavioural consequences
Lunardi and colleagues, <i>Neurotox Res</i> 2010; 17: 179–88	Rats Sprague-Dawley	PND 7	Combination: midazolam 9 mg kg <sup>-1</sup> , N <sub>2</sub> O 75%, isoflurane 0.75%	6 h then killed on PND 21	Quantitative and morphometric analysis of synapses by EM	Decreased neuropil and synapse density and morphology indicative of mitochondrial degeneration in the anaesthesia group	Sub	N/A	N/A	
Liu and colleagues, <i>Curr Neuropharmacol</i> 2011; 9: 256–61	Rats Sprague-Dawley	PND 7	Ketamine 5–20 mg kg <sup>-1</sup> in one, three, or six injections at 2 h intervals	Max 12 h then killed at 5 min or 1, 2, 4, 6, or 18 h	1. Neuronal degeneration: electron microscopy 2. NMDA receptor expression: <i>in situ</i> hybridization of NMDA-R NR1 subunit	1. Increased neuronal degeneration after 6×20 mg kg <sup>-1</sup> ketamine but not at lower doses 2. Increased NR1 subunit expression in ketamine-treated rats	FroC	N/A	N/A	Quantitative PCR for NR1, NR2A, and NR2C subunits of NMDA receptor—significantly increased in ketamine-treated rats. Microarray analysis showed altered expression of apoptotic-relevant genes and increased NMDA-R gene expression with ketamine. Suggests ketamine causes compensatory up-regulation of NMDA-R, subsequently triggering apoptosis
Istaphanous and colleagues, <i>Anesthesiology</i> 2011; 114: 578–87	Mice C57BL/6J	PND 7–8	Desflurane 12.2% or isoflurane 2.7% or sevoflurane 5.4% to achieve MAC 0.6	6 h	Apoptosis: caspase-3, NeuN	Significantly increased neuronal cell death and apoptotic activity irrespective of anaesthetic agent	Cor	N/A	N/A	Colorimetric assay for activated caspase-3—increased after anaesthetic exposure. Increased neuroapoptosis with all three agents, no significant difference between agents
Kodama and colleagues, <i>Anesthesiology</i> , 2011; 115: 579–91	Mice C57BL/6	PND 6	Desflurane 4% or desflurane 8% or sevoflurane 3% or isoflurane 2%	3 or 6 h	Apoptosis: caspase-3, TUNEL	Significantly increased apoptosis after 6 h, desflurane > isoflurane > sevoflurane. No significant difference cf. controls after 3 h	SomC, RSC, Hc	At 6 weeks age: 1. General activity—open field test 2. Anxiety-related behaviour—elevated plus-maze 3. Working memory—Y-maze  At 7 weeks age: 1. Long term memory—fear conditioning test	1. No significant anomalies 2. No significant anomalies 3. Impaired working memory in desflurane-treated mice only 4. Impaired long-term memory in all groups	Western blot for PARP (biomarker for apoptosis)—increased after 6 h desflurane > isoflurane > sevoflurane. Results suggest that neurotoxicity of desflurane > isoflurane > sevoflurane
Zhou and colleagues, <i>Indian J Pharmacol</i> 2011; 174: 64–70	Rats Sprague-Dawley	PND 7	N <sub>2</sub> O 70% + Isoflurane 0.75% in F <sub>10</sub> , 25%	6 h	1. Apoptosis: caspase-3 2. Labelling for glutamatergic, GABAergic, dopaminergic, or cholinergic neurones	Increased apoptosis in the cingulate and hippocampus (GABA/glut) and substantia nigra (dop) but not basal forebrain (chol)	BF, SN, Hc, CingC	N/A	N/A	Suggests anaesthetic exposure significantly increases neuroapoptosis of glutamatergic, GABAergic and dopaminergic but not cholinergic neurones in developing brain
Cui and colleagues, <i>Indian J Pharmacol</i> 2011; 43: 648–51	Mice Kunming	PND 5–7	Propofol 50–150 mg kg <sup>-1</sup> , 4 injections at 90 min intervals	6 h	Apoptosis: caspase-3, c-Fos	Increased apoptosis at 100–150 mg kg <sup>-1</sup> propofol	Hc	N/A	N/A	Western blot for cIcsp3 and c-Fos—results as for histology



Tu and colleagues, <i>Brain Res Bull</i> 2011; <b>86</b> : 29–35 <sup>†</sup>	Rats Sprague–Dawley	PND 7	1. Propofol 50 mg kg <sup>-1</sup> +F <sub>102</sub> , 50% 2. P + F <sub>102</sub> , 21% 3. P + F <sub>102</sub> , 18% 4. Vehicle + F <sub>102</sub> , 50% 5. V + F <sub>102</sub> , 21% 6. V + F <sub>102</sub> , 18%	7 days	1. Apoptosis: TUNEL, electron microscopy 2. Neuronal density: Nissl	Significant apoptosis in propofol+air/hypoxia groups. No significant apoptosis in propofol+oxygen and all vehicle groups	Hc	N/A	N/A	<sup>†</sup> Plus hypoxia. Propofol-induced attenuation of respiration may produce lower blood oxygen concentrations in air, resulting in neuronal degeneration. F <sub>102</sub> , 50% rescued propofol-induced hypoxia. Western blot for C/Clsp3—increased apoptosis results as for histology
Briner and colleagues, <i>Anesthesiology</i> 2011; <b>115</b> : 282–93	Rats Wistar	PND 5–90, male only for >PND 20	Propofol 40 mg kg <sup>-1</sup> then 4 × 20 mg kg <sup>-1</sup> , injections at hourly intervals (total five injections)	6 h	1. LM analysis of neuronal cytoarchitecture/dendritic tree after inophoretic injection 2. EM for synaptic density	Propofol decreased dendritic spine density when administered at PND 5–10, but increased spine density and synapse number when administered at PND 15–30	PFC	N/A	N/A	Propofol-induced modifications in dendritic spine density persisted up to PND 90
Tsuchimoto and colleagues, <i>Paediatr Anaesth</i> 2011; <b>21</b> : 1209–13 <sup>†</sup>	Mice ddN strain	PND 7	Isoflurane 1% ± REPO 50 000 IU kg <sup>-1</sup>	6 h	Neuronal density: Nissl	EPO attenuated isoflurane-induced neurodegeneration	Hc	Spatial learning/memory assessed by radial arm maze on PND 56–59	EPO improved spatial learning/memory of mice exposed to isoflurane	<sup>†</sup> Neuroprotection study. EPO attenuated isoflurane-induced neurodegeneration
Sanchez and colleagues, <i>Anesthesiology</i> 2011; <b>115</b> : 992–1002	Rats Sprague–Dawley	PND 7	Combination: midazolam 9 mg kg <sup>-1</sup> , N <sub>2</sub> O 75%, isoflurane 0.75% in F <sub>102</sub> , 24%	6 h then killed at PND 21–28	Regional distribution and ultrastructural properties of mitochondria: electron microscopy	Anaesthesia caused protracted injury to mitochondria including disturbance of regional distribution and ultrastructural appearance	Sub	N/A	N/A	Spectrophotometric analyses of mitochondrial electron transport chain activity—anaesthesia increased complex IV activity (known to increase free radical production)
Shu and colleagues, <i>Neurobiol Dis</i> 2012; <b>45</b> : 743–50 <sup>†</sup>	Rats Sprague–Dawley	PND 7	6 groups: 1. Room air 2. N <sub>2</sub> O 70% + isoflurane 0.75% for 6 h 3. Formalin 4. Formalin+ anaesthesia 6 h 5. Surgical incision alone 6. Surgery+ anaesthesia 6 h	Killed after 0 or 6 h or DVT assessment at 40 days	1. Apoptosis: caspase-3 2. Neuronal activation: c-Fos	6 h anaesthesia caused widespread CNS injury; both nociceptive stimuli (formalin, surgery) increased this injury	Cor, Th, Hc, SC	Trace fear conditioning 40 days post-insult	6 h anaesthesia caused long-term detriment in cognitive function. Both nociceptive stimuli exacerbated this	<sup>†</sup> Plus noxious stimuli. Nociceptive stimulation and prolonged anaesthesia produced significantly more apoptosis than anaesthesia alone. Western blot for TNF $\alpha$ and IL1 $\beta$ —anaesthesia plus nociceptive stimuli increased IL1 $\beta$ but not TNF $\alpha$
Boscolo and colleagues, <i>Neurobiol Dis</i> 2012; <b>45</b> : 1031–41	Rats Sprague–Dawley	PND 7	6 groups: 1. Room air 2. Combination: midazolam 9 mg kg <sup>-1</sup> , N <sub>2</sub> O 75%, isoflurane 0.75%, F <sub>102</sub> , 24% for 6 h 3. GA + EUK-134* 4. EUK alone* 5. GA + PPX* 6. PPK alone*	6 h	1. Mitochondrial morphology: EM, assessed on PND 21 2. Intracellular ROS formation: oxidant-sensing fluorescent probe HZDCF-DA, assessed immediately 3. Neuronal density: Nissl, assessed on PND 53	GA caused: 1. Mitochondrial damage, reversed by co-treatment with EUK or PPX 2. Up-regulation of ROS, diminished by EUK 3. Neuronal deletion, prevented by co-treatment with EUK or PPX	Subi	1. Cognitive impairment: radial arm maze, assessed from PND 45 2. Hyperactivity: open field test, assessed on PND 32	1. GA caused long-term cognitive impairment, completely prevented by EUK or PPX 2. GA did not cause hyperactivity	*EUK-134=synthetic ROS scavenger, PPX=pramipexole, a synthetic aminobenzothiazol derivative=mitochondrial protector. ELISA for pg 8-isoprostane, the most abundant prostaglandin-like compound formed <i>in vivo</i> from free radical-catalysed peroxidation of essential fatty acids—increased with GA, ameliorated by EUK
Huang and colleagues, <i>Brain Res</i> 2012; <b>2</b> : 164–71	Rats Sprague–Dawley	PND 7	Ketamine 25–75 mg kg <sup>-1</sup> daily for 3 days	3 days (3 doses)	Apoptosis: TUNEL	Ketamine 75 mg kg <sup>-1</sup> (but not 25–50 mg kg <sup>-1</sup> ) significantly increased TUNEL+ cells	Hc	Morris water maze test, assessed on PND 60	Ketamine 75 mg kg <sup>-1</sup> caused learning and memory impairment in adulthood	Western blot for t-PKC $\gamma$ , p-PKC $\gamma$ , t-ERK1/2, p-ERK1/2, Bcl-2—ketamine decreased p-PKC $\gamma$ , p-ERK1/2, Bcl-2 (pro-apoptotic effect). No change in t-PKC $\gamma$ , t-ERK1/2
Fang and colleagues, <i>Neurosci Bull</i> 2012; <b>28</b> : 499–508	Rats Sprague–Dawley male	PND 7	Sevoflurane 2% with O <sub>2</sub> 2 litre min <sup>-1</sup>	4 h	1. Apoptosis: caspase-3 2. Neuronal necrosis: Fluoro-Jade B 3. Progenitor proliferation: 5-bromodeoxyuridine (S-phase marker)	Increased cell death and decreased progenitor proliferation until at least 4 days post-sevoflurane	Hc	Morris water maze test assessed at 2 and 6 weeks post-sevoflurane	Spatial reference memory was not affected at 2 weeks but was affected at 6 weeks post-sevoflurane	Western blot for MK167 (mRNA Ki-67 protein, present during all active phases of cell cycle, absent from resting cells)—decreased post-sevoflurane

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Table 1 Continued

Reference	Species	Age	Anaesthetic	Duration (h)	Histology outcome	Histology results	Brain area	Neurodevelopmental outcome	Neurodevelopmental result	Other outcomes/comments
Liu and colleagues, <i>Anesthesiology</i> 2012; 117: 64–71 <sup>†</sup>	Rats Sprague–Dawley male	PND 7	Ketamine 2 mg ml <sup>-1</sup> , 5 injections at 90 min intervals ± 0.01 ml CFA (peripheral noxious stimulation)	6 h	1. Apoptosis: caspase-3, TUNEL 2. Neuronal density: NeuN	Increased apoptosis with ketamine, attenuated by concomitant noxious stimulation with CFA	CingC, SomC	N/A	N/A	<sup>†</sup> Plus noxious stimulus. Concurrent noxious stimulation with ketamine attenuated neuropoptosis. Western blot for Cl-Csp3, t-AKT, p-AKT, GSK-3β, cyclin D1 and β-actin—ketamine minimally decreased p-AKT and GSK-3β. Ketamine increased cyclin D1, response suppressed with concomitant noxious stimulus
Zhou and colleagues, <i>Mol Med Rep</i> 2012; 6: 543–6	Rats Sprague–Dawley	PND 7	Sevoflurane 2.3%	6 h, then killed 6 h post-sevoflurane	1. Morphology: H&E 2. Apoptosis: caspase-3 3. IHC for nNOS	Sevoflurane-exposed rats showed disorganized neuronal architecture, increased apoptosis and decreased nNOS expression	Hc	N/A	N/A	Previous studies have shown that sustained nNOS inhibition triggers neuroapoptosis
Popic and colleagues, <i>PLoS One</i> 2012; 7: e34396	Rats Wistar male	PND 14	Propofol 25 mg kg <sup>-1</sup> single injection	Killed after 0, 1, 2, 4, 8, 16, or 24 h	Neuronal necrosis: Fluoro-Jade B staining	No increase in neuronal necrosis seen at any time point	Cor, Thal	N/A	N/A	Western blot for BDNF, NGF, TrkA (=NGF-R), TrkB (=BDNF-R), Akt, ERK, Cl-Csp3—showed mostly down-regulation of neurotrophins, their receptors, and downstream effector kinases
Ponten and colleagues, <i>Acta Anaesthesiol Scand</i> 2012; 56: 1058–65 <sup>†</sup>	NMRI	PND 10	Ketamine 50 mg kg <sup>-1</sup> ± clonidine 10 or 40 mg kg <sup>-1</sup> pretreatment 30 min prior	Killed 24 h-post dose or tested PND 55	Apoptosis: Fluoro-Jade B	Increased apoptosis with ketamine. Pre-treatment with 40 mg kg <sup>-1</sup> but not 10 mg kg <sup>-1</sup> clonidine reversed ketamine-induced apoptosis	FroC, ParC, Hc	Spontaneous activity in a novel environment at PND 55	Reduced spontaneous activity after ketamine. Pre-treatment with 40 mg kg <sup>-1</sup> but not 10 mg kg <sup>-1</sup> clonidine reversed this	<sup>†</sup> Neuroprotection study. Clonidine pre-treatment reversed the adverse affects of ketamine in this mouse model
Wang, <i>Neuro Sci</i> 2012; 33: 535–44	Rats Sprague–Dawley pregnant	Adult pre-gest or preg	Sevoflurane 2.4% MAC in oxygen. Exposed pre-gest or at gest age 6, 10, 14, and 18 days	6 h then killed on PND 0, 7, 14, 28	1. Morphology: H&E 2. Apoptosis: caspase-3 3. IHC for nNOS	Pre-pregnancy anaesthesia did not cause increased apoptosis. Sevoflurane in pregnancy resulted in disorganized architecture, increased apoptosis, and decreased nNOS expression at PND 0, 7, 14 but not PND 28	Hc	N/A	N/A	RT-PCR for NMDA-R 1/2 expression—pre-pregnancy anaesthesia had no effect. Sevoflurane in pregnancy resulted in increased expression on PND 0, 7, 14 but no significant difference at PND 28. Western blot for PKCα, p-JNK, p-ERK, and FOS showed no effect with pre-pregnancy anaesthesia. Sevoflurane in pregnancy resulted in up-regulation of PKCα and p-JNK and down-regulation of p-ERK and FOS on PND 0, 7, 14 but no significant difference on PND 28

**Table 2** Primate studies of anaesthetic-induced neuroapoptosis. PND, post-natal day; GA, gestational age; MAC, mean alveolar concentration

Reference	Species	Age	Anaesthetic	Duration (h)	Histology outcome	Neurodevelopmental outcome
Slikker and colleagues, <i>Toxicol Sci</i> 2007; <b>98</b> : 145–58	<i>Rhesus macaques</i> (Macaca mulatta)	GA 122 days or PND 5 or PND 35	Ketamine 20 mg kg <sup>-1</sup> i.m. followed by infusion of 20–50 mg kg <sup>-1</sup> h <sup>-1</sup> i.v. to maintain light surgical plane of anaesthesia	3 or 24 h	Increased apoptosis, necrosis, and NMDA-receptor NR1 subunit expression in neonatal brains after 24 h ketamine at GA 122 or PND 5 but not PND 35 or with shorter duration ketamine	N/A
Zou and colleagues, <i>Int J Dev Neurosci</i> 2009; <b>27</b> : 727–31	<i>Rhesus macaques</i> (Macaca mulatta)	PND 5–6	Ketamine 20 mg kg <sup>-1</sup> i.m. followed by infusion 20–50 mg kg <sup>-1</sup> h <sup>-1</sup> i.v. to maintain light surgical plane of anaesthesia	3, 9, or 24 h	Time-dependent increased apoptosis and necrosis seen after ketamine 9 or 24 h, not seen at 3 h. Apoptosis prominent in neocortical areas, especially frontal cortex	N/A
Brambink and colleagues, <i>Anesthesiology</i> 2010; <b>112</b> : 834–41	<i>Rhesus macaques</i>	PND 5–6	Isoflurane, end-tidal 0.7–1.5 vol% to maintain light surgical plane of anaesthesia	5 h	Increased apoptosis. Temporal and somatosensory cortices most severely affected	N/A
Zou and colleagues, <i>Neurotox Teratol</i> 2011; <b>33</b> : 592–7	<i>Rhesus macaques</i>	PND 5–6	N <sub>2</sub> O 70%, isoflurane 1% alone or combination	8 h	Increased apoptosis with combination anaesthesia but not with N <sub>2</sub> O or isoflurane alone. Frontal and temporal cortices and hippocampus most severely affected	N/A
Paule and colleagues, <i>Neurotoxicol Teratol</i> 2011; <b>33</b> : 220–30	<i>Rhesus macaques</i>	PND 5–6	Ketamine 20 mg kg <sup>-1</sup> i.m. followed by infusion 20–50 mg kg <sup>-1</sup> h <sup>-1</sup> i.v. to maintain light surgical plane of anaesthesia	24 h	N/A	At 7 months and persistent at 3.5 yr, the ketamine group had poorer performance in learning, colour, and position discrimination tasks; slower response speed; lower motivation
Brambink and colleagues, <i>Anesthesiology</i> 2012; <b>116</b> : 372–84	<i>Rhesus macaques</i>	GA 120 days or PND 6	Ketamine i.v. 10–20 mg kg <sup>-1</sup> bolus then 10–85 mg <sup>-1</sup> kg <sup>-1</sup> h <sup>-1</sup> infusion to maintain intermediate surgical plane of anaesthesia	5 h	Widespread apoptosis in all groups. Loss of neurones 2.2× greater in fetuses than in neonates. Damage throughout fetal brain; damage maximal in basal ganglia in neonatal brain	N/A
Brambink and colleagues, <i>Ann Neurol</i> 2012; <b>72</b> : 525–35	<i>Rhesus macaques</i>	PND 6	Isoflurane, end-tidal 0.7–1.5 vol% to maintain light surgical plane of anaesthesia	5 h	Significant apoptosis seen throughout CNS in both white and grey matter. Fifty-two per cent dying cells were glia, 48% were neurones. Oligodendrocytes engaged in myelinogenesis were selectively vulnerable	N/A

### Preclinical evidence for anaesthetic-induced neurodegeneration: piglets and primates

Non-rodent mammals, including piglets and non-human primates, are also vulnerable to injury. Piglets are of particular interest as their brain development closely parallels

humans, with synaptogenesis occurring pre- and post-natally. Isoflurane-nitrous oxide induced apoptosis in a single 5-day-old piglet.<sup>12</sup> In a second piglet study, isoflurane-nitrous oxide was shown to cause more damage in intrauterine growth restricted piglets than in normal birth weight piglets (though there were no non-anaesthetized controls).<sup>45</sup> Further useful information could be gleaned by

**Table 3** Clinical observational studies of neurocognitive impairment after infant anaesthesia and surgery (IAS). NOS, Newcastle–Ottawa Score

Reference	Design	Study period	Follow-up	Sample size	Operations	Anaesthetic	Confounder adjustment	Outcomes with exposure to anaesthesia and surgery	Grade of evidence	NOS (max=9*)
DiMaggio and colleagues <sup>19</sup>	Birth case–control	1991–2001	1–2 yr	5050 control, 383 exposed	Inguinal hernia repair	N/A	Yes	Behavioural/developmental delay after IAS	B	8*
Hansen and colleagues <sup>17</sup>	Birth cohort	1986–1990	25–16 yr	14 575 control, 2689 exposed	Inguinal hernia repair	N/A	Yes	Academic performance: No difference but increased non-attainment of test scores with IAS	B	8*
Ing and colleagues <sup>21</sup>	Birth cohort	1989–1992	10 yr	2287 control, 321 exposed	Varied	N/A	Yes	Neuropsychological testing: increased expressive and receptive language deficits after IAS	B	8*
Wilder and colleagues <sup>24</sup>	Birth cohort	1976–1982	19 yr	4764 control, 593 exposed	Varied	Known	Yes	Increased learning difficulties with increased exposure to IAS	B	8*
Sprung and colleagues <sup>23</sup>	Birth cohort	1976–1982	19 yr	5357 control, 531 exposed	Varied	Known	Yes	Increased ADHD with increased exposure to IAS	B	8*
Flick and colleagues <sup>22</sup>	Case–control	1976–1982	19 yr	5007 controls, 350 exposed	Varied	Known	Yes	Increased learning difficulties after IAS	B	8*
DiMaggio and colleagues <sup>20</sup>	Sibling cohort	1999–2005		10146 control, 304 exposed	Varied	N/A	No	Increased developmental and behavioral disorders after IAS. Effect not found in more tightly controlled analysis	B	8*
Block and colleagues <sup>25</sup>	Treatment cohort	1990–2008	7–17.9 yr	287 exposed	Inguinal hernia, orchidopexy, pyloromyotomy	Known	No	Academic achievement tests: mean scores lower than population norm after IAS	B	5*
Bartels and colleagues <sup>26</sup>	MCMA twin cohort	1986–1995	12 yr	1890 control, 1248 exposed	Varied	N/A	Yes	Educational achievement: scores significantly lower than population norm for males, non-significantly lower for females after IAS. However, no effect observed between monozygotic twins	B	8*

studying piglets as their brain growth spurt, myelination, and development of electrical activity are thought to be similar to humans.<sup>46</sup>

Importantly, anaesthetic injury is also observed in the primate brain (Table 2). The initial studies involved ketamine exposure for 3, 5, 9, and 24 h at a 'surgical plane' of anaesthesia. Unlike many of the rodent studies, in primates, there was good control of physiological variables.<sup>8 10 47 48</sup> The doses used were much higher than would be used in humans. However, it is well known that animals require higher doses (on a mg kg<sup>-1</sup> basis) of some anaesthetics such as ketamine to produce anaesthesia, and these doses were necessary to simulate 'clinical' conditions. Exposure to 5, 9, or 24 h of ketamine produced robust apoptosis in the frontal cortex in 5-day-old monkeys; however, a 3 h exposure was not toxic.<sup>8 47</sup> Twenty-four hours of ketamine produced no apoptosis in 35-day-old monkeys. Although the lack of effect seen with 3 h of exposure is reassuring, the limited number of animals studied and focus on only one brain injury somewhat limits conclusions about the relative safety of just a 3 h exposure. Ketamine administration for 24 h has also been associated with long-term cognitive impairment up to 3.5 yr later<sup>10</sup> using the Operant Test Battery, a test that correlates with intelligence in children. The cognitive impairments induced in rhesus monkeys are relatively 'high level' involving both learning and concept formation, consistent with significant apoptosis in the frontal cortex. Ketamine injury is also apparent in primate fetus with exposure during pregnancy. In fact, the rhesus fetus is more vulnerable than the neonate.<sup>47</sup> The fetal stage examined in the rhesus macaque appears to correlate with the last trimester of human pregnancy or premature birth.<sup>27</sup>

Isoflurane has also been shown to induce apoptosis in the neonatal rhesus monkey brain after a 5 h exposure at an anaesthetic dose very similar to that required in humans (0.7–1.5%).<sup>48</sup> The areas particularly affected include layers II, V, and VI in the frontal, somatosensory (primary visual areas), and temporal cortices. This indicates that diverse neuronal types, in widely distributed regions throughout the cerebral cortex, are vulnerable. The authors argue that, in terms of brain development, these monkeys are comparable with 6-month-old infants, and suggesting that humans up to that age might be vulnerable.<sup>48</sup> A similar study has shown that in addition to neurones, isoflurane also produces degeneration of oligodendrocytes, cells that play a key role in myelinating axons.<sup>49</sup> A single exposure to 5 h of isoflurane led to loss of around 6.3% of the total population of myelinating oligodendrocytes; the biological consequences of reducing myelinating oligodendrocytes from the developing brain may include disruption of myelinogenesis and pathological changes in axonal structure or function. The oligodendrocyte degeneration induced by isoflurane in neonates differs from that in the preterm brain where it is loss of the *premyelinating* oligodendrocytes that occurs due to their increased vulnerability at this developmental stage of brain development.

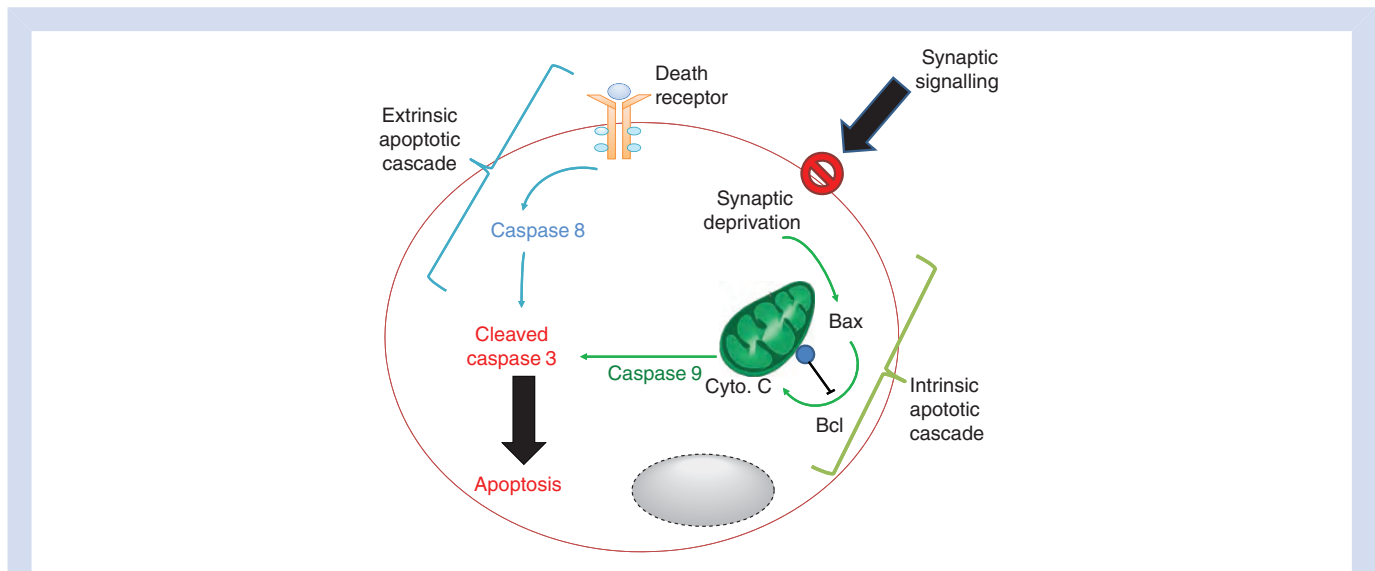
Data are accumulating regarding the diverse range of injuries induced by anaesthetics; however, significant

questions remain in how to translate these findings to humans. Critical issues include the different doses required, how to compare periods of development across species, how to interpret neurobehavioural outcomes in animals and humans, and the degree of physiological control during anaesthesia. The relatively long exposure time used in animal models to induce injury also entails some uncertainty. The rodent brain develops over a much shorter period than humans, so the relative period of anaesthesia is considerably greater in rodents. Lastly, the influence of surgery itself is unknown. An understanding of the putative mechanisms of injury is essential to knowing how to translate these findings to humans.

## Putative mechanism of the anaesthetic injury

One hypothesis for the mechanism underlying the neurotoxic effect of anaesthesia is that suppression of neurotrophic synaptic signalling leads to apoptosis of the postsynaptic neurone,<sup>50</sup> via both the intrinsic and extrinsic apoptotic pathways<sup>16 29</sup> (Fig. 1). This hypothesis directly links the putative mechanisms of anaesthesia<sup>51 52</sup> to the mechanisms of neurotoxicity in the newborn. In terms of neurodevelopment, the anaesthetic suppression of synaptic activity might make the dying neurone redundant and lead to programmed cell death, known as apoptosis. This is consistent with the known effects of synaptic signalling in neurodevelopment;<sup>50</sup> the abundance of rapid-eye-movement sleep in the neonatal period (providing an active brain in a restful baby and presumably avoiding neurone-suppressing effects of non-rapid-eye-movement sleep);<sup>53</sup> and the known effects of anaesthesia on synaptic activity.<sup>52 54</sup> One possibility is that the anaesthetic-induced suppression of synaptic activity leads to altered processing of the neurotrophin brain-derived neurotrophic factor (BDNF), leading to the cell death promoting form of the neurotrophin.<sup>55</sup> Manipulating this pathway might be one way to prevent post-synaptic neuronal apoptosis.<sup>55</sup>

Neuronal apoptosis typically occurs through two distinct signalling pathways, the intrinsic and extrinsic apoptotic pathways<sup>16 29</sup> (Fig. 1). The intrinsic pathway involves internal signals activating a mitochondrial-dependent pathway with the release of cytochrome c, activation of the pro-apoptotic factor BAX, and ultimately DNA cleavage and cell death. The loss of synaptic activity can lead to apoptosis through direct activation of the intrinsic apoptotic cascade.<sup>56</sup> The exact molecular mechanism through which anaesthetics suppress synaptic signalling is unclear, although effects on glutamate (e.g. NMDA receptors) and  $\gamma$ -aminobutyric acid (GABA) synaptic transmission seem likely.<sup>1</sup> Interestingly, the GABA<sub>A</sub> antagonist gabazine does not reverse the apoptotic effects of isoflurane *in vitro*, suggesting that chloride permeable GABA<sub>A</sub> receptors might not be solely responsible.<sup>7</sup> However, given that several GABAergic drugs (such as midazolam, propofol, thiopental, and the volatile anaesthetics) induce apoptosis, it is premature to dismiss this mechanism. A role for chloride gradients has been implicated in the toxicity, since the Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> cotransporter 1 inhibitor



**Fig 1** Proposed mechanisms of anaesthetic-induced neurodegeneration in the newborn involving the intrinsic and extrinsic apoptotic cell death pathways. Suppression of synaptic signalling is proposed to attenuate neurotrophic survival signals leading to activation of the intrinsic apoptotic cascade. This involves mobilization of the pro-apoptotic protein Bax, which in turn induces cytochrome c (Cyto. C) release from mitochondria, activation of caspase-9, and subsequent induction of caspase 3 cleavage to provoke apoptosis. Anti-apoptotic effectors such as Bcl oppose this pathway. The extrinsic pathway is stimulated by ligands binding to death receptors located on the cell membrane that cause caspase 8 activation, which then cleaves and activates caspase 3. These ligands include cytokines such as TNF- $\alpha$ .

bumetanide reverses sevoflurane-induced apoptosis.<sup>57</sup> Beyond any putative role of chloride gradients, there are several synaptic and intracellular targets that still require evaluation before the mechanism is more clearly understood. For example, suppression of pro-survival extracellular-related kinase (ERK) signalling has been associated with apoptosis from ketamine, propofol, and/or isoflurane; rescue of this pathway with lithium<sup>58</sup> or dexmedetomidine<sup>35</sup> protects against neuroapoptosis.

The extrinsic pathway is typically triggered by external stimuli such as the cytokine tumour necrosis factor (TNF)- $\alpha$ . While anaesthetic and sedative agents typically exert anti-inflammatory effects in the adult,<sup>59</sup> in the young, the effects seem largely pro-inflammatory.<sup>60</sup> This pro-inflammatory effect might not only stimulate the extrinsic apoptotic cascade but could also synergize with other inflammatory stimuli such as pain and surgery. This hypothesis requires further testing. The reason for this pro-inflammatory effect is unclear, although it might relate to altered GABA<sub>A</sub> signalling with neurodevelopment. At birth, GABA<sub>A</sub> receptor activation can be excitatory, and this switches to the adult inhibitory effect with ageing. This might mean that the immune effects of GABA<sub>A</sub> signalling switch from a pro- to anti-inflammatory effect with development.<sup>59 61</sup>

Interestingly, not all neurones are vulnerable to injury. Glutamatergic, GABAergic, and dopaminergic neurones die after anaesthetic exposure to neonatal rats; however, cholinergic neurones are resistant.<sup>62</sup> Hence, it can be hypothesized that neurones innervated by cholinergic neurones are vulnerable to anaesthetic injury as neurotrophic cholinergic signalling is suppressed. The distributed nature of these neurones

implies that this does not account for all cell death. Nonetheless, as anaesthesia is known to suppress cholinergic signalling,<sup>63</sup> it is possible that cholinergic manipulation is another way of altering apoptosis, particularly in the cortex where cholinergic innervation is extensive. Given the distinct type of anaesthesia induced by ketamine, it may not be prudent to assume that similar pathways are involved in its neuroapoptotic mechanism compared with volatile agents.

Another hypothesis that has emerged is that anaesthetics induce seizure activity to cause neurotoxicity based on work with sevoflurane in neonatal rats.<sup>57</sup> Studies are required to understand if a similar activity occurs in the human neonatal brain under anaesthesia. Despite suggestions that suppression of neural activity or the opposite—seizure activity—underpins the anaesthetic brain injury, there are limited data correlating EEG or neuroimaging activity with injury *in vivo*.<sup>57</sup> Further mechanistic studies investigating non-invasive translational markers of anaesthetic injury, such as EEG, are required.

The effect of anaesthetic exposure on hypoxic-ischaemic brain injury in the neonate is unclear. One study demonstrated that a 2 h hypoxic exposure the day before anaesthetic injury increased the subsequent apoptosis.<sup>64</sup> This suggests that neonates with prior, even mild, brain injury may be more susceptible to anaesthetic injury and thus may represent a vulnerable cohort of patients. Further preclinical study is required.

## Agents that reduce the injury

Insights might also be afforded by examining the accumulating data for agents that can protect against anaesthetic

neurotoxicity, which comes entirely from rodent studies (relevant studies are highlighted in *italic* in Table 1). One of the more promising class of agents that might provide neuroprotection against anaesthetic-induced apoptosis is the  $\alpha_2$ -adrenergic agonists, which can exert cognitive protection.<sup>7 35</sup>  $\alpha_2$ -Adrenoceptors are known to play a key role in neurodevelopment underlying the rationale for targeting this receptor. Dexmedetomidine and clonidine are the only drugs that have been shown to reduce anaesthetic-induced apoptosis and also prevent subsequent decline in cognition (although neither are completely attenuated).<sup>7 35 65</sup> The protective effect of dexmedetomidine was shown against isoflurane injury, and was attenuated in the hippocampus by an  $\alpha_2$ -adrenoceptor antagonist indicating involvement of  $\alpha_2$ -adrenoceptors.<sup>7</sup> Dexmedetomidine also reversed the cognitive impairment induced by isoflurane. Furthermore, clonidine protects against ketamine neuroapoptosis and behavioural abnormalities.<sup>65</sup> Importantly,  $\alpha_2$ -agonists do not themselves provoke apoptosis or produce neurological abnormalities by either systemic or spinal injection.<sup>7 42 65</sup>

Xenon is another candidate neuroprotective drug; xenon itself shows minimal toxicity but also can attenuate isoflurane-induced apoptosis when given with isoflurane<sup>29</sup> or before isoflurane.<sup>64</sup> This effect of xenon is particularly intriguing as it is an NMDA antagonist, similar to ketamine, but is protective rather than toxic. The expense and difficulties in administering xenon may mitigate against its development for neonatal anaesthesia however.

Melatonin<sup>5 66</sup> and  $\beta$ -estradiol<sup>14 67</sup> provide protection against anaesthetic and anti-epileptic drug-induced apoptosis in rats. Both are known to act as free radical scavengers, anti-inflammatory, and anti-apoptotic agents. They target the intrinsic apoptotic pathway,<sup>5 14 66 67</sup> which is up-regulated in anaesthetic injury,<sup>29</sup> and hence they are plausible candidates of neuroprotective drugs. Other agents such as lithium,<sup>58 68</sup> hypothermia,<sup>69</sup> L-carnitine,<sup>70</sup> xestospongine-C,<sup>71</sup> bumetanide,<sup>57</sup> and erythropoietin<sup>72</sup> can also provide protection, but further studies are required to confirm these observations and to allow clinical translation. The possibility of modulating the BDNF pathway to promote cell survival, rather than cell death, is also of particular interest,<sup>55</sup> especially if combined with anaesthetic drugs that up-regulate BDNF expression.<sup>5 29 67</sup> Most of the signalling pathways that have been manipulated so far to reduce anaesthetic-induced apoptosis involve the intrinsic apoptotic cascade, particularly modulating the Bcl-2 or Bcl-XL to Bax ratio (Fig. 1). The potential to modulate other contributing factors, including the extrinsic pathway, needs further attention. Furthermore, all of these proposed therapies need to be trialled in animal models other than rodents, given the limited clinical translation from rodent models to humans.

Environmental enrichment can improve cognitive function after neonatal exposure to sevoflurane in rats, suggesting that it might be possible to compensate for the injury.<sup>73</sup> However, anaesthetic-exposed rodents might not learn as fast as non-exposed rats,<sup>73</sup> similar to primates.<sup>10</sup> Hence, it is unclear whether this really constitutes a therapeutic non-pharmacological protective strategy.

## Potential impact of surgery on neurodevelopment

Neonatal pain, induced by surgery, is known to induce long-term behavioural effects in animals and humans,<sup>1 74</sup> emphasizing the need for adequate analgesia in the perioperative period. In neonatal rodents, brief inflammatory pain leads to increased neuronal activation and cell death, particularly in immature animals.<sup>75</sup> The combination of glial cell activation, pro-inflammatory cytokine release, and prolonged pain is likely to produce prolonged neuronal hyperexcitability in cortical and subcortical areas. Cell death might result from this prolonged excitation.<sup>75</sup>

Anaesthesia is given in the context of surgery to prevent harmful effects of painful stimulation; the impact of surgery on anaesthetic injury is thus of great clinical significance. There are only limited, and conflicting, data on the influence of surgical stimulus on the anaesthetic injury. Nociceptive stimuli (surgical incision or formalin) provided during 6 h of nitrous oxide–isoflurane anaesthesia exacerbated neuroapoptosis and long-term cognitive impairment in rat pups.<sup>76</sup> This might be driven by exacerbation of extrinsic apoptotic signalling through inflammation (Fig. 1). However, limitations of this animal model include the lack of physiological control and realistic surgical stimulation. Others have shown that tissue injury, induced by tail clamping, has no influence on sevoflurane-induced apoptosis,<sup>73</sup> while another group found that ketamine-induced neuroapoptosis was antagonized by noxious stimulation.<sup>77</sup>

The apparent inconsistencies in these studies might be explained by the differing molecular targets affected by ketamine, sevoflurane, and isoflurane–nitrous oxide. For example, ketamine is known to possess anti-inflammatory properties and does not target GABA<sub>A</sub> receptors, so the difference between the effect of noxious stimulation on the two anaesthetic regimens could relate to the different anaesthetic drugs studied. Another difference is in the degree of tissue injury and surgical stimulus. The relative toxic or protective effects of the anaesthetic might depend on the size of the injury relative to the duration and dose of anaesthetic. Hence, studies are required to address how surgical stimulation affects anaesthetic-induced injury in a large animal model where physiological variables can be better controlled.

The impact of surgery, in addition to anaesthesia, on perinatal brain injury also requires further investigation as inflammatory stimulation could exacerbate the previously incurred hypoxic–ischaemic injury.<sup>78</sup> Surgery induces significant pain that might exacerbate the excitotoxic injury of hypoxia–ischaemia by augmenting neuronal activity. Furthermore, surgical trauma is characterized by pro-inflammatory cytokine release, microcirculatory disturbance, and cell-mediated immune dysfunction, followed by a compensatory anti-inflammatory response. Also directly altering neuronal function,<sup>79</sup> these inflammatory changes might provoke further degeneration,<sup>80</sup> including activation of the extrinsic apoptotic cascade leading to further neuronal death.

## Epidemiology evidence for anaesthetic and surgical effects on neurodevelopment

There are numerous cohort and cross-sectional studies that have investigated the association between surgery, anaesthesia, and cognitive or neurobehavioural outcome, with conflicting results. A recent systematic review and Bayesian meta-analysis identified 12 retrospective epidemiology studies relevant to anaesthetic neurotoxicity in children. Seven of these were included in the analysis, involving 40 685 children.<sup>18</sup> Bayesian meta-analysis differs from the standard meta-analytic approach as it seeks to identify the odds ratio of the mean effect size and calculate the likelihood that any future study would identify the same mean effect size. A measure of uncertainty in the finding is also produced: the credible interval. The model takes into account the prior beliefs of the investigators (in this case, that anaesthesia exerts no toxic effect). These data can be 'synthesized' to produce a summary of the data, but also can be used to form a 'predictive' model for a future study and hence has value in the planning of research. Importantly, there was significant heterogeneity between the studies for both the unadjusted and adjusted outcome of adverse neurodevelopmental and behavioural outcome of the analysis for 'exposed to any anaesthesia'. The *unadjusted* odds ratio was 1.9 (95% credible interval 1.2–3.0) for the synthesized data, although for the predictive model, the odds ratio was 2.2 (95% credible interval 0.6–6.1). The *adjusted* odds ratio was 1.4 (95% credible interval 0.9–2.2) for the data synthesis, and for the predictive model, the odds ratio was 1.5 (95% credible interval 0.5–4.0). Using this technique, the authors concluded that there is a 'modestly elevated risk of adverse behavioral or developmental outcomes in children who were exposed to anaesthesia/surgery during early childhood. The evidence, however, is considerably uncertain'.

## Systematic review of epidemiology studies

We have conducted our own systematic review of the literature and provide a descriptive table to the reader where we have evaluated the methodology and grade of evidence for each study (Table 3). This information is provided, so the reader can appreciate the accumulating clinical evidence that anaesthetics and surgery could induce harm. We identified nine studies in total,<sup>17 19–25 81</sup> and we systematically graded the evidence<sup>82</sup> from each study, and also detailing their strengths and weaknesses.

## Excluded studies

To be included in the systematic review, some attempt to compare an exposed group with a non-exposed control group was required; however, we did include studies using population controls.<sup>17 25</sup> One excluded study (due to a lack of a non-exposed control group) found that the point estimate for neurobehavioural impairment was non-significantly higher for operations at a younger age (under 24 months of

age); however, the confidence intervals were wide, overlapping with one.<sup>83</sup>

One study, which was incorporated in the Bayesian meta-analysis,<sup>18</sup> was excluded as it pertained to intensive care sedation rather than anaesthesia and surgery.<sup>84</sup> This study did not find an association between the duration of sedation for mechanical ventilation in premature neonates and adverse cognitive outcome.<sup>84</sup> In contrast, another small study found that anaesthesia and surgery in premature neonates was associated with impaired mental development index and reduced brain volumes and increased white matter injury on magnetic resonance imaging at 2 yr of age.<sup>85</sup> This study was also not included in our Table 3 as it focused on preterm brain injury. It is important to note that morphine use in the neonatal intensive care unit has been associated with impaired cognitive function at 5 yr of age as assessed by the visual analysis IQ subtest.<sup>86</sup> We did not include studies that found that neuraxial labour analgesia<sup>87</sup> or anaesthesia for Caesarean section<sup>81</sup> were not associated with adverse cognitive outcomes.

## Included studies

The majority of studies included in our review had robust methodology (indicated by a Newcastle–Ottawa score of >8\*; Table 3). Nonetheless, there is heterogeneity in the approaches, degree of confounding, and endpoints analysed. Despite these limitations, the cumulative data suggest a small increase in the risk of adverse neurodevelopmental outcome with exposure to anaesthesia and surgery. All nine studies provided some evidence that anaesthesia and surgery might be associated with impairments in cognition or behaviour (Table 3). Furthermore, there is some evidence that the risk increases with increased cumulative exposure to anaesthesia and surgery.<sup>22–24</sup> Of course, given the observational nature of these studies, it is difficult to dissect the effect of anaesthesia from the effect of surgery, or the underlying pathology driving the need for surgery, or patient comorbidities. Another limitation of these studies is the limited information about perioperative care, including anaesthetic drugs, haemodynamics, postoperative care, complications, and comorbidities. Furthermore, the relevance of the anaesthetic techniques and perioperative care used in these retrospective studies to modern practice is unclear (as many of the studies date back decades to allow sufficient patient follow-up). Therefore, there is significant reason to be uncertain about the clinical data, as suggested by the Bayesian meta-analysis.

One study is worth particular attention as it compared twins where one twin was exposed to anaesthesia and surgery and the other was not.<sup>26</sup> This study found that educational achievement scores were significantly lower than the population norm for males exposed to anaesthesia but non-significantly lower for females. However, no difference was detected between monozygotic twins where one twin was exposed and another was not. The authors use these data to argue that there is not a causal relationship between



anaesthesia and surgery and adverse cognitive outcomes.<sup>26</sup> However, the limited sample size precludes any definitive conclusions. Another limitation of this study and other similar studies is the relatively coarse outcome measure. Finding no effect in a summary score such as composite school score does not exclude effects in neurobehavioural subdomains. A similar criticism can be made of another study that reported no effect of anaesthesia and surgery on academic achievement;<sup>17</sup> more subtle tests might have identified a difference. Against this, the same study suggested that anaesthesia and surgery increases the risk of not obtaining any test score, consistent with a profound deficit in cognition. Therefore, even within studies, interpretation of the data can be difficult. The other seven studies largely provide point estimates that suggest there might be harm from anaesthesia and surgery, but with confidence intervals that are too wide to allow confidence in their findings.

Given the limitations of these observational studies, it is difficult to draw any firm conclusions. At best, they suggest that an association between anaesthesia exposure and adverse outcome is possible, but this cannot be definitely confirmed or ruled out. This emphasizes the need for further high-quality prospective approaches to this problem.

### Prospective studies of the effect of anaesthetics and surgery on neurodevelopment

The significant limitations of the retrospective observational epidemiology studies mandate prospective approaches to studying the effects of anaesthesia and surgery on neurodevelopment.<sup>11 88</sup> Two important studies underway include the GAS trial and PANDA cohort study. The GAS trial is a randomized controlled trial of general anaesthesia compared with spinal anaesthesia for hernia surgery in more than 700 neonates.<sup>89</sup> Randomization is stratified by age and by site to ensure balanced groups. The aim is to discern whether general anaesthesia and awake regional anaesthesia have similar effects on cognitive function at 2 and 5 yr of age. The GAS trial is likely to complete recruitment in February 2013. PANDA (Pediatric Anesthesia and NeuroDevelopment Assessment) is a cohort study that will enrol 500 sibling matched pairs (1000 children) who underwent hernia surgery under general anaesthesia (ASA I–II) before 36 months of age.<sup>2</sup> The children will undergo a series of neuropsychological tests between 8 and 15 yr of age. The results of a recent pilot study for PANDA<sup>90</sup> suggest that it will have adequate power to detect statistically significant differences in total IQ (mean difference of four points, standard deviation of 17 points;  $\alpha = 0.05$ ;  $\beta = 0.8$ ).

### Future investigations into the neurodevelopmental effects of perioperative care

There are numerous outstanding questions, not least the clinical relevance of the large amount of preclinical evidence

for harm of anaesthetic exposure. It remains to be seen whether neuroprotective agents, effective in rodents, can provide clinical protection. Here, an important step will be translation via large animal models such as the piglet or primate that share greater similarities to human neurodevelopment.<sup>91</sup> We are a long way from clinical trials of these putative neuroprotective agents. Rather we emphasize the need for further prospective studies and translational research approaches to the problem. The prospect of 'in human' neuroimaging to detect injury must be explored further,<sup>85</sup> and also non-invasive biomarkers of injury such as the EEG. In the short term, we are not able to issue clinical guidelines.<sup>11</sup> We would not recommend reducing or increasing the anaesthetic dose and there is a lack of evidence to support neuroprotective interventions unless they are indicated for other purposes (e.g. premedication or analgesia).

### Conclusions

The increasing public exposure of research in this area is making parents increasingly aware that anaesthetic exposure could affect neurodevelopment in children. We suggest that is currently only weak evidence that anaesthesia is harmful but that withholding or significantly altering established anaesthesia techniques is likely more harmful.<sup>1 11</sup> In some circumstances, delaying surgery might be appropriate; however, surgery in children is rarely ever elective and delay can also cause harm. We recommend that any discussion over delaying an operation should involve the surgeon.

### Declaration of interest

R.D.S. has acted as a consultant for Air Liquide, Paris, France, concerning the development of medical gases and has received speaker fees from Orion (Turku, Finland) and Hospira (Chicago, USA).

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