

Anaesthesia associated developmental neurotoxicity (AADN) 2015

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The long-term cerebral effects of general anaesthesia at the extremes of age are currently enjoying attention. In children, the concern is that exposure to anaesthesia when less than 4 years of age may cause subsequent learning disabilities and behavioural changes. However, clinical equipoise exists as the available human studies are imperfect and the results of the large-scale multinational trials are not yet available. This structured narrative review summarises the overwhelming amount of information available.

'Everything should be as simple as it can be, but not simpler.'

(Albert Einstein)

'An estimated 6 million children (including 1.5 million infants) undergo surgery and anaesthesia each year in the USA alone, so the clinical relevance of anaesthetic neurotoxicity is an urgent matter of public health.'¹

Animal anaesthesia associated developmental neurotoxicity (AADN) studies: the evidence

'Studies in rodents and primates have (convincingly) shown that anaesthesia is neurotoxic to the developing brain after exposure in the neonatal period.'²

Early research demonstrating that halothane impaired rat neural synaptogenesis and learning was largely ignored.^{3,4} The current concerns were sparked by Jevtovic-Todorovic and colleagues⁵ evidence that day 7 postnatal (P7) rats exposed to a 'triple cocktail' of midazolam, isoflurane, and nitrous oxide exhibited accelerated neuronal apoptosis during synaptogenesis (Table 1) followed by impaired cognitive function (learning, memory, behaviour) in later life.

Similar findings have been observed with non-anaesthetic N-methyl-D-aspartate (NMDA) glutamate receptor antagonists,⁶ other volatile anaesthetics (sevoflurane, halothane, desflurane), xenon, propofol, barbiturates and benzodiazepines.⁷⁻¹³ The injury is worse when younger animals are exposed to larger dosages for longer periods.^{6,7,14-19} The cognitive consequences of these injuries invariably continue into the adulthood of the animal, expressed as memory impairment and autism-like behavioural changes.^{5,9,11,14,20,21}

The evidence that anaesthetics damage the developing non-human brain therefore appears robust,²² a recent review identifying 55 rodent and 7 primate studies of relevance.²

Animal AADN studies: critical evaluation

Despite animal evidence raising the question whether AADN exists in humans, critical questions have been posed about this evidence. 'The clinical significance of these observations remains controversial.'²³

Critical evaluation of the available animal studies raises many questions:

- (1) It is unclear whether it is permissible to extrapolate animal models of AADN to human infants.²⁴
- (2) Anaesthesia dosages differ significantly between animals and humans, animals requiring greater dosages due to their increased resting metabolic rates. For example, the propofol induction dose in infant mice is 200 mg/kg⁷ while ketamine levels required to maintain anaesthesia in primates are five- to tenfold those in humans.¹⁷ Although animals need higher dosages compared with humans, many studies far exceeded the required dose in animals.
- (3) The duration of exposure to anaesthetics in animal experiments far exceeds that typical in human surgery.²⁵ Rat studies have typically involved six hours of anaesthetic exposure.^{5,6,14,17,26} If the average lifespan of rats and humans is 760 and 28 105 days respectively, a six-hour rat anaesthetic extrapolates to a 222-hour child exposure.
- (4) The proviso for initiation of neurotoxicity is that anaesthesia exposure must occur during the vulnerable period of neural development. However, the time-windows for vulnerability to AADN differ between species.²⁷
 - (4.1) Human and animal brains develop very differently with rats being altricial, while humans are a precocial species.²³ Altricial and precocial are terms that refer to species that at birth are immature and relatively well developed respectively. Rats and humans undergo rapid periods of brain development after birth and in utero respectively.
 - (4.2) The associations between peak synaptogenesis in humans and animals are not well defined. Postnatal day 7 in rats corresponds with peak synaptogenesis, the most vulnerable period of neurological development in rats.⁵ The classic rat model that employs 7-day-old (P7)

Table 1: Definitions of the core pathophysiological concepts

Neurogenesis: The generation and survival of new-born neurons from neural stem/progenitor cells. ²¹
Synaptogenesis: The process of brain development that involves both neuronal development (migration, cell differentiation, synapse formation and axonal myelination) and apoptosis. ⁶⁶ In humans, synaptogenesis occurs between 28 weeks gestation and 4 years of age. ¹²⁸
Apoptosis: The process of selective neuronal suicide, an essential physiological process aimed at disposing of the 50% to 70% of redundant neurons produced during synaptogenesis. ⁶⁶
Neurotoxicity: The structural or functional alteration of the nervous system as a result of chemical, biological or physical agent exposure. ¹²⁹
Discordant: Where only one twin received an anaesthetic.
Learning disabilities: The need for individualised education, learning disability or impaired cognition. Other definitions and outcomes do, however, exist.

rats is, however, not representative of human neonatal brains, but reflects brain development between 16 and 22 weeks' gestation. Similarly, brain development in 5-day-old primates equates to human brains between 20 and 22 weeks' gestation.²⁸ These observations suggest that exposure 'in utero' may represent the most vulnerable time.

- (5) Less physiological neurodevelopmental apoptosis occurs in animal compared with human brains. (Physiological) apoptosis occurs in only 0.5% to 1% of rodent neurons but in up to 50% of human neurons.²⁴ Excess apoptosis may have a more pronounced deleterious effect in a species that does not have spare or excess neurons.
- (6) The presence of physiological trespass in animal models may limit the applicability thereof to humans. Animal studies have not used the same standards of airway control, monitoring or interventions to restore deranged physiology (hypoxia, hypotension, low cardiac output, hypoglycaemia, hypercarbia etc.) as in human anaesthesia and surgery²⁵ with some new-born animals dying during anaesthesia exposure.²
- (7) Although strongly denied, it has been suggested that rodent pups do not suckle after anaesthesia; malnutrition has been linked to a decline in brain growth and learning.²⁵
- (8) General anaesthetics do not consistently cause neural damage, a number of animal models refuting the existence of AADN. Near-term pregnant sheep exposed to four hours of midazolam-thiopentone-isoflurane anaesthesia exhibited improved foetal systemic and cerebral oxygenation with no histological neurotoxicity.²⁹ Neonatal pigs exposed to a six-hour anaesthetic (midazolam, isoflurane, and nitrous oxide) did not exhibit functional impairment or aggravated neuronal death. Sixty-day old (P60) rats exposed to isoflurane exhibited both increased neuronal differentiation and improved neurocognitive function.¹⁶
- (9) Painful stimuli may ameliorate AADN. It has been hypothesised that painful stimuli may prevent the toxic effects of anaesthetics.^{2,25} In neonatal rats, pain results in neuronal excitation and cell death: this response is blocked by ketamine²⁴ but not sevoflurane.^{10,30}

Human AADN studies: the evidence

'... the cumulative data suggest a small increase in the risk of adverse neurodevelopmental outcome with exposure to

anaesthesia and surgery ... impairments in cognition and behaviour ... the risk increases with increased cumulative exposure to anaesthesia and surgery.'²

The available human studies are precised in Table 2 with their essential findings delineated below.

- (1) In one form or another, this debate has been around for considerable time, early studies either being ignored or attributing postoperative behavioural changes solely to psychological stress.^{32,33}
- (2) Behavioural disturbances similar to post-traumatic stress disorder occur in approximately 20% of children younger than 2 to 3½ years of age following the psychological trauma associated with anaesthesia, surgery and hospitalisation. Kotiniemi and colleagues' study emphasised that behavioural response to such trauma varies widely.³⁴ This negative behaviour is however self-limiting, the majority of improvements occurring within the four weeks following surgery.^{32,34-42}
- (3) Multiple anaesthesia-surgical exposures at younger ages may increase the risk of subsequent learning disabilities.^{41,43}
- (4) Brief anaesthesia exposure, including maternal exposure during labour and birth, does not affect the incidence of subsequent learning disabilities.⁴⁷⁻⁵¹
- (5) Divergent effects on cognitive performance have been reported. Some studies show few or no consequences following early anaesthesia exposure, rather attributing all cognitive changes to genetics.⁴⁷
- (6) Neurodevelopmental impairment does occur in children who survive severe life-threatening illnesses.⁵²

Human AADN studies: critical evaluation

'The clinical data, comprising largely retrospective cohort database analyses, are (concerning but) inconclusive, in part due to confounding variables inherent in these observational epidemiological approaches.'²

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. This uncertainty emphasises the need for further high-quality studies.

- (1) Viewpoints on the aetiological mechanisms of AADN are constantly changing. Older perspectives that postoperative changes were exclusively due to psychological trauma have radically shifted with recent experimental evidence

Table 2: Human studies

1. **Wilder et al. Early exposure to anaesthesia and learning disabilities in a population-based birth cohort. *Anaesthesiology*, 2009:** This Mayo Clinic-based group interrogated medical and educational records contained in the Olmstead County Birth Cohort registry 19 years after children had been exposed to anaesthesia and surgery before 4 years of age.⁴⁴ They concluded that not single, but rather two or more, exposures were associated with an increased risk (hazard ratios of 1.0, 1.59 and 2.6) of subsequent reading, writing, and mathematical learning disabilities.
2. **Flick et al. Cognitive and behavioural outcomes after early exposure to anaesthesia and surgery. *Paediatrics*, 2011:** The Mayo Clinic group also compared the records of individuals 19 years after they had undergone surgery before age 2 years; the control group comprised unexposed individuals.⁴⁵ Multiple but not single exposure was associated with greater learning disability and impaired cognition, the incidences being similar to those observed in the Wilder study.
3. **DiMaggio et al. Early childhood exposure to anaesthesia and risk of developmental and Behavioural disorders in a sibling birth cohort. *Anesth Analg*, 2011:** These investigators evaluated the effects of anaesthesia and surgery below 3 years of age on subsequent developmental and behavioural disorders. For exposure to one, two and three or more operations, the hazard ratio for developmental and behavioural disorders increased from 1.1 to 2.9 and 4.0 respectively.⁴¹
4. **Stratmann et al. Effect of general anaesthesia in infancy on long-term recognition memory in humans and rats. *Neuropsychopharmacology*, 2014:** Stratmann and colleagues performed a 'parallel' study, investigating both humans and rats. They concluded that early anaesthetic exposure deleteriously affected both humans and rats as demonstrated by impaired memory recollection tests.⁴³
5. **Bartels et al. Anaesthesia and cognitive performance in children: no evidence for a causal relationship. *Twin Research and Human Genetics*, 2009:** This study enrolled monozygotic twins to differentiate the effects of genetics and anaesthetic exposure. At age 12 years, similar educational achievement and cognitive performance was noted in discordant twin pairs.⁴⁷ This underpowered study observed no relationship between early anaesthesia exposure and subsequent cognitive performance.
6. **Levy DM. Psychic trauma of operations in children. *Am J Dis Child*, 1945.** This aptly titled study documented abnormal postoperative behaviour in 20% of children. Postoperative emotional consequences were analogous to combat neurosis, or post-traumatic stress disorder. He recommended that, where possible, surgery be postponed until after 3 years of age, and trauma be minimised with preoperative explanations, adequate premedication and maternal contact up to and immediately after surgery.
7. **Eckenhoff. Relationship of anaesthesia to postoperative personality changes in children. *Am J Dis Child*, 1953:** Using a parental questionnaire, this researcher identified a 17% incidence of personality changes in children after ENT surgery.³⁵ The findings paralleled those of Levy in that factors aggravating the changes included children younger than 3 years and psychological trauma (inadequate premedication, unsatisfactory induction with crying, struggling, vomiting and early airway obstruction). While the anaesthetics used (cyclopropane, ethylchloride and vinyl ether) in both this and Levy's 1945 study are irrelevant to the effects of current anaesthetics on neurodevelopmental outcome, the conclusions are still valid.
8. **Keaney et al. Postoperative behavioural changes following anaesthesia with sevoflurane. *Paediatr Anaesth*, 2004.** Comparison of sevoflurane and halothane found, as expected, that the newer agent was associated with more emergence delirium.^{36,130,131} The negative postoperative behaviour decreased over time,³⁴ and was not anaesthetic agent specific. This study is considered significant collateral evidence that modern drugs like sevoflurane are safe and cause minimal neuronal changes in both rodents,^{15,58} and in humans.⁶⁵
9. **Kotiniemi et al. Behavioural changes following routine ENT operations in two-to-ten-year-old children. *Paediatr Anaesth*, 1996:** This was a study to detect personality changes following ENT surgery and not to detect potential anaesthesia-related problems.³⁷ Age less than 3½ years was associated with more postoperative behavioural problems; however, behaviour and sleeping patterns improved in a third of children, presumably due to correction of the underlying medical problem. This study also compared the effects on behaviour of one or two nights' hospitalisation with the presence of parents with treatment as day case surgery. Despite large inter-individual differences in behavioural responses, no differences between the hospitalised and day cases were detected.
10. **Kotiniemi et al. Behavioural changes in children following day-case surgery: a 4-week follow-up of 551 children. *Anaesthesia*, 1997:** In this Finnish study, parents indicated that 47% of children exhibited negative behaviour following day surgery, these changes ordinarily resolving by 4 weeks.³⁴ Negative behaviour was aggravated by poorly controlled pain on the first postoperative day, younger age and previous poor health care experience; anaesthetic technique did not account for any of the changes. The positive behavioural changes observed in 17% of children were associated with correction of an underlying medical condition.
11. **DiMaggio et al. A retrospective cohort study of the association of anaesthesia and hernia repair surgery with Behavioural and developmental disorders in young children. *J Neurosurg Anaesthesiol*. 2009:** Interrogation of New York Medicaid archives revealed more behavioural problems and developmental disorders subsequent to hernia repair before 3 years of age.⁵⁷
12. **Kalkman et al. Behaviour and development in children and age at the time of first anaesthetic exposure. *Anesthesiology*, 2009:** More behavioural abnormalities were observed if children underwent anaesthesia and surgery before rather than after 24 months of age.⁴²
13. **Hinz et al. Neurodevelopmental and growth outcomes of extremely low birth weight infants after necrotising enterocolitis. *Paediatrics*, 2005:⁵²** The infants with necrotising enterocolitis managed surgically rather than medically were significantly more likely to have specific (cerebral palsy, blindness, deafness) and global neurodevelopmental impairment. The poorer surgical group outcomes were likely a marker of the severity of the disease process.
14. **Sprung et al. Anaesthesia for caesarean delivery and learning disabilities in a population-based birth cohort. *Anesthesiology*, 2009:** The Mayo Clinic group studied the association between general and regional anaesthesia during caesarean delivery and subsequent learning disabilities compared with vaginal delivery using the Olmstead County Birth Cohort registry.⁴⁸ Sprung and colleagues concluded that the type of caesarean anaesthesia did not adversely affect long-term neurodevelopmental outcomes.
15. **Flick et al. Neuraxial labour analgesia for vaginal delivery and its effects on childhood learning disabilities. *Anesth Analg*, 2011:** Sprung and colleagues' 2009 study⁴⁸ unexpectedly indicated a lower risk of learning disability occurred following caesarean section using neuraxial anaesthesia than normal vaginal delivery.⁴⁸ The authors hypothesised this was due to less fetomaternal stress with neuraxial anaesthesia. A subsequent study by the same group found that neuraxial analgesia during labour was not associated with subsequent learning disabilities.⁴⁹
16. **Hansen et al. Academic performance in adolescence after inguinal hernia repair in infancy: a nationwide cohort study. *Anesthesiology*, 2011:** The academic performance of all Danish children undergoing inguinal hernia repair over a five-year period was compared with a randomly selected, matched population sample. After adjusting for congenital abnormalities and socio-economic differences, short-term anaesthesia exposure was not linked to later poorer academic performance.⁵¹
17. **Ing et al. Long-term differences in language and cognitive function after childhood exposure to anaesthesia. *Paediatrics* 2012; 130, e476–85:** These researchers analysed 2 868 children who were enrolled in the Western Australian Pregnancy Cohort Study. Anaesthesia exposure before age 3 years was associated with increased risk of disability in language and cognition (abstract reasoning deficit) at age 10. The effects of anaesthesia on neuropsychological outcome may be limited to specific functional areas.
18. **Block et al. Are anaesthesia and surgery during infancy associated with altered academic performance during childhood? *Anesthesiology* 2012; 117: 494–503:** These investigators observed that anaesthesia-surgery during infancy was associated with worse academic achievement compared with the population norm.

of the adverse impact of anaesthetics on cellular neurodevelopment. AADN is likely to have a multifactorial

aetiology with genetic aberrations, prematurity and low birth weight, younger age, comorbid diseases, physiological

trespass, psychological stress, and anaesthetics all contributing.⁴⁴ Healthy children are less likely to require multiple anaesthetics and procedures. Is it the presence of co-morbid disease that adversely affects children's learning opportunities and alters parents' psyche?

- (2) Surgically induced inflammation and the associated cytokine response, which has been associated with blood-brain barrier and neurological changes in adults, may also contribute to the problem.^{14,53}
- (3) A serious concern is that no human study has as yet considered the role of perioperative-altered physiology (hypoxia, hypotension, low cardiac output, hypercarbia, hypoglycaemia) as a contributor to AADN. An explicit criticism of the Wilder study is that it was performed between 1976 and 1982, a time before both routine pulse oximetry and ASA minimum monitoring standards became mandatory.^{50,54}
- (4) The relative contributions of aetiological factors in points 1 to 3 above are difficult to quantify.⁴⁰ It may also be that the direct neurotoxic effects of anaesthetics are relatively minor compared with points 1 to 3 above.⁵⁴⁻⁵⁶
- (5) The role that particular anaesthetics play in AADN is not known. What has been suggested is that modern agents such as sevoflurane are safer,⁵⁷ causing minimal neuronal changes in vitro, in rodents^{8,15,26,58-64} and in humans.⁶⁵ These findings suggest that studies which employed outdated anaesthesia agents (halothane, enflurane, methoxyflurane, nitrous oxide) are less relevant to current practice.^{44,48,66} Nonetheless, recent research contradicts these findings and suggests that sevoflurane has similar toxicity^{9,30,67-72} while a single study suggested that desflurane⁶¹ induced worse neuroapoptosis than other anaesthetics.
- (6) Despite dose-dependent and duration-dependent deleterious neurocognitive effects being identified in animal studies, no study has considered the effects of either anaesthetic duration or anaesthetic depth.⁷³ While brief anaesthetic exposure is unlikely to produce long-term learning disability,^{51,74} the aforementioned concerns may be relevant considering that excessive anaesthesia depth is associated with postoperative cognitive dysfunction in the elderly.⁷⁵ There may be similarities/overlap between AADN in very young and elderly brains.^{23,27,76}
- (7) Whether studies can be extrapolated to other population groups is not known. The Wilder study population has been critiqued for having studied a homogeneous white middle-class cohort.
- (8) The evaluation of neurodevelopmental impairment has been neither free of bias nor comparable between studies. Examples of such methodological inconsistencies include the Wilder study that interrogated learning outcomes and not disabilities,⁴⁴ possible parental bias when answering questionnaires,⁴² and that diagnosis of behavioural disorders in young children is susceptible to selection bias and misclassification.
- (9) Some studies may have enrolled sicker patients. Examples include the Sprung study where general

anaesthesia was more likely performed in emergency caesarean sections with neonates having lower gestational ages, birthweights and APGAR scores;⁴⁸ the incidence of perinatal hypoxia was not noted in the DiMaggio study.⁵⁷

- (10) The nature of the procedures, particularly that a large proportion were for otorhinolaryngeal procedures, may have biased the conclusions and results of some of the retrospective studies. The need for tympanostomy tubes has been linked to delayed language development, this measurement being an important endpoint of AADN studies.³¹
- (11) It is obviously not ethically acceptable to anaesthetise neonates solely to interrogate the effects thereof.² This ethical consideration has been addressed by studying AADN using retrospective observational clinical studies. Unfortunately, these studies have multiple problems.^{1,42,44,47,48,57,66} The results of the first randomised controlled human trial, the GAS study, are therefore eagerly awaited.

Human AADN studies currently being conducted

'The lack of robust clinical evidence 'places even greater emphasis on prospective approaches to this problem, such as the ongoing GAS trial and PANDA study.'²

- (1) 'SAFEKIDS' and SMART-Tots: Unresolved concerns with the available research prompted the US Food and Drug Administration (FDA) to launch a clinical research initiative entitled 'Safety of Key Inhaled and Intravenous Drugs in Paediatrics' ('SAFEKIDS'). SAFEKIDS evolved into a private-public partnership between the FDA and the International Anaesthesia Research Society (IARS) entitled 'Strategies for Mitigating Anaesthesia-Related Toxicity in Tots' (SMART-Tots). The SMART-Tots website indicates that the collaboration is currently funding research in non-human primates by the National Centre for Toxicological Research and has awarded generous grants for several multicentre human trials.
- (2) PANDA: The 'Paediatric Anaesthesia NeuroDevelopment Assessment' (PANDA) study (<http://www.clinicaltrials.gov/ct2/show/NCT00754897>) has enrolled 500 sibling pairs discordant for inguinal hernia surgery under general anaesthesia before age 36 months and data are currently being analysed. Long-term effects of anaesthesia on neurodevelopment will be evaluated.^{1,77,78}
- (3) GAS: The GAS study (A Multi-site Randomised Controlled Trial Comparing Regional and General Anaesthesia for Effects on Neurodevelopmental Outcome and Apnea in Infants; <http://www.clinicaltrials.gov/ct2/show/NCT00756600>) is a seven-country international randomised control trial aimed at determining whether impaired outcome after surgery can be attributed to general anaesthetic agents rather than effects of prematurity, illness or the surgical intervention itself.⁷⁸ Following general or regional anaesthesia alone (no sedation at all in the regional group) for hernia repair, neurodevelopmental outcome will be performed at 2 and again at 5 years of age. The earlier assessment with Bayley Scales for Infant Development is a secondary outcome of this

study. The later assessment, a primary outcome of this study, will use the Wechsler Preschool and Primary Scale of Intelligence Third Edition Full Scale Intelligence Quotient and additional neuropsychological tests within NEPSY II (Second edition of the neuropsychological test battery for children and adolescents).¹ Recruitment is complete and the 5 year follow-up will be completed in 2017.²³

Preliminary findings showed significant differences in mean arterial pressures between the general anaesthesia and spinal groups, general anaesthesia having lower intraoperative blood pressures. Differences in apnoea rates on arrival in recovery were also identified between the two groups, this latter finding prompting a new study of perioperative NIRS monitoring in children under 6 months undergoing general anaesthesia.⁷⁹ GAS study secondary outcomes were very recently (October 24, 2015) made available online on the Lancet website. Results were analysed for 238 of 363 and 294 of 359 infants administered awake regional and general anaesthesia respectively. Despite the difference in the preliminary results, cognitive scores did not differ at all. The authors concluded, 'For this secondary outcome, we found no evidence that just less than 1 h of sevoflurane anaesthesia in infancy increases the risk of adverse neurodevelopmental outcome at 2 years of age compared with awake-regional anaesthesia'.

- (4) Danish study: An ongoing study is comparing the academic achievements of the Danish population with that of all children who underwent surgery between 1977 and 1990 and were younger than 1 year of age at the time.⁵⁰

Meta-analyses attempt to clarify the probability of AADN

'Concerns over the effects of anaesthesia on the developing brain remain well placed; ... The uncertainty with existent epidemiological evidence is considerable.'⁴⁰

Three recent meta-analyses independently concluded that the associations between early anaesthetic exposure and adverse neurodevelopmental outcomes are tenuous due to the limitations inherent in current studies.^{2,40,80} Wang *et al.* observed an increased risk of adverse neurodevelopmental outcomes following multiple, early anaesthesia exposures. This meta-analysis also indicated that following single exposure, anaesthetic dose and duration was not important.⁸⁰ Sanders *et al.*'s meta-analysis indicated only a small increase in AADN risk, this risk increasing with increased cumulative anaesthesia exposures.²

DiMaggio and colleagues performed a Bayesian meta-analysis of the current literature. Standard and Bayesian meta-analyses differ with the latter seeking to identify the odds ratio of the main endpoint, in this case AADN. DiMaggio and colleagues' Bayesian meta-analysis calculated an AADN odds ratio and 95% credible interval of 2.0 and 0.7–4.7 respectively. These data were used to estimate that, for the USA, the population-attributable risk of an anaesthesia-related learning or a behavioural disorder was 2.6%. For comparison, the population-attributable risk of smoking-related cardiovascular disease approximates 10.9%. Their two main conclusions were, first, that 'concerns over the effects of anaesthesia on the developing brain remain well placed' despite the considerable variation in results and

uncertainty caused by the currently available research and, second, 'what can reasonably be learned from continuing to analyse existing data sources is becoming increasingly limited'.⁴⁰

Possible cellular mechanisms of AADN

'Since exposure to GAs often cannot be avoided when a child's well-being is in danger, a considerable effort has been made in recent years to elucidate the mechanisms of anaesthesia-induced developmental neuroapoptosis.'⁷⁷

Current research is attempting to elucidate the subcellular, non-anaesthetic AADN mechanisms; detailed knowledge thereof may facilitate development of preventative measures.^{21,23} Despite significant overlap between AADN-related mechanisms, the main themes involve anaesthesia-induced calcium dysregulation, accelerated neuroapoptosis, altered neurogenesis, teratogenicity, neuroexcitation, and inflammation.

Anaesthetic-induced neuronal and mitochondrial calcium dysregulation likely has multiple underlying mechanisms, including:^{81,82}

- (1) anaesthetic activation of agonist- (NMDA) and voltage-dependent calcium channels;⁸³
- (2) isoflurane activation of GABA-A receptors leads to chloride efflux, neuronal membrane depolarisation and opening of voltage-dependent calcium channels. This has been termed the 'reversed transmembrane chloride gradient';⁸⁴ and
- (3) isoflurane, sevoflurane, desflurane and propofol all activate inositol triphosphate receptors, which stimulates calcium-induced calcium release (CICR) from the endoplasmic reticulum.⁸⁴

Intracellular calcium overload damages intracellular organelles, particularly mitochondria, endoplasmic reticulum and lysosomes. Increased cytosolic calcium increases lysosomal activity and accelerates autophagy.⁸⁵ Endoplasmic reticulum damage inhibits protein synthesis and impairs neuronal growth and repair.^{81,86}

Anaesthetic agents also cause mitochondrial calcium overload. The consequent mitochondrial injury is revealed histologically as organelle enlargement, compromised structural integrity and reduced organelle density, and functionally as impaired ATP production.⁷⁸ Mitochondrial damage is also a potent activator of the intrinsic apoptotic pathway.⁸⁶ Elevated mitochondrial calcium concentrations abolish the normal mitochondrial transmembrane potential, and greatly increase mitochondrial permeability transition pore activity, with cytosolic translocation of cytochrome C, a potent activator of the intrinsic apoptotic pathway. Increased mitochondrial calcium concentrations also activate certain proteins within the mitochondria that activate the intrinsic apoptotic pathway.⁸⁶

Accelerated apoptosis of neurons that would normally survive was formerly considered the crucial AADN mechanism,^{5,87} Neuroapoptosis may be initiated via one of two discrete (intrinsic or extrinsic) pathways.^{2,14,88} Elevated cytosolic calcium activates calpain, a calcium-dependent protease, which initiates the intrinsic apoptotic pathway neuroapoptosis.^{89,90} Other mechanisms activating the intrinsic apoptotic pathway are mitochondrial damage (see above) and neurotrophic hypothesis.

This latter hypothesis suggests that the anaesthesia-induced interruption of sustained (neurotrophic) neuronal synaptic activity leads to postsynaptic neurons deeming they are redundant with initiation of intrinsic apoptosis.^{2,91,92} It has been suggested that surgical stimulation may counteract this 'use it or lose it' mechanism.^{55,78} Extrinsic apoptotic pathway activation typically follows external stimuli such as inflammation with associated tumour necrosis factor release. It is of relevance that, as opposed to their anti-inflammatory effects in adults, anaesthetics promote inflammation in children. Furthermore, isoflurane activates the neurotrophin receptor that initiates actin depolymerisation, causing synaptic loss and accelerated neuronal apoptosis.⁹³

Developmental delays and altered neurogenesis have recently also been observed in the absence of accelerated apoptosis.^{2,94} In this respect, multiple cellular vulnerabilities exist where anaesthetics may interfere with neurogenesis:^{83,85,93-102}

- (1) Anaesthesia-induced impaired calcium oscillation: Well-ordered calcium oscillation is important in normal neural developmental processes such as gene expression, synaptogenesis, differentiation, axonal growth, and dendritic arborisation.⁸³
- (2) Delayed axon polarisation: Axon polarisation is a critical development process whereby neurons develop axon- and dendrite-specific components. This process is delayed by both isoflurane and propofol.⁹⁵
- (3) Impaired dendritic spine development: Dendritic spines are protrusions on neuronal dendrites that receive and transmit synaptic signals. Systematic dendritic spine development and activity are essential for normal synaptogenesis and normal neuronal circuit assembly.⁹⁷ Dissimilar anaesthetics affect dendritic spine development differently. Modern volatile agents (sevoflurane, desflurane) increase, whereas ketamine consistently reduces, dendritic spine density respectively.¹⁰³ Propofol's effect depends on the developmental stage, decreases and increases in dendritic spine density occurring in younger and older neonatal rats respectively.⁹⁸
- (4) Brain-derived neurotrophic factor (BDNF): BDNF either promotes survival or accelerates neuronal apoptosis, depending on its arrangement. BDNF is secreted from neurons as proneurotrophin (proBDNF). Neuronal activity causes release of tissue plasminogen activator, which cleaves plasminogen to plasmin. In turn, plasmin cleaves proBDNF to BDNF, the latter promoting synaptogenesis and neuronal survival. Anaesthetic inhibition of neuronal activity is accompanied by less release of tissue plasminogen and less plasmin formation. This fosters dominance of proBDNF, which impairs synaptogenesis and accelerates neuroapoptosis.^{2,99} The possibility of pharmacologically influencing BDNF expression to favour cell survival to ameliorate AADN has been mooted.^{14,88,99,104}
- (5) Impairment of neural precursor (progenitor) cell proliferation: Isoflurane reduces proliferation of neural precursor cells in the rat hippocampus.¹⁰⁰
- (6) Stem cell loss: Isoflurane-exposed infant rats had a reduced pool of hippocampal stem cells; the extents of

stem cell loss and memory loss correlate well with each other.¹⁰²

- (7) Glial cell cytoskeletal damage: Isoflurane damages the cytoskeleton of immature astroglial cells.¹⁰¹
- (8) Teratogenicity: Anaesthetic administration to pregnant animals indicates that the foetus is at significant risk for AADN. For example, the offspring of halothane- or enflurane-exposed pregnant mice learn more slowly than controls and exhibit behavioural changes.¹⁰⁵ Better understanding of inter-species neurodevelopmental timeframes is needed.^{28,47,105}

Anaesthesia-induced neuroexcitation and convulsions have been observed following sevoflurane anaesthesia in rats.^{70,106} The implications of this observation are currently not clear.

Neuroprotection against AADN

'Does the Emperor have no clothes?'⁵⁵

The concept of employing neuroprotective agents for AADN has been extrapolated from strategies used in neuro- and cardiac surgery, traumatic brain injury and hypoxic ischaemic encephalopathy.²⁶ Hypoxic ischaemic encephalopathy may be a more relevant model as the pathophysiology overlaps with that of AADN, as, in both, the predominant mechanism of injury is neuroapoptosis.¹⁰⁷ Neuroprotectants are currently in the preclinical (animal research) stage. Human studies are still lacking. The possibilities are numerous:

- (1) Erythropoietin: Erythropoietin is used in neonatal encephalopathy.¹⁰⁸⁻¹¹² Its protective effects stem from its inhibition of the final common neuronal apoptosis pathway involving caspase-3 and also because it activates cell-survival signals.¹⁰⁸⁻¹¹² It has recently been shown to protect against neonatal rat sevoflurane neurotoxicity.¹¹³
- (2) Dexmedetomidine: Not only does high-dose dexmedetomidine not induce neuronal apoptosis,¹¹ but it also attenuates isoflurane- and ketamine-induced apoptosis.^{10,11,114} This is interesting as alpha-2 adrenoreceptors are involved in neurodevelopment, and also relevant as this class of drugs is used clinically in paediatric anaesthesia. Dexmedetomidine may possibly attenuate AADN even if administered post-insult.^{10,11,26,114}
- (3) 17 β -Oestradiol: Oestradiol limits both NMDA-antagonist and GABA_A-agonist induced murine neurotoxicity via activation of a pro-survival protein that limits apoptotic signalling.¹¹⁵ Its physiological role may be neuroprotection during delivery. Long-term administration may alter neuronal development.¹¹⁶
- (4) Xenon: Xenon is a peculiar NMDA antagonist. Although it has been implicated as pro-apoptotic in some studies, others have shown that it is neuroprotective and limits anaesthesia-related neuroapoptosis (Brosnan).^{88,117} It also limits ischemic-hypoxic encephalopathy induced neuroapoptosis,^{26,117} in this respect acting synergistically with both dexmedetomidine,¹¹⁸ and also hypothermia.¹¹⁹ Xenon is exorbitantly expensive and not freely available for clinical use.
- (5) Melatonin: Melatonin stabilises mitochondria and reduces the release of caspase-9 and cytochrome-C.

Melatonin's actions are dose dependent with 1 and 20 mg/kg limiting anaesthesia-induced apoptosis by 30–40% and 75–90% respectively.^{26,82} Its favourable side effect profile and hypnotic and analgesic properties make melatonin a strategically useful drug that would potentially target AADN. Human studies are still outstanding.

- (6) Lithium: Propofol and NMDA antagonists suppress extracellular signal regulated protein kinase (ERK) phosphorylation.¹²⁰ Lithium counteracts this with reversal of both propofol- and ketamine-induced neuroapoptosis.¹²⁰ Lithium's side effect profile limits its use in paediatric anaesthesia.
- (7) Free radical scavengers: Apoptosis is associated with the mitochondrial electron transport chain upregulating its production of reactive oxygen species.¹²¹ Free radical scavengers and anti-oxidants protect the mitochondria from such damage.^{122,123}
- (8) Omega-3-polyunsaturated fatty acids: Supplementing the diets of pregnant rats with fish oil ameliorated the deleterious effects caused by neonatal sevoflurane exposure.¹²⁴
- (9) Bumetanide: In neonatal rats, prior treatment with the Na-K-Cl cotransporter inhibitor, bumetanide, was effective in inhibiting the deleterious neurobehavioural effects of sevoflurane but not isoflurane.⁷⁰
- (10) Avoiding hypnotic anaesthetics: A recent editorial suggested that the concept of anaesthesia (immobility, analgesia and lack of recall) is not relevant in neonates, and that large doses of opioids alone would suffice.⁵⁵

Perspective on neuroprotective agents

The wisdom of administering drugs with unknown toxicity to protect from a hypothetical condition is questionable. It has been suggested that the therapeutic benefits and neurotoxic side effects of neuroprotective drugs could be inseparable companions. The explanation offered is that general anaesthetics are neuroprotective during (focal) brain ischaemia because they decrease metabolic demands and limit excitotoxicity. However, the more effective an agent is at suppressing neuronal activity, the more likely it may cause apoptosis of developing neurons. Indeed, there appears to be a fine homeostatic balance between pro-apoptotic and pro-survival proteins in the brain. In an interesting study, Milanovic and colleagues demonstrated that in neonatal rat brains exposed to propofol, the increase in apoptotic proteins was accompanied by a concomitant increase in pro-survival proteins.¹²⁵

Non-pharmacological therapy

The hypothesis suggests that employing neuroplasticity constructively may ameliorate the toxic effects of anaesthetics.¹²⁶ The concept is exemplified by studies demonstrating that short-term memory impairment following murine sevoflurane exposure was reversed by 'immediate' or 'delayed' environmental enrichment.³⁰ Environmental enrichment consists of voluntary exercise, social interaction and increased environmental complexity. The process possibly re-engages neurons that would otherwise undergo post-anaesthesia apoptosis. A significant advantage of this technique is that it does not incur additional pharmacological risks.¹²⁶ Human reports employing such therapeutic strategies have as yet not been published.

Approach for clinicians

'It is unlikely that definitive clinical studies absolving general anaesthetics of neurotoxicity will become available in the near future, requiring clinicians to use careful judgement when using these profound neurodepressants in vulnerable patients.'²³

As anaesthesia safety progresses, minor concerns increase in importance. In this respect, the likening of anaesthesia to sleep suggests a fully reversible condition with no long-term adverse sequelae: unfortunately this may not be true. Clinicians are in a difficult position, needing to weigh the risks of surgery and AADN despite the current lack of consensus and firm evidence. These concerns must be communicated to parents, surgical and medical colleagues in a clear, non-anxiety-provoking manner. This latter overtly simple instruction is a difficult brief, facilitated by practitioners keeping up to date with current study findings and expert opinions. Advice could follow the following path:

- (1) No data currently support delaying surgery.
 - (1.1) Newer data show that one cannot define the time scale of the 'brain growth spurt' reliably.
 - (1.2) One should be cognisant that very few procedures performed in young children are truly elective.
- (2) If surgery does proceed, the following points need consideration:
 - (2.1) Modern surgery cannot be separated from anaesthesia for practical and humane reasons.^{11,15} Indeed, well-conducted anaesthesia and effective postoperative analgesia will likely minimise a child's psychological trauma.
 - (2.2) In medicine, benefits are often associated with some degree of risk. The gains likely to be achieved by treating a surgically correctable problem must be weighed against risks of anaesthesia. The latter risks include the largely unproven entity of human AADN.
 - (2.3) Single anaesthetics-surgeries appear safe. It is perhaps reassuring that a single 'brief' anaesthetic exposure does not appear to harm the developing brain.^{50,51}
 - (2.4) If multiple procedures are likely to be needed, unnecessary delays may compromise the future well-being of the child leaving little choice but to continue.
- (3) Anaesthesia practice: If surgery does proceed, anaesthesiologists must adopt a non-reactive, measured approach. Currently, the United States Food and Drug Administration (FDA) also agrees there is no evidence to suggest changes in anaesthetic practice or omitting particular anaesthetic agents, almost all anaesthetic agents having been implicated in AADN.¹²⁷ Attention needs to be given to maintenance of physiological homeostasis. Meticulous record keeping is of utmost importance.

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Received: 19-05-2015 Accepted: 30-11-2015