

# Anaesthesia-induced developmental neurotoxicity

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Keywords: anaesthesia, paediatrics, development, neurotoxicity, anaesthesia-induced developmental neurotoxicity

## Abstract

Recently, concerns regarding anaesthesia-induced developmental neurotoxicity (AIDN) have emerged. The aim of this review was to summarise the available information on AIDN, and to assist the practising anaesthetist in making safe decisions with regard to the administration of anaesthesia in the young child.

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South Afr J Anaesth Analg 2012;18(5):242-247

## Introduction

Anaesthesia-induced neurodegeneration is a recognised entity that occurs at the extremes of age. In the elderly, postoperative cognitive dysfunction can follow anaesthesia. However, in recent times, the effects of anaesthesia on the young brain have sparked great controversy.

## What do we know?

### Apoptosis

The neuronal cells in normal term and premature babies' brains undergo apoptosis.<sup>1</sup> For this reason, babies' brains are more sensitive to insults and may respond to these with accelerated apoptosis.<sup>1</sup> Insults may take the form of hypoxia, hypoglycaemia or exposure to anaesthetic agents. There are two mechanisms of apoptosis. The first, known as the intrinsic or "mitochondrial pathway," is caused by mitochondrial dysfunction which is followed by the release of cytochrome c and caspase-9. The extrinsic or "death receptor" pathway involves the adaptor protein fas-associated protein with death domain, the death-induced signalling complex and caspase-8. Either way, the final culprit in the pathway is caspase-3 which causes structural changes in the cellular DNA and ultimately results in apoptosis.<sup>2</sup>

### Nature of neurotoxicity

The period in which the nervous system is most at risk is during the "growth spurt" of the brain, which occurs in

humans from the sixth month of gestation to a few years of age. Therefore, children may have an adverse outcome when exposed to an agent that would otherwise be harmless in an older patient.<sup>3</sup> Experimental studies have shown accelerated apoptosis, alterations in dendrite morphology and ultra-structural alterations to the spinal cord.<sup>4</sup> The dendritic changes are characterised by a reduction in synaptic activity or as enhanced excitotoxicity due to N-methyl-D-aspartate (NMDA) receptor upregulation.<sup>4</sup> Recent work has also shown that general anaesthetics influence neuroplasticity and affect the normal synaptogenesis that characterises the developing brain.<sup>5</sup> The areas of the brain that are implicated include the cortex, thalamus and hippocampus.<sup>6</sup> In humans, this is thought to translate into difficulties with speech, learning and language, but behaviour may remain unaffected.<sup>5</sup> Gamma-aminobutyric acid (GABA) type A agonists, e.g. benzodiazepines, have also been postulated to be harmful. Interestingly, ethanol possesses both NMDA antagonism and GABA-mimetic effects. Its adverse effects on the developing brain are well described.<sup>7</sup>

### Specific agents

#### *N-methyl-D-aspartate antagonists*

It is well known that ketamine induces neurotoxicity when administered intrathecally.<sup>2</sup> Numerous studies have shown conclusively that ketamine enhances neuroapoptosis in the developing brain, in particular via the mitochondrial pathway.<sup>2</sup> However, there is scepticism as to whether or not this is clinically relevant. To date, the majority of

studies have been performed on rat pups. It has been shown that ketamine toxicity is both concentration- and time-dependent.<sup>2</sup> In many studies, the doses of ketamine that were used were much larger and administered over longer periods than standard ketamine regimens. In some studies, ketamine infusions that exposed the rat pup to an equivalent of several weeks of anaesthesia were used.<sup>1</sup> Others exposed the subjects to a bolus dose of 140 mg/kg, far in excess of standard anaesthetic dosages.<sup>1</sup> On the other hand, in one study, rat pups were exposed to a single bolus dose of ketamine at subanaesthetic concentrations for rats (10-50 mg/kg subcutaneously). The results showed a fourfold increase in neuroapoptosis.<sup>8</sup> Other studies were more reassuring initially. A primate study by Slikker et al showed no pathological microscopic changes resulting from ketamine if it was given for a short period (less than three hours). However, longer exposure periods resulted in greater toxicity.<sup>9</sup> The same group performed a follow-up study and demonstrated that the animals that were exposed to ketamine for 24 hours showed long-term cognitive deficits at the age of three years. They concluded that “these observations demonstrate that a single 24-hour episode of ketamine anaesthesia, occurring during a sensitive period of brain development, results in very long-lasting deficits in brain function in primates and provide proof of concept that general anaesthesia during critical periods of brain development can result in subsequent functional deficits”.<sup>10</sup>

On the other hand, ketamine is recognised as an anti-inflammatory agent. Studies have shown better outcomes in children with septic shock who were sedated with ketamine infusions in the intensive care unit.<sup>11</sup> Similarly, low doses of ketamine have been shown to have anti-inflammatory effects. Ketamine may actually be protective.<sup>4</sup>

Results of studies that were carried out in the setting of traumatic brain injury (TBI) suggest that ketamine is neuroprotective.<sup>12</sup> Glutamate, an NMDA receptor agonist, is released excessively in TBI. By antagonising the effects of glutamate, ketamine can prevent pathological excitation and calcium release. Experimentally, ketamine has been shown to protect against hypoxic, ischaemic, mechanical and chemical insults.<sup>12</sup> It appears that the key to understanding the bipolar effects of ketamine is whether or not the glutamate secretion is physiological. In TBI, the glutamate secretion is excessive. Therefore, blockade is protective. The blockade of normal glutamate function may be harmful in normal brain physiology.

### **Isoflurane**

Evidence is convincing with regard to toxicity,<sup>7</sup> as well as neuroprotection and effective preconditioning<sup>6</sup> by the volatile anaesthetics. Isoflurane at 0.75%, a concentration well below minimum alveolar concentration (MAC), has been shown to produce neuroapoptosis.<sup>7</sup> Exposure for one

hour only can be harmful.<sup>13</sup> It is important to note that there is minimal evidence that sevoflurane produces neurotoxicity in rodents.<sup>6,14</sup> One study showed that sevoflurane induces apoptosis in human lymphocytes, but to a far lesser degree than isoflurane at an equipotent concentration.<sup>15</sup>

### **Nitrous oxide**

There is no evidence that nitrous oxide (N<sub>2</sub>O) causes neurotoxicity. However, it is an antinociceptive agent that acts on the descending inhibitory pathway. As this pathway is immature in the neonate, it is postulated that N<sub>2</sub>O's analgesic properties are less effective in the very young patient.<sup>6</sup>

Jevtovic-Todorovic et al published a landmark study in 2003 entitled “Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits”.<sup>7</sup> The study revealed that combined exposure to isoflurane, N<sub>2</sub>O and midazolam produced widespread neurodegeneration. The dosages of agents were comparable to N<sub>2</sub>O 50% and isoflurane 0.5%. The midazolam dose was equivalent to a “sedating” dose for rats, as confirmed in previous studies.

Some of the results are worrying, for example:

- When agents were given alone, only isoflurane exposure resulted in histological changes. Midazolam alone and N<sub>2</sub>O alone both produced no structural changes.
- When agents were given in combination, toxicity appeared to be cumulative. Combining isoflurane with an additional drug produced more widespread changes than isoflurane alone. The combination of all three agents produced even greater detrimental effects.

However, what was not stressed was that the rats were exposed to six hours of anaesthesia in excess of a standard paediatric case, and that midazolam was administered in doses of 3 mg/kg, 6 mg/kg and 9 mg/kg, far in excess of standard anaesthetic doses. Although this seems to be the “sedating dose” for rats, the question is whether or not this data can be extrapolated to humans?

### **Withholding analgesics and anaesthetics during surgery**

Initially, when the evidence for neurotoxicity was discovered, some believed that the answer was merely to withhold anaesthetic agents in children and proceed with surgery with minimal drugs. However, there is overwhelming evidence against this practice, which is both unethical and against the norms of standard patient care.<sup>1,4</sup> Experiencing significant noxious stimuli early in life is associated with hyperalgesia and neurodevelopmental changes later on in life.<sup>6</sup> In fact, humans in the neonatal period are the most sensitive to noxious stimuli, because of the immaturity of the descending inhibitory pathway.<sup>6</sup>

### Long-term follow-up of children exposed to anaesthetic agents early in life

Numerous studies have shown that children who underwent surgery in early life have poorer developmental outcomes, emotional disturbances and behavioural problems.<sup>4</sup> However, healthy children tend not to require surgery in the neonatal period. Studies were performed on children who underwent laparotomies or repairs of tracheo-oesophageal fistulae. Both these patient groups are more likely to be premature and have multiple risks for complications, such as hypoxia, hypotension, and sepsis, which may also contribute to an adverse neurological outcome.

Sprung et al examined the effects of anaesthesia during birth.<sup>16</sup> They studied children who were born by normal vaginal delivery (NVD) versus Caesarean section conducted under regional anaesthesia, and Caesarean section carried out under general anaesthesia. Interestingly, the study found that children born via Caesarean section under regional anaesthesia had a lower risk of learning difficulties compared to those born by NVD. There was no difference between children born via NVD and Caesareans conducted under general anaesthesia.<sup>4,16</sup> It is important to note that although this study was published in 2009, the deliveries that were examined were from 1976-1982. The majority of the general anaesthesia cases were performed with thiopentone, N<sub>2</sub>O and halothane. Hardly any cases involved ketamine or isoflurane.

DiMaggio et al<sup>17</sup> performed a retrospective study that examined the incidence of developmental problems in children who were exposed to anaesthetics before the age of three years. The study group were children who had undergone inguinal hernia repair. It was found that these children were twice as likely to have developmental problems. There are some flaws in this study. There was a much higher incidence of “secondary birth-related diagnoses” in the study group (77% vs. 32.9%, *p*-value < 0.0001). Diagnoses included low birthweight (31.6%), perinatal hypoxia (16.5%), infection or haemorrhage (14.4%). Although the authors claim that the data were adjusted for these factors, their contribution cannot be known for certain. Furthermore, the control group comprised children who had not undergone inguinal hernia repair. However, they may have undergone anaesthesia for another reason, even before the age of three years. This study offers a good example to those who believe that it is not the anaesthetic itself that causes problems, but rather the underlying reason for requiring anaesthesia in the first place.

Kalkman et al<sup>18</sup> performed a pilot study on children who received anaesthesia for urological procedures before the age of 24 months versus those whose first exposure was after the age of 24 months. Children at high risk of acquiring learning disabilities were excluded at the outset, e.g. those who had experienced congenital heart disease, peripartum

adverse events and admission to the neonatal intensive care unit. The authors found a difference between the two groups. The children who were exposed at a younger age were more likely to exhibit learning difficulties. These differences were not statistically significant because their numbers were small. This study is important as it is one of the few studies in which all subjects were exposed to anaesthesia.

What is clear is that the adverse effects of anaesthetic agents are cumulative. The greater the exposure to anaesthesia, the higher the risk of learning disability for the child. Wilder et al<sup>19</sup> examined a large cohort of 5 357 children, 593 of whom had received general anaesthesia before the age of four years. They showed that a single exposure was insignificant, but that children exposed to two or more anaesthetics had a progressively greater incidence of learning disabilities. Although these data appear promising, the subjects in this study were children who were all born between 1976 and 1982. During this time, the majority of anaesthetics were carried out under N<sub>2</sub>O and halothane, with minimal exposure to either ketamine or isoflurane.

There is concern that the reasons for developmental problems in children are multifactorial. Both genetics and the environment play a role. Studies using monozygotic twins ensure that other influencing factors are removed and almost all that remains is the absence or presence of exposure. Twin studies work on the following premise. Some twins are not exposed (the “control” group). Some twins are discordant, i.e. one twin is exposed, whilst the other is not. The concordant group refers to the set in which both twins are exposed. A large retrospective twin study was reported by Bartels et al from The Netherlands.<sup>20</sup> The group found no difference in behavioural problems in discordant monozygotic twins.<sup>20</sup>

### Agents that may be protective against anaesthesia-induced developmental neurotoxicity

- 17β-oestradiol: During pregnancy, foetuses are exposed to high concentrations of maternal oestradiol. This plays a physiological role in protecting the baby’s brain from injury during the vulnerable period of delivery. Supplementation with oestradiol is protective against the harmful effects of GABA agonists, NMDA antagonists, sodium-channel blockers and oxygen toxicity.<sup>3,6</sup> Whether or not oestrogen supplementation is a feasible and safe option remains to be seen.
- Lithium<sup>4</sup>
- Xenon:<sup>4</sup> Xenon is an NMDA antagonist that has been shown to be neuroprotective when given before, during or after an insult. This is interesting, as other NMDA antagonists appear to have the opposite effect.<sup>6</sup>
- Dexmedetomidine:<sup>4</sup> Dexmedetomidine is neuroprotective to rat pups in the simulated hypoxic ischaemic encephalopathy scenario.<sup>6</sup> It is important to note that

there is evidence that it may be helpful following the insult and does not have to be administered pre-emptively. It has also been shown to reduce isoflurane-induced apoptosis. Even at very high dosages, dexmedetomidine does not induce apoptosis.<sup>6,21</sup>

- Erythropoietin: Evidence supports the neuroprotective effects of erythropoietin (EPO) following NMDA antagonist-induced toxicity.<sup>6,22</sup> The efficacy of EPO in animal models is also well established in the context of hypoxic ischaemic injury and TBI.<sup>22</sup>
- Melatonin: Melatonin is an antioxidant and mitochondrial stabiliser that provides a dose-dependent neuroprotective effect.<sup>6,23</sup> 1 mg/kg (subcutaneously) produces a 30-40% reduction in apoptosis (p-value < 0.05) and 20 mg/kg reduces the apoptosis by 75-90% (p-value < 0.001).<sup>23</sup> Melatonin's favourable safety profile may mean that this drug may be used in the future.

## What do we still need to determine?

### Extrapolation of results from animal studies

It is very unclear whether or not the information that is available on rat pups can be extrapolated to human babies.<sup>1</sup> In general, the translation of animal data to human populations is highly controversial. The scientific contribution of animal research is undisputed. However, the clinical relevance is unclear. Biological differences are viewed by some as too great.<sup>24</sup> Others view animal studies as vitally important, as they allow the manipulation of environmental and genetic factors which are impractical or unethical in humans.<sup>25</sup> Perel et al conducted a study that compared six clinical scenarios. They used clinical trials with a clear outcome (benefit or harm) in humans and compared them with the outcomes of equivalent studies in animals. Their results were interesting. Some animal studies affirmed the effects in humans. Others showed the opposite outcome and the remainder were inconclusive.<sup>24</sup>

Poor concordance between animal studies and clinical trials is thought to be due to bias, random error or the failure of animal models to accurately represent human disease.<sup>24</sup> One review of 76 animal trials by Hackam and Redelmeier showed that only approximately 10% of the studies were randomised and only about 20% were blinded.<sup>26</sup> Other problems include differences in inducing disease or injury, variations in drug-dosage schedules, the use of animals who are otherwise free of co-morbidity, or the effects of ageing, improper or absent randomisation, small studies with low power and studies in which the duration of follow-up may be inappropriate for extrapolation to humans.<sup>27,28</sup> In anaesthesia-induced developmental neurotoxicity (AIDN) research, seven-day-old rat pups are generally used, but the equivalent age in humans is stated to be anything between a preterm baby and a two-year-old child.<sup>6</sup>

Interestingly, animal studies are not subjected to the same rigorous standards as human studies. Perhaps registering animal trials, in a similar way to clinical trials, would improve the quality of the research.<sup>24</sup> The suggestion is that animal studies need to be interpreted with extreme caution.

### Physiological vs. pathological apoptosis

Regarding apoptosis, it is unclear whether or not the neurons would have died anyway as a normal part of the development of the brain.<sup>1</sup> The contribution of the surgical stress response to neurodegeneration is still unclear.

### Confounding effects of other physiological stressors

What about hypotension, hypoxia, hypoglycaemia, pain and inflammation?<sup>29</sup> Many studies did not monitor the animals for such complications,<sup>1</sup> although in some studies repeated arterial blood gas analysis and temperature monitoring were performed.<sup>7,30</sup> Authors who are opposed to this argument remind us that the histological features of hypoxic ischaemic damage are excitotoxic and not apoptotic.<sup>30</sup> Yet others disagree and state that hypoxic ischaemic damage in children is usually apoptotic (vs. necrotic in adults).<sup>6</sup> Certain studies did not provide any nutritional support to their animal subjects, which is far from the perioperative scenario in humans.<sup>1</sup> However, some researchers have ensured that the rat pups were fed prior to induction and that both the control groups and study groups underwent the same degree of maternal and nutritional deprivation.<sup>30</sup>

### Providing anaesthesia without surgical stress

It is understood that the administration of analgesics and anaesthetics in the absence of noxious stimuli can be harmful. What has not been examined experimentally are the effects of these agents in a simulated surgical situation.<sup>1,29</sup>

There is also emerging evidence that intensive brain stimulation, following exposure to a general anaesthetic, can lead to an improvement in outcome. It is thought that agents can halt normal brain development, but if the patient is stimulated the brain can be "kick started" to act normally and so AIDN can be prevented or ameliorated.<sup>5</sup>

## What evidence are we awaiting?

### The GAS study

A multi-site randomised controlled trial comparing regional and general anaesthesia for effects on neurodevelopmental outcome and apnoea in infants (GAS) is currently underway.<sup>31</sup> Patients aged between 27 and 60 weeks (postgestational age) who presented for inguinal hernia repair were randomised into receiving either a general anaesthetic or a regional procedure alone. The end-points are the neurodevelopmental outcome (as assessed by intelligence quotient tests at the age of five years) and the incidence of postoperative apnoea. The study is expected to be concluded by mid-2017.

*The Pediatric Anesthesia and NeuroDevelopmental Assessment study<sup>32</sup>*

The Pediatric Anesthesia and NeuroDevelopmental Assessment (PANDA) study is a large multicentre trial that will compare the neurocognitive function in groups of siblings. Each sibling pair will consist of one child unexposed to anaesthesia (the control), whereas the other child will have had an inguinal hernia repair before the age of three years.

**What should we do with the information that we have?**

There is a large body of available research, but none of it has drawn definitive conclusions regarding AIDN.

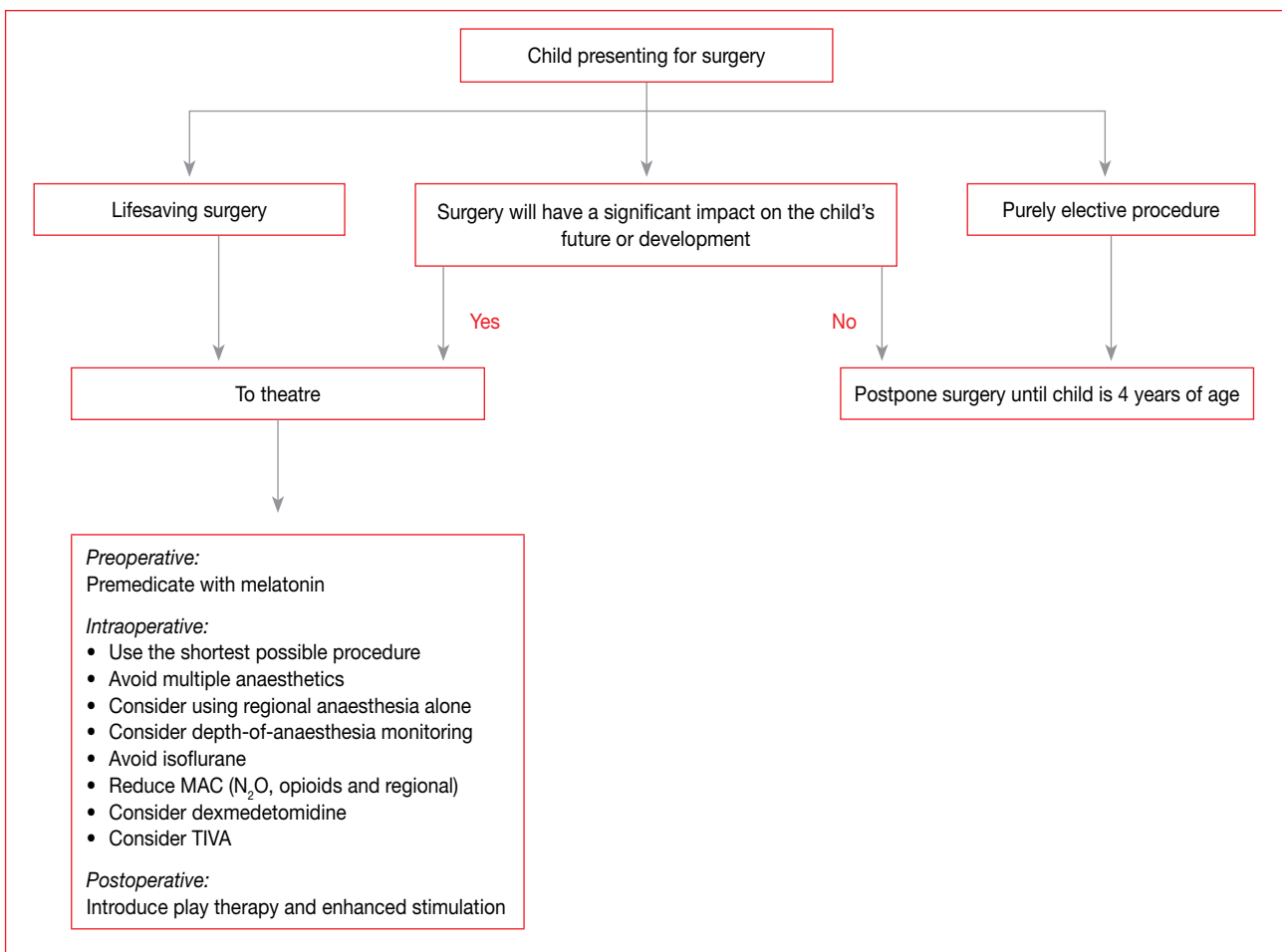
Extrapolating from the evidence, the following points are recommended:

- Surgery before the age of four years should be avoided, unless absolutely necessary.
- Repeated anaesthetic exposure, i.e. an attempt to group surgical procedures under one anaesthetic if possible, should be avoided.
- The use of a regional technique alone should be considered.

- Prolonged exposure to anaesthesia should be avoided. This can be achieved by good theatre preparation, the use of experienced surgeons and minimal delays between induction and skin incision.
- Isoflurane should be avoided, if possible. Sevoflurane should be used instead.
- Techniques to reduce MAC should be used, such as regional anaesthesia or agents such as N<sub>2</sub>O and opioids. There is no evidence of neurotoxicity from opioids in the developing brain.<sup>33</sup> Conversely, opioids ameliorate the surgical stress response and are thought to be protective. General anaesthesia can also be combined with regional anaesthesia to reduce MAC.
- Total intravenous anaesthesia (TIVA) should be considered.
- The use of dexmedetomidine should be considered.
- There is insufficient evidence to withhold ketamine. However, consideration should be given to avoiding high doses or long-term infusions of ketamine.

Other methods that are not standard practice, but which are unlikely to cause harm include:

- Melatonin premedication at a dose of 0.1 mg/kg.<sup>34</sup>
- Play therapy or attempts at educational play in the postoperative period, in order to rehabilitate the child's brain.



**Figure 1:** Suggested plan for administration of anaesthesia to children who are younger than four years of age



Figure 1 is a flow diagram that features a suggested plan for administration of anaesthesia to children who are less than four years of age.

## Conclusion

It is premature to change our current practice just yet. However, the information that is available provides cause for concern. The anaesthetist should remain cognisant of the risks and benefits of both surgery and anaesthesia and then act accordingly. Once the results of the GAS and PANDA studies become available, perhaps clearer answers will be available.

For further reading, see SmartTots.<sup>35</sup> The SmartTots Programme is an American public-private partnership that funds and coordinates research into AIDN.

## Conflict of interest

There was no conflict of interest.

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