

SPECIAL ARTICLE



## Anaesthetic neurotoxicity and neuroplasticity: an expert group report and statement based on the BJA Salzburg Seminar

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### Editor's key points

- The issue of neurotoxic and neuroplastic effects of general anaesthesia is extremely important.
- The authors have provided an overview of preclinical evidence of long lasting neuronal effects of general anaesthesia.
- Neuronal modulation by general anaesthesia can negatively affect cognition, especially in the young and the elderly.
- This article provides up-to-date knowledge, and direction for future developments and research, in this area.

Although previously considered entirely reversible, general anaesthesia is now being viewed as a potentially significant risk to cognitive performance at both extremes of age. A large body of preclinical as well as some retrospective clinical evidence suggest that exposure to general anaesthesia could be detrimental to cognitive development in young subjects, and might also contribute to accelerated cognitive decline in the elderly. A group of experts in anaesthetic neuropharmacology and neurotoxicity convened in Salzburg, Austria for the BJA Salzburg Seminar on Anaesthetic Neurotoxicity and Neuroplasticity. This focused workshop was sponsored by the *British Journal of Anaesthesia* to review and critically assess currently available evidence from animal and human studies, and to consider the direction of future research. It was concluded that mounting evidence from preclinical studies reveals general anaesthetics to be powerful modulators of neuronal development and function, which could contribute to detrimental behavioural outcomes. However, definitive clinical data remain elusive. Since general anaesthesia often cannot be avoided regardless of patient age, it is important to understand the complex mechanisms and effects involved in anaesthesia-induced neurotoxicity, and to develop strategies for avoiding or limiting potential brain injury through evidence-based approaches.

**Keywords:** anaesthesia, general; anaesthetics; cognitive disorder; neurotoxicity syndromes; postoperative complications

There is growing concern that exposure to general anaesthetics can lead to subsequent learning impairment and

memory deficits and behavioural abnormalities in young subjects, and to accelerated cognitive decline in the elderly.

A group of physicians and scientists who are experts in anaesthetic neuropharmacology and neurotoxicity convened in June, 2012 at the BJA Salzburg Seminar on Anaesthetic Neurotoxicity and Neuroplasticity in Salzburg, Austria for a workshop sponsored by the *British Journal of Anaesthesia*. The group met for several days of intense discussions and workshops to review and challenge currently available evidence from animal and human studies, and to consider the direction of future research. The discussions identified the following points as being crucial for the evolving understanding of anaesthetic neurotoxicity.

## Anaesthesia-induced neurotoxicity and neuroplasticity in the developing brain

### Anaesthesia-induced developmental neuroapoptosis

The panel recognized that two critical factors determine anaesthetic neurotoxicity: the stage of brain development at the time of exposure, and the degree of anaesthetic exposure, which includes both exposure frequency and cumulative anaesthetic dose. The specific anaesthetic drug utilized, health status, or the specific procedure all represent possible secondary factors. Animal studies provide clear evidence that the severity of pathomorphological changes indicative of extensive neuroapoptosis or impaired synaptic development coincides with extensive synapse formation (i.e. synaptogenesis).<sup>1</sup> It is noteworthy that the peak period of synaptogenesis does not occur at the same time in all brain regions even within the same species. Thus, different brain regions are vulnerable at different developmental periods. For example, exposure to a variety of anaesthetics causes severe apoptosis at postnatal day (PD) 7 in rat thalamus, hippocampal region CA1, and neocortex while other neuronal populations, such as dentate gyrus, are not substantially affected at this developmental time point (AW Loepke et al., unpublished observations).<sup>2</sup> However, following anaesthetic exposure at PD 21, substantial neuroapoptosis was observed in dentate gyrus, while neocortical vulnerability had dramatically subsided.

Even within the same brain region, vulnerability may not be uniform. For example, at PD 7 anaesthesia-induced neuroapoptosis within neocortex is highest in superficial layers II and III.<sup>3</sup> Regional differences in anaesthetic toxicity could reflect regional differences in synaptogenesis during early stages of brain development. Moreover, different neuronal subtypes may vary in their vulnerability to anaesthetics, as demonstrated, for example, by greater susceptibility of glutamatergic and GABAergic neurones than cholinergic neurones in neocortex of 7-day-old rats.<sup>4</sup>

### Effects of general anaesthetics on neuronal network assembly

In addition to anaesthesia-induced developmental neuroapoptosis, the effects of general anaesthetics on neuronal network assembly were also discussed. To this regard, several research groups have shown that anaesthesia exposure during the early stages of the brain growth spurt rapidly

leads to a significant and persistent decrease in the number of synapses in several brain regions in rodents.<sup>5–7</sup> In contrast, these same drugs induce a lasting increase in the number of synaptic contacts when administered at later stages (between PD 15 and PD 30) of the peak synaptogenic period.<sup>7,8</sup>

$\gamma$ -Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain, and GABA subtype A (GABA<sub>A</sub>) receptors are primary targets for most clinically used general anaesthetics. Activation of GABA<sub>A</sub> receptors can generate a depolarizing or excitatory response during early stages of brain development. It has been suggested that changes in the morphology and density of synaptic spines, a histological correlate of synaptogenesis, correlate with excitability in the developing brain. During initial phases of the brain growth spurt, GABA acts as an excitatory neurotransmitter, and GABA<sub>A</sub> receptor-mediated membrane depolarization is a key regulator during early stages of both excitatory and inhibitory synaptogenesis in developing neurones.<sup>9,10</sup> At later stages of the peak synaptogenic period, there is a functional shift towards the hyperpolarizing and thus inhibitory effects of GABA<sub>A</sub> receptor-mediated neurotransmission. The switch from excitatory to inhibitory GABAergic neurotransmission correlates temporally with changes in the neurone-specific chloride potassium symporter responsible for establishing the chloride gradient in neurones and maintenance of low intracellular chloride concentrations necessary for inhibitory chloride influx. Neuronal potassium-chloride cotransporter (KCC) expression undergoes a developmental switch from the NKCC1 sodium-potassium-chloride cotransporter, an immature form that promotes neuronal excitability, to a mature form, KCC2, that promotes neuronal inhibition.<sup>11</sup> KCC2 expression increases markedly during the second postnatal week in the rodent cerebral cortex,<sup>12</sup> and from the 30th gestational week in humans.<sup>13</sup> It is, thus, tempting to speculate that anaesthesia-evoked developmental toxicity depends, at least partly, upon the expression levels of KCC2 in the central nervous system (CNS). It is also recognized that in the normal adult neurones, a large and persistent increase in GABA<sub>A</sub> receptor conductance can cause a biphasic response such that the initial membrane hyperpolarization is followed by depolarization. The depolarization is due to the accumulation of intracellular chloride and the resulting collapse of the chloride gradient as well as efflux of bicarbonate ions.<sup>14</sup> During periods of intense GABA<sub>A</sub> receptor activation (as occurs during exposure to most anaesthetics) such events could lead to increased excitability of neurones even in the presence of adult levels of the co-transporter KCC2.

### Anaesthetic effects on neurogenesis

When the volatile anaesthetic isoflurane was administered for 35 min every day for 4 days to both very young and adult rats and mice, the young but not adult rodents showed impaired memory performance, and the deficits became more pronounced as the animals grew older.<sup>15</sup> Memory deficits were paralleled by a decrease in the hippocampal stem cell pool and persistently reduced neurogenesis. Neurogenesis

continues throughout life in two discrete brain regions, the dentate gyrus of the hippocampus and the subventricular zone. In the hippocampus, the formation of new neurones is thought to be important for memory and learning. The isoflurane-induced loss of stem cells and reduction of neurogenesis occurred without any overt signs of cell death. The underlying mechanisms remain to be identified. One possibility is that dying cells are cleared by microglia before markers of cell death can be detected.<sup>16</sup> Another possibility is that under pathological conditions progenitor cells differentiate into glial cells instead of neurones.<sup>17</sup> A third possibility is that the normal, age-related disappearance of hippocampal neural stem cells,<sup>18</sup> the appearance of new astrocytes, and the decline in the production of new neurones may be accelerated by isoflurane. Regardless of the underlying mechanism, it remains to be shown why the isoflurane-induced loss of stem cells and reduction of neurogenesis occurred in young but not in adult brains.

### Role of neurotrophic factors in anaesthetic effects on synaptic density

It appears that neurotrophic factors in general, and brain-derived neurotrophic factor (BDNF) in particular, are involved in isoflurane- and propofol-induced reductions in synapse density in the developing hippocampus. A model that has been widely used to study neurotoxicity involves the *in vitro* exposure of primary cultured hippocampal neurones to either 1.4% isoflurane or 2  $\mu$ M propofol for 4 h. Both propofol and isoflurane cause significant reductions in synaptic density. This is accompanied by activation of RhoA and the growth factor receptor p75<sup>NTR</sup> as part of a cascade leading to actin depolymerization, loss of microtubules, and impairment of axonal transport. When isoflurane was co-administered with Pep5, an inhibitor of the p75 receptor, the effects of isoflurane on synaptogenesis were reduced.<sup>5</sup> It is noteworthy that disruption of microtubules after propofol exposure leads to inhibition of BDNF trafficking. The microtubule system is crucially important in transporting not only metabolic elements essential for neuronal survival and development, but also for the strategic transport of cellular organelles (mitochondria in particular) from the soma to remote compartments such as axons and dendritic spines where their presence is required to ensure proper neuronal function and formation of circuits.

### The role of mitochondria and reactive oxygen species in anaesthesia-induced neurotoxicity

In the immature brain, anaesthetics and sedatives that promote GABA<sub>A</sub> receptor activation result in elevated intracellular calcium, which leads to disturbances in mitochondrial membrane potential and bioenergetics causing neuronal dysfunction and death.<sup>19 20</sup> In particular, propofol, sevoflurane, and isoflurane (in combination with nitrous oxide and midazolam) increased the production of reactive oxygen species (ROS).<sup>20 21</sup> As hyperoxia worsens ischaemic brain damage

in both immature and mature animals,<sup>22 23</sup> avoidance of unnecessary hyperoxic ventilation or administration of antioxidants while under anaesthesia may help protect against anaesthetic neurotoxicity.<sup>24</sup> Oxidative stress and mitochondrial dysfunction in both the heart and the brain can also be reduced by anaesthetic preconditioning,<sup>25–27</sup> possibly mediated by stimulation of sub-toxic ROS production and subsequent expression of antioxidant gene products.<sup>28</sup>

Even under normoxic conditions, many anaesthetics cause a significant increase in ROS that leads to increased neuronal lipid peroxidation and neuronal deletion in vulnerable brain regions such as the subiculum, a part of the hippocampus proper.<sup>21</sup> To assess the functional importance of ROS up-regulation in anaesthesia-induced cognitive impairment, the protective role of ROS scavengers was explored. Exposure of rat pups to general anaesthesia at PD 7 caused significant cognitive deficits. However, if pups were also treated with either EUK-134, a synthetic mimic of superoxide dismutase and catalase, or R(+)-pramipexole (PPX), a mitochondrial protectant and ROS scavenger, cognitive development was almost indistinguishable from that of animals not exposed to the anaesthetic.<sup>21</sup> The results suggest that early protection around the time of anaesthesia exposure using agents that can prevent mitochondrial damage and ROS up-regulation could be very important in alleviating anaesthesia-induced developmental cognitive impairment. Similar protection with antioxidants or mitochondrial enhancers has been noted for the neurohistological effects produced by anaesthetics.<sup>29</sup>

### Nitrous oxide neurotoxicity

Neurotoxicity induced by nitrous oxide-induced block of N-methyl-D-aspartate (NMDA) receptors is manifest by massive swelling of neuronal organelles including mitochondria and endoplasmic reticulum.<sup>30</sup> Nitrous oxide also increases plasma homocysteine caused by oxidation of methionine synthase. As levels of homocysteine can be easily measured in blood, they can be used as biomarkers of nitrous oxide-induced modulation of methionine synthase activity. After an 8 h exposure to nitrous oxide, an eight-fold increase in blood homocysteine levels could be detected.<sup>31 32</sup> This increase was prevented by continuous infusion of vitamin B12, which is an enzyme co-factor of methionine synthase. Future research aims to assess how and whether this effect is relevant to short-term neurocognitive outcomes, long-term neurocognitive outcomes, or both.

### Role of glial cells in developmental anaesthetic neurotoxicity

Glial cells are crucially important during early stages of brain development, and are important anaesthetic targets,<sup>33 34</sup> so the role of anaesthesia in modulating glial function and growth was considered. Using primary astroglia cultures from E18 rat embryos, it was found that isoflurane causes marked reduction in glial fibrillary acidic protein (GFAP) and  $\beta$ -tubulin staining, suggestive of isoflurane-induced impairment of

astroglial cytoarchitecture similar to that in previously published reports. However, unlike previous findings, which suggested that isoflurane impairs astroglial proliferation, newer evidence did not find an effect on astroglial survival, cytochrome c release, caspase-3 activation, or proliferation.<sup>35</sup> The apparent discrepancy was attributed to several factors: age of the cultures, duration of exposure, and dose. Interestingly, the impairment in astroglial proliferation was found in primary astroglial cultures obtained from 1- to 2-day-old rat pups that were exposed to isoflurane for 24 h (as opposed to 4 h) and at a concentration of 3% (as opposed to 1.4%).<sup>36</sup> Nevertheless, the common finding, regardless of the dose or duration, is that isoflurane exposure impairs glial cytoarchitecture in immature astrocytes, which could result in impaired morphological development and proliferation.

### Role of surgery, inflammation, and pain in anaesthesia-induced developmental neurotoxicity

Although skin incision and formalin injection are painful stimuli, they cannot simulate true surgical conditions where, in addition to intense nociception, the role of inflammation, infection, blood loss, and fluid shifts can be substantial. Available evidence suggests that surgical stimulation worsens isoflurane-induced developmental neuroapoptosis and anaesthesia-induced cognitive deficits.<sup>37</sup> However, concurrent inflammatory peripheral noxious stimulation with ketamine anaesthesia attenuated the increased neuroapoptosis compared with ketamine anaesthesia without noxious stimulus.<sup>38</sup> Although the mechanisms underlying these contrasting effects remain to be deciphered and are likely complex, these emerging findings suggest that surgery is an additive if not synergistic propagator of anaesthesia-induced developmental neurotoxicity.<sup>37</sup> For example, the key pro-inflammatory factor interleukin 1 beta (IL-1 $\beta$ ), which is elevated during surgery, increases the trafficking of GABA<sub>A</sub> receptors to the surface of neurones in the hippocampus.<sup>39</sup> The resulting increase in surface expression of GABA<sub>A</sub> receptors on neurones might increase neurotoxicity associated with activation of these receptors.

### Role of complement activation in anaesthesia

Molecules of the inflammatory cascade, including complement, play a vital role in the establishment and modification of synaptic connections during development.<sup>40–41</sup> Along these lines, emerging evidence suggests that isoflurane activates the complement cascade and inflammatory pathways via modulation of C1q+ and C3 and by inducing a variety of cytokines and chemokines. Activation of the complement cascade occurs in the absence of apoptosis or overt changes in microglial number or morphology; effects on C1q appeared after relatively short exposure to isoflurane (as short as 2 h). These results suggest that anaesthetic effects could be much more complex than activation of apoptosis during synaptogenesis (Culley DJ and Crosby C, unpublished observations).

### Developmental anaesthetic neurotoxicity in non-human primates

Exposure of young rhesus monkeys to ketamine at PD 5/6 for 24 h induces significant apoptosis as detected with caspase-3, Fluoro-Jade and silver staining.<sup>42</sup> In a recent study, rhesus monkey neonates (PD 5 or 6) were exposed to 1% isoflurane combined with 70% nitrous oxide for 8 h and control monkeys were exposed to room air only. One day later, a positron emission tomography (PET) imaging agent ([<sup>18</sup>F]N-2-(2-fluoroethoxy)benzyl)-N-(4-phenoxy-pyridin-3-yl)acetamide ([<sup>18</sup>F] FEPPA) that labels cellular markers indicative of neuronal damage and brain cell death was injected for microPET/computed tomography imaging over the next 2 h, at 7 and 21 days, and at 6 months after anaesthetic exposure. On days 1 and 7 after exposure, uptake of [<sup>18</sup>F] FEPPA was significantly increased in several brain regions, but by day 21 and at 6 months uptake was not different from controls. These data suggest that PET imaging can be used to describe the time course of anaesthetic-induced neurotoxicity in a minimally invasive manner in the same animal.<sup>43</sup> Exposure to ketamine causes significant upregulation of NMDA receptors, in particular the NMDA receptor subunit NR1. Young cultured rat fore-brain neurones exposed to ketamine showed a similar and dose-dependent up-regulation of mRNA for the NR1 subunit and, when NR1 subunit up-regulation was blocked with NR1 antisense RNA, ketamine-induced cell death was prevented.<sup>44</sup>

In addition to pathomorphological indices of ketamine-induced developmental neuroapoptosis, there is emerging evidence that cognitive behaviour of non-human primates is impaired as well. Assessment with an Operant Test Battery (OTB) revealed that ketamine causes long-term (perhaps permanent) impairment of learning and memory. Ketamine-treated primates also suffer from lower motivation scores, which could, at least in part, account for lower learning scores. However, effects on motivation do not lessen concerns regarding long-term behavioural sequelae of early exposure to general anaesthesia.<sup>45</sup>

### The window of vulnerability to anaesthetic-induced damage

Time windows of vulnerability must be carefully assessed when comparing vulnerability to anaesthetics between species. For example, brain maturation in monkeys at gestational age 120 days (during their last trimester *in utero*) is generally accepted as comparable with that in the first week of postnatal life in humans (~0–6 days of age). On the other hand, brain maturation at PDs 6 and 35 in monkeys corresponds to that of ~6 and 12 months of age, respectively, in human infants.<sup>46</sup> When non-human primates were exposed to isoflurane, ketamine, or propofol either *in utero* (120 days of gestational age) or postnatally (6 days of age), the patterns of neuronal damage in the brain differ in accordance with previously noted findings that brain region vulnerability depends on the stage of development. For example, in non-human primates the fetal apoptosis pattern after ketamine anaesthesia was more widespread and involved the cortex, basal ganglia,



thalamus, amygdala, cerebellum, and brainstem, whereas the neonatal apoptotic pattern after the same ketamine anaesthetic seemed to be more pronounced in cortical and basal ganglia grey and white matter compared with other brain regions.<sup>47</sup>

In neonatal monkeys, there were agent-specific differences in the severity of neurotoxicity—isoflurane was more damaging than propofol, and propofol was more damaging than ketamine in both white and grey matter using caspase-3 staining.<sup>48</sup> Agent-specific differences in the degree of anaesthetic neurotoxicity were also age-dependent. Ketamine was more toxic in fetal compared with neonatal brain, whereas isoflurane was more damaging to the neonatal than fetal brain. When apoptosis was studied in white matter after 5 h of isoflurane anaesthesia, significant caspase activation was noted in premyelinating and myelinating oligodendrocytes in neonatal monkeys.<sup>49</sup> In contrast, astrocytes were not found to be injured by the anaesthetic exposure.<sup>49</sup> At PD 35, monkeys did not show neuronal cell death after 24 h of ketamine anaesthesia.<sup>50</sup>

When comparing neurotoxicity of different anaesthetics (i.v. and inhalation) the doses of the different agents require normalization for potency. A clinically relevant way to approach this issue is to administer equipotent doses such that the depth of anaesthesia is comparable. The approach used thus far has been titration of anaesthetic administration to achieve the lack of response to a profoundly noxious stimulation to all four extremities without causing any motor response or an increase in arterial pressure or heart rate of >10% from baseline, with assessments made every 30 min.

### Preliminary results from human studies to assess neurocognitive effects of early anaesthetic exposure

General anaesthetics such as nitrous oxide, sevoflurane, and isoflurane given to children at <12 months of age seem to impair recollection when these children are 6–11 yr old. Recollection is an important component of recognition memory and is supported by anatomic brain structures that are affected by anaesthesia-induced cell death. When the outcomes of spatial tasks were explored, young boys were more affected than young girls, although difficulties with the colour recognition task were detected equally in boys and girls. The performance was worse when children were exposed for a longer time (several hours). It is noteworthy that this preliminary study, which included 28 children, failed to find a difference between single and multiple exposures in terms of deficits in recognition memory (Stratmann *et al.*, unpublished observations). When recognition memory was tested in a rat model of anaesthetic neurotoxicity, anaesthesia-treated rats exhibited significant impairment of recollection similar to humans; but no deficits were detected in another component of recognition memory called familiarity. As familiarity is dependent on a properly functioning perirhinal and parahippocampal cortex, the hippocampus and functionally connected areas might be more sensitive to anaesthesia-induced damage. Additional

large-scale prospective, randomized clinical trials will be needed to re-examine these findings.

### Clinical outcome studies

Although preclinical data regarding anaesthesia-induced neurotoxicity during early stages of brain development have been very convincing and highly reproducible in a variety of species, similar effects in humans are much less certain because of inherent difficulties in outcome studies.<sup>51</sup> Truly definitive studies would require subjects to be exposed to anaesthetics without medical necessity. This is not possible because of obvious ethical issues with designing prospective randomized clinical trials in very young children. Some of the retrospective clinical studies looking at long-term behavioural effects of anaesthesia were collected from patient cohorts that were exposed to anaesthesia in the 1970s and early 1980s when sophisticated monitoring (pulse oximetry in particular) was not routinely available. Consequently, concerns exist that respiratory or haemodynamic disturbances might not have been detected and remedied in a timely fashion and, thus, potentially could have contributed to the observed cognitive deficits. However, hypercarbia or hypoglycaemia, although intuitively presumed to worsen anaesthesia-induced developmental neurotoxicity, was not observed to affect the degree of anaesthesia-induced neuroapoptosis in some animal studies.<sup>52 53</sup>

Concerns regarding potential developmental neurotoxicity in humans have to be considered with some delicacy. Surgery cannot be performed without anaesthesia and is frequently performed in very young children to treat life-threatening conditions, to avert dangerous health conditions, or to improve quality of life. The known health risks of not treating these conditions have to be weighed against the potential adverse effects of surgery with anaesthesia. Given the fact that current clinical studies do not unequivocally demonstrate impairment of behavioural development, it is possible that more harm could be inflicted if necessary or timely treatment is withheld because of concerns regarding early life exposure to anaesthesia.

## Anaesthesia-induced neurotoxicity and neuroplasticity in the aging brain

### The role of anaesthesia in postoperative cognitive decline in the elderly

Available data suggesting an association between cognitive decline and anaesthesia exposure in humans are based mainly on case reports rather than on large-scale prospective clinical trials. The problem centres on the reliability of diagnosis—postoperative cognitive decline (POCD) is not a clearly defined clinical diagnosis. In the clinical environment, the concern is based on the subjective assessment of a patient or his/her family members. Nevertheless, even after short surgical or diagnostic procedures, up to 47% of elderly patients will demonstrate cognitive decline 24 h after anaesthesia.<sup>54</sup> At the time of hospital discharge, cognitive decline has been

demonstrated in 31–47% of patients, whereas at 3 months post-procedure ~10% of patients still have evidence of cognitive decline.<sup>55–57</sup> Several risk factors have been suggested, such as advanced age, lower educational level, longer duration of anaesthesia, and higher severity of surgery (e.g. vascular, orthopaedic, and cardiac). There has been no correlation between POCD and the type of anaesthesia (i.e. regional or general), nor was there a clear correlation with deep or light anaesthesia or the specific anaesthetic agents used. For example, some studies suggest that deep anaesthesia (as determined using bispectral index monitoring) is associated with higher incidence of POCD<sup>58</sup> whereas others suggest no association between the depth of anaesthesia and POCD.<sup>59</sup> A pilot study by Zhang and colleagues<sup>60</sup> suggested that perhaps isoflurane might be more likely to cause POCD when compared with desflurane although large-scale studies would be necessary to confirm this notion.

Current clinical trials focus on the comparison of POCD post-cardiac surgery on-pump or off-pump.<sup>61</sup> Some of the preliminary findings suggest that off- and on-pump cardiac surgeries result in approximately the same cerebral oxygen levels with no difference in long-term POCD with respect to the anaesthetic agent used or the dose administered (Absalom *et al.*, unpublished observations). There was no correlation between slight hypoxaemia/hypothermia and POCD. Some studies suggest that hypothermia (core body temperature of 32°C), which necessitates re-warming at the end of surgery, causes disturbed cerebral autoregulation and can result in cerebral oedema, thus worsening cognitive recovery postoperatively.

### Effects of anaesthetics on cognitive function in adult animals

Studies of adult mice and rats have shown that exposure to inhaled anaesthetics causes memory deficits that persist even after the anaesthetics have been eliminated. Memory deficits for both anterograde and retrograde memory can persist much longer than expected based on pharmacokinetic properties. Further, age might exacerbate post-anaesthetic memory loss.<sup>62–63</sup> For example, robust deficits in anterograde memory are evident in adult mice for up to 48 h after a single brief (1 h) exposure to isoflurane at 1 minimum alveolar concentration.<sup>64–65</sup> Only certain types of memory are vulnerable to anaesthetics. Working memory (required to perform short-term tasks such as remembering a phone number immediately after locating it in the phonebook) generally remains intact,<sup>65</sup> while long-term memory is impaired. There is some inconsistency here, however, as most studies find working memory is disrupted while reference memory is intact.<sup>63–66–67</sup> Specific subtypes of GABA<sub>A</sub> receptors that generate a tonic inhibitory conductance in principle neurones are known to be particularly sensitive to inhaled<sup>68</sup> and i.v.<sup>69</sup> anaesthetics might play a key causal role; preemptive inhibition of  $\alpha 5$  GABA<sub>A</sub> receptors before exposure to the anaesthetic prevented memory deficits in mouse models.<sup>64–65</sup> Also, administration of a drug that inhibits  $\alpha 5$  GABA<sub>A</sub> receptors after exposure fully restored memory performance.

### Effects of anaesthetic exposure on Alzheimer's disease in the elderly

Approximately 8.5 million Alzheimer's disease (AD) patients and a much greater number of senior patients who are vulnerable to AD will need surgical care under anaesthesia annually around the world. Tau is a highly soluble and hydrophilic microtubule-associated protein known to play an important role in AD dementia and neurodegeneration as it is essential for A $\beta$ -mediated neurotoxicity.<sup>70–71</sup> Tau pathology correlates with cognitive decline in AD patients.<sup>72–73</sup> Tau undergoes very minimal phosphorylation in the adult brain (<5%), but in AD brain tau protein is close to 100% phosphorylated and aggregates.<sup>74</sup>

Most anaesthetics promote tau hyperphosphorylation in rodents, albeit indirectly by inducing hypothermia.<sup>71–72</sup> Hypothermia has a profound effect on tau phosphorylation via reducing phosphatase activity.<sup>73</sup> Interestingly, repeated exposure to hypothermic anaesthesia results in both tau detachment from microtubules and increased aggregation.<sup>75–76</sup> However, anaesthetics *per se* can have an effect independent of hypothermia as normothermic administration of i.v. anaesthetics such as propofol<sup>77</sup> or sedatives such as dexmedetomidine (Whittington RA, unpublished observation), result in persistent tau hyperphosphorylation in mice. Repeated normothermic administration of volatile anaesthetics to mice results in either persistent tau hyperphosphorylation with sevoflurane,<sup>78</sup> increased A $\beta$  aggregation with halothane<sup>79</sup> or isoflurane,<sup>80</sup> cognitive impairment, or both as determined using the Morris Water Maze test (sevoflurane and isoflurane). This is particularly important as the combination of both A $\beta$  oligomerization and tau hyperphosphorylation is necessary for AD pathology. On the other hand, a very recent study has demonstrated that surgery *per se* can also promote tau but not A $\beta$  pathology independently of anaesthesia.<sup>81</sup> It is, therefore, of concern that general anaesthetics, surgery, or both can promote both processes.

### Agent-specific differences in neurotoxicity

Potential differences between isoflurane and desflurane on mitochondrial dysfunction have been examined in mouse models. Isoflurane but not desflurane can induce ROS accumulation, open the mitochondrial permeability transition pore, decrease mitochondrial membrane potential, reduce ATP levels, release cytochrome c, induce caspase-3 activation, and lead to impairment of learning and memory.<sup>19–82</sup> Although the clinical relevance of these differences between isoflurane and desflurane remains to be determined, a pilot human study suggests that surgery under anaesthesia with isoflurane, but not desflurane, can lead to cognitive dysfunction in patients.<sup>60</sup>

### Protection for cognitive dysfunction by overexpression of heat-shock protein 72

Overexpression of heat shock protein 72 (Hsp72), which can protect the brain from ischaemia, can also prevent memory loss after orthopedic surgery under isoflurane anaesthesia

in adult male mice.<sup>83</sup> Although the mechanism for this protection remains to be deciphered, it is likely multifactorial. Wild-type mice suffered from memory impairment up to 7 days post-surgery because of effects of surgery, anaesthesia, or both, whereas Hsp72 transgenic mice that overexpress Hsp72 protein were significantly less impaired in both hippocampal-dependent and hippocampal-independent forms of memory.<sup>83</sup>

## Conclusions

Mounting evidence exists from preclinical studies that general anaesthetics are powerful modulators of neuronal development and function. Although evidence from clinical studies in paediatric and geriatric anaesthesiology is emerging, it is important for this line of research to be expanded. As general anaesthesia often cannot be avoided regardless of patient age, it is important to understand the complex mechanisms and effects involved in anaesthesia-induced neurotoxicity, and to develop strategies for avoiding or limiting potential brain injury. Studies towards those ends will permit more definitive conclusions about potential neurotoxicity in humans, and facilitate the establishment of recommendations to guide clinical practice as definitive clinical data are likely to be elusive.

## Authors' contributions

All authors contributed to the conception and writing of this manuscript.

## Declaration of interest

H.C.H. is an Editor for the *BJA* and for *Anesthesiology*; all others none declared. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the US FDA.

## Funding

A.R.A., National Institute of Academic Anaesthesia and Dutch Association of Anesthesiologists; K.B., Swedish Research Council, the Swedish Childhood Cancer Foundation, the Swedish Cancer Foundation, governmental grants from Agreement Concerning Research and Education of Doctors (ALF), the StratNeuro Network at the Karolinska Institute, the Stockholm and Gothenburg Freemasons' Pediatric Research grants; D.C., William F. Milton Fund, National Institutes of Health (Bethesda, MD, USA) K08 GM077057; G.C., National Institutes of Health R01 GM088817; G.F., National Institutes of Health 2P01 1HD16596-26, US Air Force FA8650-11-2-6D04, US Army W81XWH-13-1-0016 and W81XWH-09-2-0187; R.G.G., National Institutes of Health GM49831 and NS053898; H.C.H., National Institutes of Health GM58055 and NS56315; KFH HE4554/5-1 DFG (German Research Foundation); V.J.-T., NIH/NICHD HD 44517 and American Recovery and Reinvestment Act supplement HD 44517S, John E. Fogarty Award TW007423-128322, March of Dimes National Award and Harold Carron endowment. V.J.-T. was an Established Investigator of the American Heart Association; D.M., Medical

Research Council, Alzheimer's Society-Bupa Foundation, BJA/RCoA, AAGBI, Westminster Medical School Research Trust, Action Medical Research and SPARKS, UK and European Society of Anesthesiology, Brussels; B.A.O., Canada Research Chair in Anesthesia, and Canadian Institutes of Health Research; E.P., Canadian Institutes for Health Research (MOP-106423, PCN-102993), Natural Sciences and Engineering Research Council (354722), Canada Foundation for Innovation (23905), and Research Scholar Career Awards (16205, 20048) from the Fonds de la Recherche en Santé du Québec; S.G.S., Boston Children's Hospital Endowed Chair in Pediatric Neuroanesthesia; G.S., John Severinghaus Research Award – Department of Anesthesia and Perioperative Care, UCSF; L.V., Swiss National Science Foundation; Z.X., National Institutes of Health R21AG038994, R01 GM088801, and R01 AG041274, Investigator-initiated research grant from Alzheimer's Association, Chicago, IL, USA, and Cure Alzheimer's Fund, Wellesley, MA, USA.

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Handling editor: R. P. Mahajan