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A holistic approach to anesthesia-induced neurotoxicity and its implications for future mechanistic studies

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ABSTRACT

The year 2016 marked the 15th anniversary since anesthesia-induced developmental neurotoxicity and its resulting cognitive dysfunction were first described. Since that time, multiple scientific studies have supported these original findings and investigated possible mechanisms behind anesthesia-induced neurotoxicity. This paper reviews the existing mechanistic literature on anesthesia-induced neurotoxicity in the context of a holistic approach that emphasizes the importance of both neuronal and non-neuronal cells during early postnatal development. Sections are divided into key stages in early neural development; apoptosis, neurogenesis, migration, differentiation, synaptogenesis, gliogenesis, myelination and blood brain barrier/cerebrovasculature. In addition, the authors combine the established literature in the field of anesthesia-induced neurotoxicity with literature from other related scientific fields to speculate on the potential role of non-neuronal cells and to generate new future hypotheses for understanding anesthetic toxicity and its application to the practice of pediatric anesthesia.

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1. Introduction

2016 marked the 15th anniversary since Jevtovic-Todorovic first presented evidence supporting anesthesia-induced developmental neurotoxicity (Jevtovic-Todorovic et al., 2003; Jevtovic-Todorovic et al., 2001). Since that time, multiple animal species (mice, rats, guinea pigs, and non-human primates) have demonstrated neurotoxicity after being exposed to the most common anesthetics (Paule et al., 2011; Rizzi et al., 2008; Shen et al., 2013a; Shen et al., 2013b; Zou et al., 2011). The actual neurotoxic effect appears to depend upon the stage of neuronal development, the drug dosage, and the duration of anesthetic exposure (Gutierrez et al., 2010; Ikonomidou et al., 1999; Stratmann et al., 2009; Zhu et al., 2010). These findings have remained consistent across multiple inhalational and intravenous agents, including nitrous oxide, desflurane, isoflurane, sevoflurane, ketamine, midazolam and propofol (Creeley et al., 2013; Istaphanous et al., 2011; Jevtovic-Todorovic et al., 2003; Young et al., 2005). Despite years of intense research, the exact mechanism behind anesthesia-induced neurotoxicity and any possible implications these findings hold for the practice of pediatric anesthesia remains equivocal. Multiple excellent reviews have been published on this topic, but the majority of previously published literature has focused on anesthetic toxicity with respect to neurons (Jevtovic-Todorovic et al., 2003; Lee et al., 2015; Lei et al., 2012).

It is estimated that the adult male human brain is composed of 86 billion neurons and 85 billion non-neuronal cells (Azevedo et al., 2009). Non-neuronal cells can be further divided into glial cells (astrocytes, oligodendrocytes, microglia and ependymal cells) and the brain vasculature/blood brain barrier (BBB). In this review, we present the first holistic approach to anesthesia-induced developmental neurotoxicity by comprehensively evaluating both neuronal and non-neuronal contributions during critical periods in neonatal development. Because very little literature exists on anesthesia-induced developmental brain toxicity in non-neuronal cells, we will incorporate recent publications from other relevant fields, including neuroanatomy, developmental neurobiology, and neuropathology to generate new hypotheses focused on possible toxic effects to developing non-neuronal brain cells after anesthesia.

2. Neurodevelopmental vulnerability

Pathological insults to the brain, including toxins, trauma, and hypoxia have varying neurocognitive effects depending upon when the events occur during development (Rice and Barone, 2000; Semple et al., 2013). Increased neurodevelopmental vulnerability appears to coincide with key periods in neurocellular development, including proliferation, migration, differentiation, apoptosis, gliogenesis, myelination, and synaptogenesis (Rice and Barone, 2000). Although the exact timing of these events with respect to conception and birth varies across species, the general progression of these events appears to be relatively maintained (Bayer et al., 1993; Clancy et al., 2007; Rice and Barone, 2000; Semple et al., 2013). The majority of animal studies on

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anesthesia-induced developmental neurotoxicity have utilized rodent models that have focused on the first 2-weeks of life, a period of time critical for multiple steps in neurodevelopment, especially apoptosis, synaptogenesis, gliogenesis, and myelination (Fig. 1, Relative Timing of Major Events in Brain Development - Rat versus Human).

2.1. Neural apoptosis

Normal development produces an excess of neurons that are systematically culled via apoptosis, resulting in the eventual elimination of 50% or more of the initial neuronal pool (Bandeira et al., 2009; Stiles and Jernigan, 2010). This period of critical apoptosis occurs at the end of gestation in humans, but remains an active process into the second week of life in rodents (Bandeira et al., 2009). Exposures that alter the physiological balance of neuronal apoptosis during this vulnerable period can detrimentally effect normal brain development as observed after exposure to multiple neurotoxins, including ethanol and lead (Liesi, 1997; Oberto et al., 1996).

Concern surrounding anesthesia-induced neuronal apoptosis first arose in 1999 when Ikonomidou et al. demonstrated that injection of an *N*-methyl-D-aspartate (NMDA) antagonist in postnatal day (PND) 0, 3, and 7 rat pups produced diffuse neuronal apoptosis (Ikonomidou et al., 1999). The resulting neurodegeneration varied both by age of the pup and by individual regions within the brain. The authors hypothesized that NMDA antagonists blocked normal glutamate stimulation via NMDA receptors, leading to neuronal apoptosis (Ikonomidou et al., 1999). Subsequent studies utilizing ketamine also demonstrated apoptosis, especially in those animals receiving multiple, high doses during periods of species specific developmental vulnerability (Hayashi et al., 2002; Rudin et al., 2005; Scallet et al., 2004; Slikker et al., 2007; Young et al., 2005). Research indicated that ketamine upregulation of NMDA receptor expression altered intracellular calcium homeostasis, possibly producing the observed apoptosis (Liu et al., 2013; Shi et al., 2010). Similar to NMDA receptor antagonists, activation of gamma-aminobutyric acid (GABA) receptors via inhalational agents (isoflurane) promoted neurotoxicity in hippocampal culture cells and was also associated with excessive neuronal influx of calcium (Zhao et al., 2011).

Anesthesia-induced neuronal apoptosis may also occur secondary to changes in calcium homeostasis within mitochondria (Sztark et al., 1995; Yang et al., 2013). Neurons exposed to anesthesia displayed structurally damaged mitochondria, accumulation of reactive oxygen species (ROS), and upregulation of caspase expression (Boscolo et al., 2012; Yon et al., 2005; Zhang et al., 2010). Although the actual mechanism for ROS upregulation was not identified, the administration of either a ROS scavenger or a mitochondrial protectant were each individually sufficient to prevent anesthesia-induced neuronal apoptosis and the previously observed cognitive impairment (Boscolo et al., 2012).

2.2. Neurogenesis

Traditionally, developmental neurobiologists believed that neural proliferation occurred prenatally with the exception of the cerebellum, olfactory bulb, and the hippocampus (Bayer, 1989). More recent studies have shown that in addition to periods of fetal neurogenesis, the brain undergoes region specific periods of large-scale neurogenesis after birth, especially during the first week of life, (Fig. 2, Postnatal Neuronal Proliferation by Brain Region in the Rat) (Bandeira et al., 2009). Toxic exposures during periods of neuronal proliferation can impede normal brain development with subsequent implications for cognitive function in adulthood (Coleman et al., 2012). Both propofol and isoflurane have been shown to alter neurogenesis in the hippocampus, especially in neonatal brains (Erasso et al., 2013; Huang et al., 2016; Stratmann et al., 2009). In addition, studies done at 2-weeks of age in male rats showed decreased neurogenesis and a reduction in the hippocampal stem cell pool after isoflurane exposure (Zhu et al., 2010). Interestingly, the window of neuronal vulnerability to anesthesia may be less dependent on a specific period of neuronal development and instead, may apply to any cells actively undergoing neurogenesis, even in the adult brain (Hofacer et al., 2013).

Brain-derived neurotrophic factor (BDNF) is an important neurotrophin that regulates neurogenesis (Binder and Scharfman, 2004). Exposure to a combination of isoflurane, nitrous oxide, and midazolam decreases BDNF expression levels in the thalamus (Lu et al., 2006). This study focused on BDNF mediated neuroapoptosis, but the
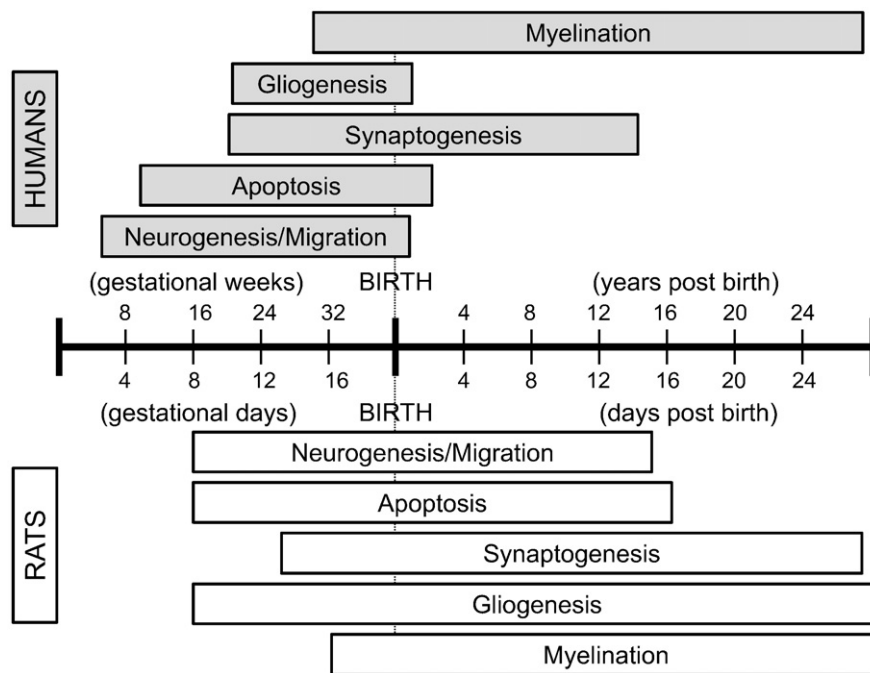

Fig. 1. Relative timing of major events in brain development - rat versus human: although specific time points vary across species, the sequence of individual events during brain development are preserved. The period immediately after birth is vital for multiple aspects of brain development in both humans and rats.
(Graph adapted from Lenroot and Giedd, 2006, Rice and Barone, 2000, and Semple et al., 2013.)
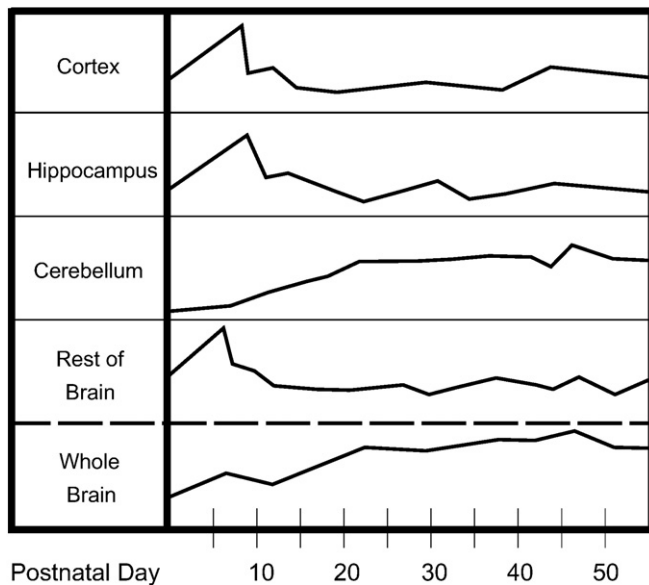
Fig. 2. Postnatal neuronal proliferation by brain region in the rat: graphical representation of relative number of total neuronal cells at different time points within individual brain regions across the first 2 postnatal months of life. Total numbers of neuronal cells increase slowly across the entire brain and cerebellum, while the cortex, hippocampus and other brain regions undergo significant neuronal proliferation during the first week followed by selective cell loss in the second week.
(Graph adapted from Bandeira et al., 2009.)

findings also have implications for BDNF regulated neurogenesis. Although studies in neonates are lacking, in adult animals BDNF expression stabilizes GABA receptors via phosphorylation of Y365/7 (Yang et al., 2008). Upregulation of BDNF promotes neurogenesis in the adult rodent hippocampus and the frontal cortex in a Y365/7 dependent manner (Boscolo et al., 2012; Yang et al., 2008). Therefore, anesthesia-induced changes in BDNF levels have the potential to directly regulate developmental neurogenesis, making it a novel target for future mechanistic studies.

2.3. Neural migration and differentiation

The majority of neuronal migration and differentiation occurs prenatally, but like neuronal proliferation, both processes also still occur within the rodent brain during the first postnatal week (Stiles and Jernigan, 2010). Rats exposed to isoflurane and desflurane on PND 2 showed decreased neuronal levels within the hippocampal dentate gyrus 5 days later (Drobish et al., 2016). The authors were unable to differentiate whether this was due to changes in migration, proliferation, or a combination effect, but studies in other neurotoxic exposures, including neonatal phenytoin, would support the hypothesis that anesthesia likely effects both processes (Ohmori et al., 1999).

Similar to anesthesia-induced apoptosis, changes in calcium homeostasis have also been implicated in altered neuronal migration and differentiation. Calcium serves as an important secondary messenger in the regulation of neuronal differentiation and neuronal growth cone formation (Henley and Poo, 2004; Rosenberg and Spitzer, 2011). According to the calcium set point hypothesis, growth at the neuronal cone depends upon a specific intracellular calcium level and alterations within this level can alter normal cone development (Kater and Mills, 1991). Therefore, GABA-mediated calcium perturbations, as discussed above, can also alter neurite growth. This has been demonstrated in a murine tissue culture model in which isoflurane altered axonal guidance in developing neurons (Mintz et al., 2013).

2.4. Synaptogenesis

Successful neuronal function and cognitive development depends upon the formation of synapses between individual neurons. This process occurs postnatally, peaking during the second postnatal week in rodents, and is altered by early developmental exposure to anesthesia (Jevtovic-Todorovic, 2012; Semple et al., 2013). Repeated exposure to midazolam or ketamine from PND 8 to 12 in transgenic green fluorescent protein-M mice produced a statistically significant decrease in both dendritic spinal density and spinal length on PND 13, although this difference was no longer present by PND 30 (Tan et al., 2009). Conversely, experiments exposing rodents to either midazolam, propofol, or isoflurane between PND 15 and 20 increased dendritic spine density (Briner et al., 2010; De Roo et al., 2009). The observed effect had resolved by PND 30 and was not present if the exposure occurred on PND 30 (De Roo et al., 2009). In a study of dendritic spine development in neonatal hippocampal slice cultures, blockade of synaptic transmission resulted in increased dendritic spine formation between PND 20 and 22, but not at earlier developmental time periods (Kirov et al., 2004). Similarly, propofol exposure in adult neuronal cultures caused neurite retraction that was ameliorated with GABA receptor antagonists (Turina et al., 2008). At least in culture, propofol appears to prevent both chemical and electrical synapse formation (Woodall et al., 2003).

These studies indicate that blockade of neuronal transmission, possibly via GABA receptors, might be partially responsible for alterations in synaptogenesis during early developmental exposure to anesthesia. Although inhibitory in mature neurons, GABA receptor activation results in depolarization and excitation in immature neurons due to increased expression levels of the chloride import channel NKCC1 (Ben-Ari, 2002). The transition from NKCC1 expression to the chloride export channel KCC2 gradually increases in hippocampal slice cultures between PND 5 and PND 14 and is believed to be responsible for the transition of GABA from an excitatory to an inhibitory neurotransmitter (Ben-Ari, 2002; Khirug et al., 2005). Interestingly, the developmental switch between anesthesia-induced synaptic loss versus increased synaptic density corresponds with rodent hippocampal expression of the potassium-chloride co-transporter KCC2 (Briner et al., 2010; De Roo et al., 2009; Khirug et al., 2005; Tan et al., 2009). Therefore, it would be worthwhile testing whether expression of KCC2 and the subsequent transition of GABA to an inhibitory neurotransmitter accounts for age-dependent differences in synaptic density after anesthesia.

3. Gliogenesis

While glial cells account for half of the cellular population within the brain, the importance of glial cells in promoting and maintaining neuronal development has only recently been recognized (Barres, 2008; Zuchero and Barres, 2015). Glial cell proliferation occurs mostly postnatally between the second and third week of life in rodents (Fig. 3, Postnatal Gliogenesis by Brain Region in the Rat). (Bandeira et al., 2009). If periods of cellular proliferation increase susceptibility to anesthesia-induced neurotoxicity, then the contradictory findings between exposures in the first and second weeks of life in rodents may be secondary to glial specific anesthetic toxicity versus the more traditionally considered neuronal toxicity. The major subsets of glial cells will be considered with respect to anesthetic-induced neurotoxicity, including reviewing the periods of developmental vulnerabilities for these cell types.

3.1. Astrocytes

Astrocytes are the largest subset of glial cells in the mammalian brain and like neurons, they undergo temporal and region specific periods of proliferation, differentiation, and migration (Molofsky and Deneen, 2015). Astrocyte proliferation and differentiation occurs during the second postnatal week in rodents (Semple et al., 2013). During this

Intracellular calcium levels in astrocytes change both spontaneously and in response to neuronal activity, resulting in the release of "gliotransmitters" (Parri et al., 2001). Although controversial, calcium induced astrocyte gliotransmitters are believed to regulate vascular smooth muscle and neuronal activity (Bazargani and Attwell, 2016). A single preliminary paper indicates that anesthesia may have significant effect on astrocytic intracellular calcium fluctuations. Mice were exposed to ketamine/xylazine, isoflurane, or urethane followed by calcium imaging. The authors found that all anesthetics suppressed calcium signaling, both spontaneous and induced, and that these changes were uniform throughout the astrocytic soma and process (Thrane et al., 2012). Inhibition of astrocyte calcium signaling could play a role in anesthesia-induced neurotoxicity and requires additional investigation.

Astrocytes also play an important role in promoting and supporting synaptogenesis. Pfieger and Barres showed that the development of functional synapses in neuronal cultures required the presence of astrocytes; cultures without astrocytes formed synapses that lacked the ability to efficiently transmit electrical signals (Pfrieger and Barres, 1997). In addition to their role in synaptogenesis, astroglia also participate in cerebral blood flow regulation and promote neuronal survival in culture (Banker, 1980; Zonta et al., 2003). Abnormalities in astroglial function have been identified in multiple neurodevelopmental disorders, including autism spectrum disorder, Fragile X syndrome, and Rett syndrome (Yang et al., 2013). Therefore, anesthetic-induced changes in astroglial development have the potential to alter synaptogenesis and angiogenesis, interfere with efficient neurotransmission, and lead to neuronal cell loss.

3.2. Oligodendrocytes

The timing of oligodendrocyte proliferation, differentiation and migration closely parallels astrocytes with immature pre-oligodendrocytes appearing during the first postnatal week in rodents and maturing during the first 2 to 3 weeks (Semple et al., 2013). Mature oligodendrocytes initiate myelination of neurons on PND 10 and the process peaks around PND day 20 in rodents (Wiggins, 1986). Due to the high energy demands and the excessive production of ROS secondary to the creation of myelin, oligodendrocytes are extremely sensitive to pathological insults (Bradl and Lassmann, 2010).

Although very little literature exists on anesthesia-induced toxicity in oligodendrocytes, the studies that are available were performed in non-human primates. The authors exposed 6-day old macaques to clinically relevant doses of isoflurane and brains were harvested 3-h post exposure. Histological analysis was significant for apoptotic cells, 52% of which were identified as oligodendrocytes. It was estimated that isoflurane exposure targeted about 6.3% of the total oligodendrocyte population. In addition, the most susceptible oligodendrocytes were those undergoing myelinogenesis (Brambrink et al., 2012). The authors repeated the study with clinical doses of propofol in neonatal macaques and isoflurane in fetal macaques with similar results (Creeley et al., 2013; Creeley et al., 2014). Due to the narrow window during which myelination occurs and the large percentage of total oligodendrocytes lost, the findings could have a profound impact on later cognitive function (Jiang et al., 2015). Unfortunately, the authors did not follow up with additional studies evaluating for long-term effects on neuronal myelination or the adult oligodendrocyte population; questions that require additional investigation in the future.

3.3. Ependymal cells

Very little has been published on either the developmental role of ependymal cells or whether the developmental function of these cells is altered after exposure to anesthesia. Ependymal cells localize to the cerebral ventricles and spinal canal, where they differentiate and proliferate during the embryonic and early postnatal period (Bruni, 1998). A single study published in 2012 using an ex vivo brain slice model found
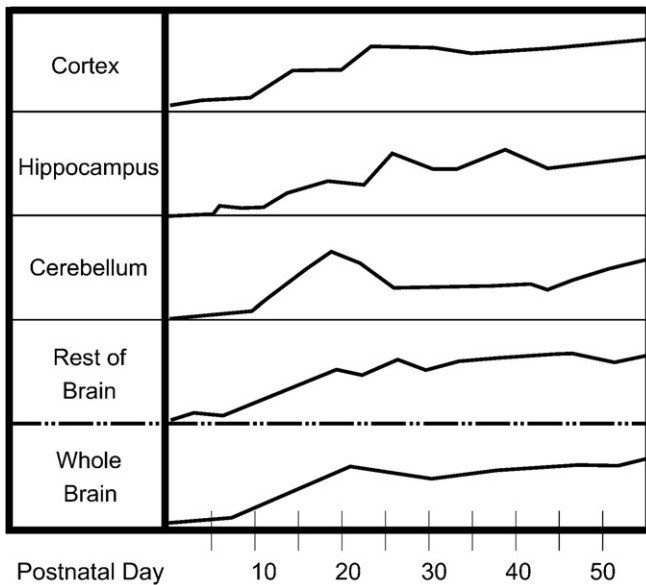

Fig. 3. Postnatal gliogenesis by brain region in the rat: graphical representation of relative number of total glial cells at different time points within individual brain regions across the first 2 postnatal months of life. At birth, the total number of glial cells remains small. Gliogenesis begins in earnest during the second postnatal week in most brain regions and continues throughout the first month of life.
(Graph adapted from Bandeira et al., 2009.)

time, astrocytes migrate from the lateral ventricles radially and into the neocortex (Marshall et al., 2003). In the rat hippocampus, PND 7 astrocytes are heterogeneous in appearance, lacking their characteristic processes (Bushong et al., 2004). Over the next 3 weeks, individual astrocytes upregulate Glial Fibrillary Acidic Protein (GFAP) expression, extend multiple fine processes, and establish connections with thousands of neuronal synapses (Bushong et al., 2004).

Immature and mature astrocytes are not just morphologically dichotomous; they also have functional differences as well. Injection of immature cultured astrocytes from kittens into the brains of adult cats restores optical plasticity, including axonal sprouting and removal of nonfunctional synapses (Muller and Best, 1989). Molecular analysis of immature versus mature astrocytes identified multiple receptors that are not expressed until PND 21 through 28, including the Glutamate Type 1 Transporter (GLT1) and the Glutamate and Aspartate Transporter (GLAST); although actual levels varied dramatically based on specific brain region (Furuta et al., 1997).

Multiple studies have shown that anesthetic exposure alters normal astrocyte development in the early postnatal period. Immature astrocytes exposed to 3% isoflurane for 24 h in tissue culture exhibited changes in morphology resembling delayed astrocyte maturation (Lunardi et al., 2011). This effect was specific to immature astrocytes; isoflurane had no visible affect on mature astrocyte cultures. The authors felt these changes were due to alterations in actin cytoskeleton development (Lunardi et al., 2011). Similar work with sevoflurane exposure in tissue culture and PND 7 rats, demonstrated decreased expression of GFAP and GLAST that lasted up to 2-weeks post treatment. In addition, the authors reported decreased total numbers of astrocytes (Wang et al., 2016). It was felt these changes represented delayed astrocytic maturation and decreased proliferation after sevoflurane exposure (Wang et al., 2016). Sevoflurane exposure has been shown to delay and decrease the extent of astroglial activation in an adult rat intracerebral hemorrhage model as well (Karwacki et al., 2005). Similarly, astroglia exposed to isoflurane and then co-cultured with naïve neurons were unable to promote and support normal axonal outgrowth (Ryu et al., 2014). These studies indicate that inhalational anesthetic exposure delays astrocyte maturation and function during a critical period of brain development.

that halothane exposure decreased the rate of ciliary beat in ependymal cells (O'Callaghan and Sikand, 2012).

Perhaps a more compelling area of investigation with respect to anesthesia-induced neurotoxicity involves the role of ependymal cells and tight junctions in maintaining the cerebrospinal fluid (CSF) and brain parenchymal barrier (Jimenez et al., 2014). This barrier controls the flow of chemicals between the CSF and brain. Prior studies have demonstrated that isoflurane exposure in adult rats down regulates expression of occludin with resulting impairment in tight junction function (Cao et al., 2015). This raises the question of whether exposure to anesthesia during early development has negative implications for maintaining the CSF/brain parenchymal barrier.

3.4. Microglia

Microglia, in comparison to the other glial cells, are unique in their origin and development. Derived from the yolk sac, microglial progenitors appear around embryonic day 9 in rodents, but migration and proliferation continues through the third postnatal week (Semple et al., 2013). Microglial differentiation is characterized by a distinct morphological transition from an amoeboid shaped cell to the traditional ramified microglia during the first 3 postnatal weeks (Orlowski et al., 2003; Perez-Pouchoulen et al., 2015). At the end of development, microglia constitute about 10 to 15% of all glial cells and serve as the resident immune cells in the brain (Nayak et al., 2014). Microglia appear to perform multiple functional roles during early development, including promoting both neuronal survival and apoptosis, phagocytic clearance of apoptotic neurons, and supporting synaptogenesis (Nayak et al., 2014). Dysfunctional microglia during development, with resulting changes in neurogenesis, neuronal apoptosis, and failed synaptogenesis, have been linked to multiple developmental diseases, including autism and schizophrenia (Edmonson et al., 2016; Inta et al., 2016). The shared pathology between developmental disorders like autism and anesthesia-induced neurotoxicity highlight the need for future studies into microglial function after anesthetic exposure.

In addition, microglia are believed to be the major source of pro-inflammatory cytokines within the brain. Inflammatory responses, likely mediated through microglia, are believed to play a key role in many neurodevelopmental diseases (Amor et al., 2010; Edmonson et al., 2016). Inhalational exposure has been associated with increased expression levels of multiple pro-inflammatory cytokines. Isoflurane exposure in adult mice has been shown to upregulate TNFα, IL-6, and IL-1β (Wu et al., 2012). Interestingly, the majority of cells positive for TNFα were identified as neurons by immunohistochemistry (Wu et al., 2012). Sevoflurane exposure in PND 6 mice resulted in cognitive impairment and increased expression of IL-6 and TNFα in the brain (Shen et al., 2013a). Similarly, sevoflurane exposure on PND 6 in Alzheimer's Disease transgenic mice upregulated TNFα mRNA and protein expression levels (Lu et al., 2010). Isoflurane in combination with nitrous oxide produced increased levels of apoptosis and IL-1β expression in 7-day old rat pups after nociceptive pain activation via formalin paw injections (Shu et al., 2012). In contrast, lipopolysaccharide (LPS) induced expression of IL-1β by microglia in cell culture was inhibited by isoflurane (Tanaka et al., 2013). During a second study, the addition of sevoflurane or isoflurane to microglia BV-2 cultured cells stimulated with LPS had minimal effect on cytokine responses, but the addition of propofol decreased LPS induced expression of IL-6, TNFα, and IL-1β (Ye et al., 2013). The effect of anesthesia on microglia, both as mediators of neuroinflammation as well as their developmental roles in apoptosis, neurogenesis and synaptogenesis require future additional studies.

4. Blood brain barrier and cerebrovasculature

The cerebrovasculature is necessary for successful brain development, but its role in anesthesia induced neurotoxicity remains unexplored. During development, the growing cerebrovasculature closely matches neuronal growth to ensure the resulting blood supply can meet neuronal demands for oxygen and energy (Lacoste et al., 2014). The resulting neurovascular unit is made up of neurons, astrocytes, pericytes and endothelial cells that together guide brain vessel growth (Hawkins and Davis, 2005). Vascular plasticity has been demonstrated in the murine barrel cortex, where vascular density steadily increases until PND 14 (Lacoste et al., 2014). During this time period, the degree of angiogenesis is dictated by the overall neuronal activity (Lacoste et al., 2014). The direct mechanism controlling vascular plasticity in response to neuronal activity is unknown, but possible targets include astrocytes and secretion of vascular growth factors. As discussed above, there is significant evidence that anesthesia modifies astrocytic function, but no studies currently explore the subsequent effect this has on vascular plasticity. As for vascular growth factors, one potential target is Vascular Endothelial Growth Factor (VEGF). Clinical studies have shown that VEGF expression levels decrease after exposure to nitrous oxide during urological surgery (Hakimoglu et al., 2014). Therefore, future studies investigating changes in vascular plasticity during early developmental exposure to anesthesia are needed.

Alterations in cerebral blood flow versus cerebral oxygen demand during anesthesia also have the potential to contribute to neurotoxicity. Under normal, non-anesthetized conditions, cerebral blood flow maintains a linear relationship with cerebral oxygen demand, a marker for neuronal activity (Hoge et al., 1999). Anesthetics have varying effects on this relationship, but in adults, sevoflurane has been shown to reduce cerebral blood flow disproportionately to cerebral oxygen demand, resulting in a significantly decreased oxygen extraction fraction and indicating possible metabolic abnormalities (Kaisti et al., 2003). This may be further compounded in infants and children where Magnetic Resonance Imaging has demonstrated a negative value for blood oxygen level-dependent (BOLD) signals in visually stimulated children and rats under sedation (Born et al., 2002; Kozberg et al., 2013). These studies did not attempt to evaluate the relationship between anesthesia and BOLD signaling, but they may indicate that oxygen consumption increases disproportionately with respect to blood flow in children compared to adults (Born et al., 2002; Kozberg et al., 2013). This in combination with the altered flow-metabolism coupling of sevoflurane raises concerns about meeting oxygen extraction requirements in neonates exposed to anesthesia during key periods of neuronal development. Cerebral blood flow is believed to be regulated by the neurovascular unit, especially neurons and astrocytes, and glutamate may be an important neurotransmitter in this process (Attwell et al., 2010; Bandopadhyay et al., 2001). The loss of blood flow/neuronal activity coupling in neonates and the possible effect of anesthesia on the cellular regulators of blood flow (as described in the sections above) dictate the need for additional investigation in this area.

The blood brain barrier (BBB) is an important component of maintaining normal brain homeostasis and damage to the barrier has the potential to prevent normal synaptogenesis, angiogenesis, neurogenesis and synaptic transmission (Zlokovic, 2008). Isoflurane anesthesia in cats alters blood brain permeability in a dose dependent manner, resulting in increased cerebral brain volumes (Tetrault et al., 2008). Similar studies in rats exposed to isoflurane produced BBB dysfunction with decreased levels of the tight junction protein occludin (Cao et al., 2015). Taken together, these studies provide evidence that BBB dysfunction may also contribute to anesthesia-induced neurotoxicity.

5. Conclusion

Despite extensive literature to support the belief that exposure to anesthesia early in development alters the normal function of multiple cellular components in the brain, the clinical implications of these findings remain uncertain. Retrospective clinical studies were relatively inconclusive, with some, but not all studies, supporting findings of cognitive changes after early childhood exposure to anesthesia (Andropoulos et al., 2014; Bartels et al., 2009; DiMaggio et al., 2009;
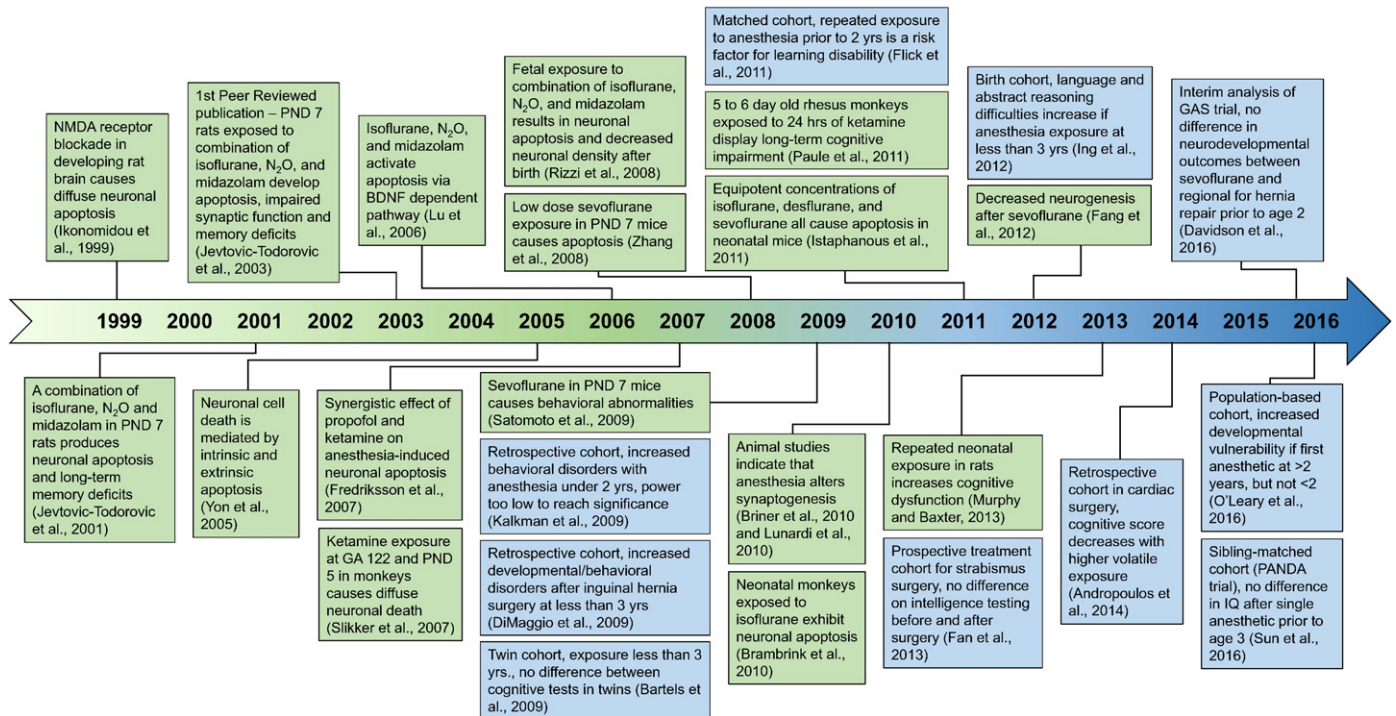
Fig. 4. A timeline - 15 years of anesthetic neurotoxicity: over the last 15 years, the number of animal models supporting anesthesia-induced neurotoxicity has rapidly increased (green). Year 2009 marked a shift in the literature towards increasing clinical trials in pediatrics (blue). Abbreviations: NMDA N-methyl-D-aspartate receptor, N₂O Nitrous oxide, PND Postnatal day, GA Gestational age, yrs Years, hrs Hours, GAS General Anesthesia compared to Spinal Anesthesia Trial, PANDA Pediatric Anesthesia & Neurodevelopment Assessment.

Fan et al., 2013; Flick et al., 2011; Graham et al., 2016; Ing et al., 2012; Kalkman et al., 2009; O'Leary et al., 2016; Sprung et al., 2009; Sun et al., 2016; Wilder et al., 2009) (see Fig. 4, A Timeline - 15 Years of Anesthetic Neurotoxicity). This debate has prompted a few, rigorously designed clinical trials, many of which are just now starting to publish their results. This past fall, the General Anesthesia compared to Spinal (GAS) randomized control trial released the results of its 2-year interim analysis, stating … "we found no evidence that just less than 1 h[our] of sevoflurane anesthesia in infancy increases the risk of adverse neurodevelopmental outcome at 2 years of age compared with awake-regional anesthesia" (Davidson et al., 2016). And while final results will not be available until the 5-year mark, we would argue that, even with completion of these trials, the clinical significance of anesthesia-induced neurotoxicity will be undecided.

We have attempted to make evident through this review, that anesthesia-induced neurotoxicity is likely secondary to a compilation of multiple individual effects, instead of one single pathological mechanism. This is probably best conceptualized by reviewing the many therapeutic agents currently described in animal models, each with a drastically different physiological target, but many of which appear to ameliorate the effects of early developmental exposure to anesthesia (Lei et al., 2012; Zhou and Ma, 2014). Like the "Double Hit Hypothesis" in cancer, anesthesia induced-neurotoxicity is born of a perfect storm that combines all the cellular changes of brain development after anesthetic exposure. By embracing an increasingly holistic view of anesthesia-induced neurotoxicity we will have the ability to better understand its clinical implications. Most importantly, this approach will require incorporating not just the combined effects of anesthesia on all cellular brain components, but also the implications of those effects on both healthy subjects and those with significant preexisting medical conditions. Only then will we truly be able to answer the question of whether anesthesia-induced neurotoxicity is a real phenomenon, not just for healthy children receiving minor procedures, but also in the children at highest risk, especially those with pre-existing neurodevelopmental abnormalities.

Transparency document

The Transparency document associated with this article can be found, in online version.

Acknowledgements

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