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Neurodevelopmental Implications of the General Anesthesia in Neonate and Infants

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Abstract

Each year, about six million children, including 1.5 million infants, in the United States undergo surgery with general anesthesia, often requiring repeated exposures. However, a crucial question remains of whether neonatal anesthetics are safe for the developing central nervous system (CNS). General anesthesia encompasses the administration of agents that induce analgesic, sedative, and muscle relaxant effects. Although the mechanisms of action of general anesthetics are still not completely understood, recent data have suggested that anesthetics primarily modulate two major neurotransmitter receptor groups, either by inhibiting N-methyl-D-aspartic acid (NMDA) receptors, or conversely by activating γ-aminobutyric acid (GABA) receptors. Both of these mechanisms result in the same effect of inhibiting excitatory activity of neurons. In developing brains, which are more sensitive to disruptions in activity-dependent plasticity, this transient inhibition may have longterm neurodevelopmental consequences. Accumulating reports from preclinical studies show that anesthetics in neonates cause cellular toxicity including apoptosis and neurodegeneration in the developing brain. Importantly, animal and clinical studies indicate that exposure to general anesthetics may affect CNS development, resulting in long-lasting cognitive and behavioral deficiencies, such as learning and memory deficits, as well as abnormalities in social memory and social activity. While the casual relationship between cellular toxicity and neurological impairments is still not clear, recent reports in animal experiments showed that anesthetics in neonates can affect neurogenesis, which could be a possible mechanism underlying the chronic effect of anesthetics. Understanding the cellular and molecular mechanisms of anesthetic effects will help to define the scope of the problem in humans and may lead to preventive and therapeutic strategies. Therefore, in this review, we summarize the current evidence on neonatal anesthetic effects in the developmental CNS and discuss how factors influencing these processes can be translated into new therapeutic strategies.

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Keywords

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2. Introduction

In medicine, pain is one of the most primary symptoms to avoid or treat. Whether the therapeutic approach involves opioids, non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, or in extreme cases, general anesthesia, the minimization of pain is always one of top priorities. This is the case both in humans and in animal models, in which the Institutional Animal Care and Use Committee (IACUC) explicitly requires the maximum reduction of pain in surgeries (Koch, 2003). It is not surprising that anesthesia has become such a prevalent component of the field of medicine, with a dedicated subset of specialists whose area of expertise is the administering and monitoring of anesthesia. In developed nations, general anesthesia comes standard with surgeries for both children and adult patients, although the decision to administer anesthesia to neonates remains contentious and situational. At least for adults, the complications are usually very minor, including nausea and vomiting in a small subset of patients, and mortalities only occur in 1:100,000 cases (Jenkins and Baker, 2003). The complications are far outweighed by the benefits of anesthesia, which spans beyond just analgesic effects. Anesthesia also encompasses sedation and muscle relaxation, which are both highly relevant both in and outside the realm of surgery. In neonates, the anesthetic effects may have long-term neurodevelopmental sequelae, which is the primary focus of this review.

Newborns receive anesthesia for a variety of reasons. Sometimes neonates need to undergo hernia repair or open chest surgery to fix congenital heart defects or pulmonary defects (Menghraj, 2012). Other times, newborn patients need to be anesthetized in order to be immobilized prior to receiving an MRI scan. Nevertheless, general anesthesia is usually reserved as a last resort in neonates. In fact, most neonatal males receiving circumcision surgeries remain unanesthetized. Aside from cost, general anesthesia carries potentially permanent drawbacks. There is an emerging body of evidence that indicates adverse long-term neurological effects of general anesthesia in the young susceptible brain (Flick et al., 2011; Flick et al., 2014; Ing et al., 2014; Sprung et al., 2012; Vutskits et al., 2012; Wilder et al., 2009). It is clear, both in animal models and to an extent through retrospective studies in human patients, that the effects of anesthesia are sensitively correlated with the age of exposure. In terms of behavior deficits, general anesthesia is most detrimental in the two extreme age groups, neonates/infants and the elderly. Both are similar in that they possess nervous systems that are more fragile. The developing brain is primed to undergo apoptosis in order to prune away redundant neurons and establish healthy neural circuitry, while the aged brain faces accelerated neurodegeneration due to senescence and buildup of deleterious byproducts, such as beta-amyloid (Aβ) protein. Both of these two groups are more susceptible to the neurodegenerative insults carried by general anesthesia, leading to widespread apoptotic neurodegeneration and learning impairments that are not otherwise observed in the adult age group (Culley et al., 2003; Erasso et al., 2013; Perouansky and Hemmings, 2009; Stratmann et al., 2009b). Interestingly, the effects are the stark opposite in

the adult brain. Instead of inducing learning deficits, general anesthetics can actually reversibly enhance the learning function of 4–5 month old mice via an upregulation of the N-methyl-D-aspartic acid (NMDA) receptors, which promotes long-term potentiation (Rammes et al., 2009).

It is worth emphasizing that while animal studies have conclusively implicated the neurodegenerative effects related to early general anesthesia exposure, retrospective studies within human patient populations have not been able to conclusively and reproducibly demonstrate similar deficits. Furthermore, general anesthesia still confers many benefits, such as pain relief and muscle relaxation, which in some cases are essential to neonates undergoing surgery or medical imaging. This review aims to objectively compare the current body of evidence available for both animal and human studies in order to provide an updated assessment of the effects of general anesthesia on neurodevelopment, as well as to briefly provide the implications of these studies towards clinical practice.

3. The Mode of Action of General Anesthetics

3.1. Brief history of general anesthetics

The term "anesthesia" was first used in 1846 by Oliver Wendell Holmes, a Greek surgeon, to describe a patient who, after inhaling ether vapor, underwent surgery without any apparent suffering (Kissin, 1997; Nuland, 1989). General anesthesia is a combination of medicines that is inhaled or injected intravenously in order to induce a state of unconsciousness (also termed hypnosis) throughout the whole body (Grasshoff et al., 2005; Mashour et al., 2005). Under anesthesia, general anesthetics bring about a reversible loss of consciousness and loss of pain (analgesia) during the surgery or operative procedure. Additionally, general anesthesia also causes forgetfulness (amnesia), immobility, loss of control of autonomic responses (e.g. reflexes), and relaxation of muscles throughout the body. Volatile anesthetics, such as sevoflurane, isoflurane, and desflurane, induce a similar physiological response in the body as intravenous anesthetics (Sonner et al., 2003). The major differences between volatile and intravenous anesthetics are that volatile anesthetics are inhaled and generally possess a much shorter induction and emergence phase, resulting in faster recovery times. Furthermore, they demonstrate an ease of monitoring, making them the more common anesthetic agent of choice used in clinical practice. This is the case not only in adult patients, but in pediatric patients as well. Volatile anesthetics have been widely selected for induction of anesthesia in pediatric patients and for maintenance of anesthesia in adult patients (Palmer, 2013).

3.2. Mechanism of general anesthetics

Although anesthetics are commonly used in clinical practice, the mechanisms of action for induction of anesthesia are still not completely understood. The earliest theory for anesthetic mechanism is the lipid solubility/anesthetic potency (Meyer-Overton) correlation, in which the anesthetic potency of general anesthetics strongly correlates with their solubility in a solvent representing the hydrophobic interior of the cell membrane (Rudolph and Antkowiak, 2004). However, recent findings have suggested that there are the exceptions to the rule. For example, non-immobilizers with similar chemical structures and lipid solubility

didn't show general anesthetic properties (Koblin et al., 1994). In addition, enantiomers with the same solubility exert different anesthetic potencies (Franks and Lieb, 1991). These results have suggested that other mechanisms of action should be studied to provide a more thorough explanation for the induction of anesthetics.

In the last decade, the other promising mechanism for induction of anesthetics is the effect of anesthesia on neurotransmission in the central nervous system. Previous data have shown that two major neurotransmitter receptor classes are involved in induction of anesthetics. One is the glutamate receptor, NMDA receptor, which is known to mediate excitatory signals, while the other is the γ-aminobutyric acid (GABA) receptor, which is known to transmit inhibitory signals (Cechova and Zuo, 2006; Olsen and Li, 2011). Most classes of general anesthetics, such as volatile anesthetics and benzodiazepines and barbiturates, act by enhancing GABA receptor activity, in particular $GABA_A$ receptor. Specific sites (such as S270) of $GABA_A$ receptors can be a crucial factor to induce the anesthetics-mediated $GABA_A$ receptor activity (Nishikawa and Harrison, 2003). Additionally, $GABA_A$ receptor antagonists were able to reverse the effects of anesthesia, indicating that the $GABA_A$ receptor is a key receptor for the mechanism of action of anesthesia induction (Nelson et al., 2002).

$GABA_A$ receptor enhancement, however, explains only half of the story of general anesthesia. Evidence has proposed that NMDA receptors are also involved in the induction of anesthetics. Certain classes of general anesthetics, such as ketamine, can act as NMDA receptor antagonists (Arhem et al., 2003; Chiao and Zuo, 2014). The knockout or blockade of NMDA receptors reduced the anesthetic potency after exposure to nitrous oxide or ketamine (Harrison and Simmonds, 1985; Sato et al., 2005). Recent reports have shown that voltage-gated channels and neurotransmitter transporters can also be important factors for induction of anesthetics (Arhem et al., 2003; Lee et al., 2010; Sonner et al., 2003). Physiological factors such as sex and weight may additionally impact drug sensitivities and metabolism related to anesthesia induction (Mawhinney et al., 2013).

3.3. General anesthesia in neonates

Neonates undergoing a wide variety of procedures may require general anesthesia. There are two main considerations in the selection of general anesthetics: 1) hemodynamic control – generally volatile anesthetics or propofol are the preferred agents of choice, but if resuscitation is required, ketamine and opiates may be selected in order to protect cardiac output, and 2) postoperative pain relief, in which a combined regiment of regional and general anesthetics would be optimal. Volatile anesthetics, propofol, and ketamine are agents that are commonly administered to neonates. Propofol is a short-acting hypnotic agent that is administered intravenously and can be used for both induction and maintenance of general anesthesia. Propofol has largely supplanted the barbiturate sodium thiopental as a drug of choice for induction of anesthesia, due to its extremely rapid induction and recovery times. Benzodiazepines, such as midazolam and diazepam, are psychoactive drugs that are commonly used as anti-epileptics and anxiolytics, but are also used for their sedative purposes for anesthesia. Their primary mechanism of action is enhancement of $GABA_A$ receptor activity. Volatile anesthetics, such as isoflurane, desflurane, and sevoflurane, are

inhalable agents that are commonly used amongst neonates and infants given their rapid induction and recovery times. They have been extensively studied in both retrospective analyses and animal models in order to assess its effects on the neuroapoptosis within the developing brain, and whether this leads to future long-term cognitive and learning deficits. Ketamine is commonly used for induction and maintenance of anesthesia, as well as for post-operative pain. Its primary mechanism of action is through noncompetitive antagonism of NMDA receptors, but it also acts weakly on opioid receptors (Kohrs and Durieux, 1998).

4. Differences between the Adult and Neonate Brain

4.1. The developing brain

The developing brain is in a dynamic state of establishing and strengthening neural connections; most of this neural network assembly occurs via activity-dependent mechanisms. The neural activity during periods of maximal synaptogenesis and neuronal pruning may contribute to the neural network organization (Ikonomidou et al., 1999; Poo, 2001). This process is especially prevalent during the developmental window in which axons are growing and finding appropriate targets, and corresponding synapses are being formed, including the late gestational phase to the early neonatal phase. The time points of these windows are different across species. In rodents, such as mice and rats, this range includes the late embryological phase until postnatal day 10–17 (P10–17) (Petit et al., 1988). In nonhuman primates, such as macaques, the most critical time window is from just before birth until around P5–7 (Malkova et al., 2006). In humans, this can range from late gestation until ~3 years of life (Dobbing and Sands, 1978). Even afterwards, children's brains are still susceptible because of the ongoing synaptic plasticity during learning and memory formation, although the level of plasticity is not as high as during the first 2 years of life. Anesthetic agents interrupt this activity mainly through either N-methyl-D-aspartate (NMDA) glutamate receptor blockade or enhancement of the inhibitory gamma-aminobutyric acid (GABA$_A$) receptor. As a result, the anomalous disruption of the neural activity causes widespread neuroapoptosis and chronic impairments in synaptogenesis later in life (Ikonomidou et al., 1999; Perouansky and Hemmings, 2009).

4.2. Cardiovascular limitations

Aside from the developing nervous system, there are major differences between neonates/infants and adults in other organ systems as well that are indirectly connected to brain function and development. The cardiovascular system is a prime example. In the adult heart, the cardiac output is highly flexible to adjust for changes in both the blood supply and energy demand of the body. The output can be increased by either intensifying heart contractility or accelerating heart rate. In the developing heart, however, the modulation of cardiac output is not as multimodal, due to the lower ventricular compliance and limited contractility of the neonatal heart (Barash, 2009). As a result, neonates depend more on the heart rate in order to modulate their cardiac output. This physiological difference makes neonates more susceptible to the global systemic depressant effects of general anesthesia. Newborns have more difficulties in compensating for the hypoxemia-induced slowed heart rate, or bradycardia, following general anesthesia induction. This could further exacerbate the neurotoxic effects associated with anesthesia, because the brain requires

disproportionately more oxygenation than the rest of the body, and the sudden and prolonged reduction in perfusion may create adverse long-term effects on the developing brain.

4.3. Minimal Alveolar Concentration (MAC)

The MAC is an important consideration when administering inhalational anesthetics. The MAC is the equivalent of the anesthetic "potency" and is defined as the minimum concentration of anesthesia in the lung alveoli that will prevent movement in 50% of patients. There are many factors affecting MAC, including gender, age, and weight. In the extremely young age range, however, the MAC is exquisitely sensitive to age. In general, neonates have a lower MAC requirement than that of infants (Barash, 2009). In premature babies, the MAC is even lower (LeDez and Lerman, 1987). Because minimizing the amount and duration of anesthesia is a priority in neonates/infants, understanding the MAC at various ages is essential and will contribute to reducing neurodevelopmental delays or damage.

5. Neuroprotection and Cytotoxicity in Neonatal Anesthesia

5.1 Neuroprotective effects of neonatal anesthesia

In previous reports from adult animals and patients, more and more data have shown that general anesthetics have a neuroprotective effect through prevention or reduction of apoptosis, neurodegeneration, traumatic brain injury, and ischemic injury (Burchell et al., 2013; Schifilliti et al., 2010; Wells et al., 1963; Yokobori et al., 2013; Yu et al., 2010). Sevoflurane preconditioning protects neurons and blood-brain-barrier against brain ischemia (Anrather and Hallenbeck, 2013; Gidday, 2010; Wang et al., 2011; Yu et al., 2011). Isoflurane administration shows a strong neuroprotective effect in various adult stroke models including subarachnoid hemorrhage or middle cerebral artery occlusion (MCAo), although there were controversial data about the long-lasting effects (Altay et al., 2012; Kawaguchi et al., 2000; Sakai et al., 2007). The neuroprotective effect occurs both when anesthetics are administered before (preconditioning) or after stroke (postconditioning) (Anrather and Hallenbeck, 2013; Chen et al., 2011; Dezfulian et al., 2013).

However, in the neonatal brain, most papers suggest that only preconditioning administration of anesthetics, but not postconditioning, shows a neuroprotective effect in experimental strokes. In neonatal rats, isoflurane (1–1.5%) preconditioning (24 hr before stroke) exhibited a reduction in brain tissue loss via inhibition of nitric oxide synthase (NOS) or activation of P38 mitogen-activated protein kinases (Dobbing and Sands, 1973; Zhao and Zuo, 2004; Zheng and Zuo, 2004). Consistent with this result, another study showed that preconditioning with 1.5% isoflurane not only reduced brain mass but also improved motor function after stroke (Zhao et al., 2007). On the other hand, only one paper showed that prolonged exposure to Isoflurane ameliorated infarction severity in the rat pup model of neonatal hypoxia-ischemia (Chen et al., 2011). Although there have been minimal investigations into neuroprotection of general anesthetics in human pediatric population, many clinicians have accepted the data supporting beneficial effects of anesthetic

preconditioning in stroke and heart attacks, and they are beginning to implement these strategies in the clinics for the pediatric population.

Various mechanisms have been proposed for the neuroprotection induced by anesthetic agents. For one, anesthetics reduce metabolic rate, resulting in better tolerance to ischemia by allowing for more efficient use of available oxygen and nutrients (Warner, 2000). In addition, anesthetics cause inhibition of glutamate receptors, modulation of calcium concentration, activation of mitochondrial adenosine-5′-triphosphate (ATP) sensitive potassium channels, and preservation of calcium/calmodulin dependent protein kinase II (CaMKII) levels (Bickler and Fahlman, 2006; Jevtovic-Todorovic et al., 1998; McMurtrey and Zuo, 2010).

5.2. Cytotoxic effects of neonatal anesthesia

Based on recently published papers, it is well-accepted that general anesthetics such as isoflurane, sevoflurane, ketamine, and propofol in neonates can lead to significant degeneration of developing neurons in various animals, including mice, rats, and non-human primates (Fredriksson et al., 2004; Fredriksson et al., 2007; Jevtovic-Todorovic et al., 2003; Loepke et al., 2009; Lu et al., 2010; Pesic et al., 2009; Rizzi et al., 2008; Satomoto et al., 2009; Shen et al., 2013; Slikker et al., 2007; Tao et al., 2014; Yon et al., 2005; Young et al., 2005; Zhang et al., 2014). Although the patterns of neonatal anesthesia-induced neurodegeneration are not consistently identified, the vulnerable areas have been implicated. They may include the primary visual cortex, temporal/somatosensory cortices, frontal cortex, and hippocampus (Brambrink et al., 2010; Rothstein et al., 2008). In the following sections, we will summarize the possible mechanisms of anesthesia-induced impairments of neuronal development.

5.2.1. NMDA and GABA in neonatal anesthesia toxicity—Given their critical roles in both neurodevelopment and the mechanism of action of general anesthesia, we will first review neonatal neurotoxicity related to GABA and NMDA receptors. As we mentioned above, GABA receptors and NMDA glutamate receptors are the major inhibitory and excitatory receptors, respectively. Previous findings have shown that some anesthetics such as ketamine are antagonists of the NMDA receptor (Liu and Sharp, 2012; Olney, 2002). Ketamine exposure caused a significant increase of NR1 expression and neuronal cell death in perinatal rhesus monkeys (Slikker et al., 2007). In rat pups, exposure to multiple injections of ketamine caused neuronal cell death in the frontal cortex and increased NR1 mRNA and proteins (Slikker et al., 2007). These findings suggests that antagonism of NMDA receptors may induce compensatory response such as upregulated NR1 expression and increased glutamate level during development, consequently leading to neuronal damage due to excitotoxicity. To further corroborate this theory, another study found that repeated exposure to isoflurane, a volatile anesthetic that enhances GABA$_A$ activity, during the neurodevelopmental stage increased glutamate levels in the posterior cortex, suggesting a compensatory response (Kulak et al., 2010).

During development, NMDA receptors play key roles in the establishment and maturation of synapses (Colonnese et al., 2005). Another report demonstrated that MK-801, another

common antagonist of NMDA receptors, triggered widespread apoptotic neurodegeneration in the developing rat brain, suggesting that a tonic basal activity of the NMDA receptor and appropriate Ca^{2+} homeostasis are essential for neuronal survival in the developing brain (Ikonomidou et al., 1999). Taken together, these findings suggest that the anesthesia-induced neurodegeneration might be in part due to inhibition of NMDA receptor, especially during the critical stage in neonates in which activity-dependent synaptic plasticity mediates neuronal survival (Habas et al., 2006; Hack et al., 1993; Jiang et al., 2005; Lafon-Cazal et al., 2002).

GABA is typically an inhibitory neurotransmitter. However, in the immature nervous system of neonates, its properties are reversed and it acts as an excitatory neurotransmitter. Generally, $GABA_A$ receptor activation leads to an influx of Cl^- into the cell, resulting in hyperpolarization. As mentioned above, the hyperpolarization via the regulation of $GABA_A$ receptor activation can lead to neuroprotection in stroke model. However, in the developing brain, activation of $GABA_A$ receptor results in Cl^- efflux and depolarization of the neuron due to high intracellular concentration of Cl^-. General anesthetics such as propofol and benzodiazepines can act as $GABA_A$ receptor agonist (Franks and Lieb, 1994). Consequently, $GABA_A$ receptor agonism leads to an increase of calcium concentration following excess depolarization in developmental stage, resulting in excitotoxicity-induced neurodegeneration.

5.2.2. Oxidative stress and mitochondrial damage in neonatal anesthesia toxicity—Another possible mechanism of anesthetic-induced neurotoxicity in neonates is an excess production of reactive oxygen species (ROS). It was recently reported that anesthesia containing a sedative dose of midazolam followed by a combination of nitrous oxide and isoflurane administration causes 30% upregulation of ROS, accompanied by a downregulation of the scavenging enzyme superoxide dismutase (Boscolo et al., 2013). An imbalance between mitochondrial fission with dynamin-related protein 1 (Drp-1) and fusion with mitofusin-2 (Mfn-2) leads to disturbed mitochondrial morphogenesis and then neurodegeneration. In addition, the impaired mitochondrial morphogenesis lead to increased autophagy activity, decreased mitochondrial density, and long-lasting disturbances in inhibitory synaptic neurotransmission (Sanchez et al., 2011). It was suggested that dysfunction of complex IV in the electron transport chain causes electron leakage from the mitochondria, thereby increasing ROS production, which reacts with macromolecules, including DNA, lipids, and protein, causing cellular injury. In another animal study, the synthetic ROS scavenger, EUK-134, effectively inhibits anesthesia-induced ROS production, resulting in decreased neurodegeneration associated with neonatal anesthetic exposure (Boscolo et al., 2012). Also, it was reported that exposure to sevoflurane (3%) for 6 hr increased cleaved caspase-3 and PARP, markers of apoptotic cell death, and 4-hydroxy-2-nonenal, a marker of lipid peroxidation and oxidative stress (Yonamine et al., 2013). A co-administration of 1.3% hydrogen gas and sevoflurane significantly reduced oxidative stress, resulting in reduced neurodegeneration. These results suggest that neonatal anesthetic exposure could lead to cellular damages through impaired mitochondrial morphogenesis, integrity, and function, leading to induction of oxidative stress during

neurodevelopment, suggesting that mitochondrial impairment and subsequent ROS release may be key factors in anesthesia-induced neurodegeneration in the developing brain.

5.2.3. Inflammation in neonatal anesthesia toxicity—Inflammation may be a critical response in various brain pathologies (Chen et al., 2014b; Hall and Pennypacker, 2010; Hosaka and Hoh, 2014; Zhou et al., 2014). However, a relationship between neonatal inflammation and anesthesia has still not been thoroughly investigated. Accumulating data reveals that the commonly used anesthetic agents can induce neuroinflammation. However, although recent report showed that 3% sevoflurane for 2 h daily for 3 days induced cognitive impairment and neuroinflammation, most of studies were carried out in adult (Shen et al., 2013; Wu et al., 2012). In adult animals, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-1β, the proinflammatory cytokines, are upregulated by isoflurane exposures. Furthermore, isoflurane induces activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) through enhancement of NF-κB transcription. Interestingly, the isoflurane-induced NF-κB activation is cell-type dependent, occurring in microglia, but not neurons, indicating that isoflurane specifically targets microglia to induce inflammation (Zhang et al., 2013). Administration of isoflurane has been shown to increase the expression of the pro-inflammatory cytokine, IL-1β, in the cortex of rat pups (Lu et al., 2010). Sevoflurane, another volatile anesthetic, increases TNF-α levels, caspase activation, apoptosis, tau phosphorylation, glycogen synthase kinase 3β activation, and Aβ protein levels in an Alzheimer's disease model of transgenic neonatal mice (Dong et al., 2009; Jiang and Jiang, 2015; Tao et al., 2014). Moreover, mice subjected to formalin-induced nociceptive pain along with prolonged anesthesia exposure produced significantly more apoptosis than prolonged anesthesia alone in neonates (Shu et al., 2012). However, some reports did not observe noticeable inflammation associated with anesthetics (El Azab et al., 2002; Helmy and Al-Attiyah, 2000; Lahat et al., 1992; Schneemilch and Bank, 2001). Although some of the data remain controversial, these findings should facilitate future studies of the potential effects of anesthetics on neuroinflammation in neonates, as well as the underlying mechanisms. Also, it remains to be determined if the inflammation is due to surgery alone or due to both surgery and anesthesia.

5.2.4. Calcium imbalance in neonatal anesthesia toxicity—Imbalance of calcium homeostasis can induce neuronal degeneration (Song and Yu, 2014). It is well known that preservation of Ca^{2+} is an important factor in maintenance and stability of cytoskeletal components, synapse formation, and production of neurotransmitters. Previous reports have shown that Ca^{2+} modulators such as MK-801, BAPTA (a Ca^{2+} chelator), and thapsigargin (an endoplasmic reticulum (ER) Ca^{2+}-ATPase inhibitor) could lead to disruption of Ca^{2+} homeostasis, impacting on neuronal maturation (e.g. growth cone expansion, neurite length, and neurite complexity) (Ringler et al., 2008). In addition, the elevated neuronal cell death induced by ketamine may involve a compensatory upregulation of NMDA receptor subunits and subsequent overactivation of the glutamatergic system (Liu et al., 2011; Slikker et al., 2007; Wang et al., 2005). Increased glutamate release leads to greater intracellular Ca^{2+} levels in the neurons, resulting in excitotoxicity-mediated neuronal death (Choi, 1994; Ramanantsoa et al., 2013). Although there are a few reports in neonates, studies in adult have shown that volatile anesthetics including isoflurane and sevoflurane could activate

Ca^{2+} ATPase by increasing membrane fluidity, inhibiting multiple voltage-gated Ca^{2+} calcium currents, and inducing significant release of Ca^{2+} from the ER, which increases cytosolic and mitochondrial Ca^{2+}, all of which contributes to neuronal degeneration (Study, 1994; Yang et al., 2008; Zhang et al., 2008a).

In developing brains, the activation of $GABA_A$ receptor can depolarize the membrane potential, resulting in Ca^{2+} influx and enhance the current from $GABA_A$ receptor-mediated voltage-dependent Ca^{2+} channels (Allene and Cossart, 2010). Isoflurane leads to increase Ca^{2+} level by increasing $GABA_A$ receptor activity, resulting in activated caspase-3 levels and increased neuronal degeneration in the immature hippocampal pyramidal neurons (Zhao et al., 2011). In addition, isoflurane also increased cytosolic Ca^{2+} released from the ER via activation of inositol triphosphate (IP3), a downstream signal of GABA receptors (Wei et al., 2007). Taken together, these observations suggest that anesthesia-mediated calcium imbalance is an ionic mechanism underlying neonatal anesthesia toxicity.

5.2.5. Effect of general anesthesia on non-neuronal cells—While its neurotoxic effects on neurons in the cortex and hippocampus have been well-characterized, general anesthesia also impairs other cell types aside from neurons, such as oligodendrocytes and astrocytes (Brambrink et al., 2012; Culley et al., 2013; Ryu et al., 2014). Of these cells, astrocytes are particularly important as supportive cells for immature neurons during development. For one, they help release neurotrophins such as brain-derived neurotrophic factor (BDNF) that promote neuronal survival and synaptic strengthening. Furthermore, astrocytes provide structural support to mediate neuronal migration and axon growth. An interference of astrocyte function would result in abnormalities with neuronal maturation and neural circuitry assembly. Recent studies have shown astrocytes are not immune to anesthetic agents. Administration of isoflurane into co-cultures of astrocytes and neurons resulted in 30% reduction in axon growth, possibly as a result of decreased BDNF release (Brambrink et al., 2012). Furthermore, isoflurane disrupts the cytoskeletal network of astrocytes, which could also contribute to its dysfunction during brain development (Culley et al., 2013). Isoflurane also may impede axon growth and guidance, possibly through a $GABA_A$ receptor mediated mechanism (Mintz et al., 2013). Because many of the other anesthetics act through an almost identical mechanism of enhancing the activity of $GABA_A$ receptor, this finding may be extrapolated into other agents as well, such as barbiturates, benzodiazepines, and propofol.

5.2.6. Effects of combined administration of anesthetics—While many studies investigate anesthetic agents individually in order to maintain consistency and maximize simplicity of the experimental design, the reality in the clinics is that oftentimes different agents may be administered together in concert. For example, propofol may be used for induction, but an additional agent such as inhalable anesthetics may also be used. Therefore, in order to maximize face validity of anesthesia exposure, it may be important to assess combination regiments of general anesthesia. However, this makes comparisons of different groups more difficult, especially in animal models, in which the dosage and durations are all operated on a different scale than in humans. These studies, however, are particularly beneficial because they holistically assess the long-term neurodevelopmental effects of the

combination regiment, which would have a stronger effect than the data possibly obtained from studying an individual agent.

Several studies have investigated the effects of inhalable anesthetics in conjunction with other anesthetics, such as the benzodiazepine midazolam, and propofol. Midazolam, propofol, and isoflurane are all common anesthetic agents that are administered to either neonates or infants. When administered to neonatal P7 rats, the rats developed significantly greater levels of apoptosis throughout the brain, as well as hippocampal synaptic dysfunction, and associated learning and memory impairments (Jevtovic-Todorovic et al., 2003). Another study used the combination of midazolam, isoflurane, and nitrous oxide, and also showed significant neurodegeneration, possibly as a result of mitochondrial instability and heightened autophagy activity (Sanchez et al., 2011). Midazolam, as well as other benzodiazepines, is commonly used as an anti-epileptic drug as well, but because of its sedative effects, it can be used to augment general anesthesia. Benzodiazepines, such as midazolam and diazepam, have been shown to affect neurodevelopment, including disruption of synaptic integrity and synaptic transmission and induction of apoptosis (Bittigau et al., 2002; Fredriksson et al., 2004; Fredriksson et al., 2007; Jevtovic-Todorovic et al., 2003; Young et al., 2005). However, the cognitive deficits are less characterized. When midazolam or other anti-epileptic drugs are given at the dosages necessary to control seizures, they seem to have long-term cognitive deficits and cause a decreased brain size. When midazolam is given to children prior to surgeries, however, they have much fewer negative behavioral changes in the first week following the procedure (Kain et al., 1999).

5.2.7. Other possible mechanisms in neonatal anesthesia toxicity—Recent report showed that neonatal mice pups exposed to ketamine decreased levels of phosphorylated extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) and AKT, a serine/threonine protein kinase (Straiko et al., 2009). Also, sevoflurane administration resulted in greater hippocampal neuronal death, upregulation of Protein kinase C α (PKCα) and phosphorylated c-Jun N-terminal kinase (p-JNK), and downregulation of p-ERK and FOS protein levels in the hippocampus of P0, P7, and P14 rats, suggesting that sevoflurane induces developmental neurotoxicity in rats through regulation of PKCα, p-JNK, p-ERK, and FOS proteins (Wang et al., 2012). Phosphorylated ERK and AKT helps neuronal survival through activation of various factors such as CaMKII and BDNF and inactivation of glycogen synthase kinase 3β (GSK-3β) (De Koninck and Schulman, 1998; Lu et al., 2006). These results indicate that some anesthetics may downregulate factors that mediate neuronal survival in developmental stage, resulting in premature neuronal degeneration.

We and other groups have shown important roles of hypoxia inducible factor-1α (HIF-1α) as a homeostatic pathway against hypoxic ischemic injury (Ogle et al., 2012; Souvenir et al., 2014; Wang and Semenza, 1993, 1995). When HIF-1α is unhydroxylated, the stable protein accumulates, and translocates to the nucleus, resulting in promotion of cell survival, angiogenesis, anaerobic metabolism, and neurogenesis (Forsythe et al., 1996; Gidday, 2010; Semenza, 1994; Yu et al., 2013). However, during normal oxygen perfusion, prolyl hydroxylase (PHD) constitutively hydroxylates HIF-1α on two conserved proline residues (Epstein et al., 2001), mediating the interaction of HIF-1α with the E-3 ubiquitin ligase von Hippel Lindau (Ivan et al., 2001; Jaakkola et al., 2001). Therefore, under normal oxygen

tension, hydroxylated HIF-1α is poly-ubiquitinated and targeted for proteasomal degradation (Sutter et al., 2000). Isoflurane increases HIF-1α expression in primary cultured neurons and in the developing rat brain (Jiang et al., 2012). In that study, HIF-1α knockdown prevented neural degeneration via inhibition of isoflurane-induced increase in cleaved caspase-3 and poly-(ADP-ribose) polymerase (PARP).

6. Behavioral and Cognitive Impairments Associated with Neonatal Anesthesia

The following sections will focus on these four major groups of anesthetics that are commonly administered to neonates and infants: volatile anesthetics, ketamine, benzodiazepines, and propofol. We will review current literature about these agents to assess their neurodevelopmental effects, including learning, memory, and social behavior.

6.1. Learning and memory deficits and underlying cellular mechanism

Neonatal mice or rats are typically defined as newborn mice/rats that are within the postnatal day 10 range. Most neonatal models of anesthesia exposure utilize P7 mice or rats in order to represent the state of maximal synaptogenesis and susceptibility within the developing brain. In animal models, isoflurane induces widespread apoptosis in many areas of the developing brain, leading to a wide array of phenotypic effects, from cognitive deficits to social abnormalities (Istaphanous et al., 2013; Johnson et al., 2008; Kong et al., 2012; Sanchez et al., 2011). In studies investigating the effects of isoflurane in P7–14 rats, it is clear that both the duration of exposure and frequency of exposure play a critical role in determining the extent of neurodevelopmental damage. For example, in an experiment using P7 rats, 4-hr exposure to isoflurane was needed to develop impairments in spatial reference memory and spatial working memory (Stratmann et al., 2009a). However, if the exposure frequency was increased from a one-time 2-hr exposure to four repetitive sessions of 2-hr exposures, then the pups developed learning and memory deficits (Murphy and Baxter, 2013). The frequency appears to be more important than the duration, because a 4-time exposure of just 35-min duration was enough to induce deficits in object recognition and associative memory in P14 rats (Zhu et al., 2010).

Aside from isoflurane, desflurane and sevoflurane are two other commonly used inhalational anesthetic agents, and both of them have been implicated to cause apoptosis in mouse and rat pups (Kodama et al., 2011; Lee et al., 2014; Zhang et al., 2008b; Zhou et al., 2012). In fact, desflurane has been shown to induce more robust levels of apoptosis and working memory deficits than isoflurane or sevoflurane (Kodama et al., 2011). The cognitive profiles of the animals reflect this difference in cellular damage. When comparing isoflurane and sevoflurane, desflurane exposure resulted in a much greater immediate deficit in cognitive function as assessed by performance on a working memory task (Kodama et al., 2011). General anesthesia also affects fear and associative memory. Exposure to isoflurane or sevoflurane resulted in suppression of the fear response despite training to either tone or context (Dutton et al., 2002; Satomoto et al., 2009; Stratmann et al., 2009b). More specifically, isoflurane induces anterograde amnesia and interferes with the animal's ability to form novel memories (Dutton et al., 2002).

Ketamine is a common general anesthetic that is administered to neonates and infants intravenously, and displays similar dosage-, duration-, and frequency-dependent apoptotic effects as inhalable anesthetics (Hayashi et al., 2002; Scallet et al., 2004; Soriano et al., 2010; Zou et al., 2009). In P7 rats, the frequency of administration again appears to be the most important factor. In one study, a single dosage of 25, 50, or 75 mg/kg of ketamine was not sufficient to induce differences in apoptosis, but repeated injections (every 90 min over 9 hr) resulted in frequency-dependent increases in neurodegeneration (Hayashi et al., 2002). Another study found that repeated doses of just 20 mg/kg were sufficient to increase levels of neurodegeneration (Scallet et al., 2004). In P5 macaques, the duration threshold for increasing apoptosis appears to be between 3–9 hr of continuous ketamine infusion. When the duration exceeded this range, significantly greater levels of apoptosis were observed in the frontal cortex.

Importantly, the cellular degenerative effects may translate into long-term behavioral changes as well. P10 mice exposed to ketamine displayed disrupted spontaneous activity and decreased learning into adulthood (Fredriksson et al., 2007; Viberg et al., 2008). Another study showed that P10 mouse pups display the most profound degeneration in the parietal cortex following ketamine administration, and later performed markedly worse on acquisition learning and retention memory tasks such as the radial arm maze-learning task and the circular swim maze-learning task (Fredriksson et al., 2004). These cognitive deficits were also observed in non-human primates. Both macaques on gestational day 120–123 and P5–6 macaques received a single dose of ketamine displayed deficits in operant training learning, as well as color and position tasks, and had slower response speeds as adults (Paule et al., 2011).

General anesthesia may show impacts on activity-dependent synaptogenesis and neurogenesis. For one, anesthetics modulate excitability, albeit only on the order of hours, via glutamatergic or GABAergic pathways. If they are administered during the critical period during neural development in which glutamatergic/GABAergic signaling are crucial for the synthesis and strengthening of synapses, or activity-dependent plasticity, then anesthetics may have an effect on neural circuitry assembly. In fact, certain general anesthetics, such as barbiturates, ketamine, and propofol, can increase dendritic budding and synaptogenesis in developing pyramidal neurons, possibly through a homeostatic plasticity mechanism (De Roo et al., 2009). Although the behavioral effects of these synaptic enhancements are unclear, it is possible that having too many spines and synapses may interfere with the synaptic pruning and strengthening that occurs during the early developmental phase. However, other anesthetic agents, such as halothane, can impair neural circuitry assembly by decrease the number of synaptic inputs, and stalling the timeline of synaptogenesis (Quimby et al., 1974; Uemura et al., 1985). Certain volatile anesthetics cause an adverse effect on synaptogenesis, and this may be partially due to a rapid and pathological increase in dendritic spine density, which could interfere with synaptic contact formation (Briner et al., 2010). Isoflurane also results in impairments in mitochondrial morphology and membrane integrity, which could contribute both to increased apoptosis, as well as reduced synaptic transmission (Sanchez et al., 2011). Finally, isoflurane may adversely affect neuroregeneration, by decreasing the neural stem cell pool and attenuating neurogenesis (Stratmann et al., 2009b). While the mechanism is not

elucidated, ion channel activity is critical for proper neural stem cell proliferation and differentiation (Chen et al., 2014a). Therefore, disruption of excitatory channels may interfere with neurogenesis during development, as well as possibly more delayed effects, such as learning and memory deficits later in life.

6.2. Abnormalities in social memory and social activity

In addition to the learning and memory impairments associated with neonatal anesthesia, more investigations have begun to probe into the associated aberrances in social memory and behavior as well. A 6-hr exposure of neonatal mice to sevoflurane (3%) caused not only learning deficits but also abnormal social behaviors, such as impaired social memory and decreased interaction in spite of normal olfactory activity (Satomoto et al., 2009). Consistent with this finding, a recent *in vivo* animal study showed that 6 hr sevoflurane exposure in the hippocampus of P5 mice caused the reduced expressions of oxytocin and arginine vasopressin levels, which are known to be key regulators of social activity (Heinrichs and Domes, 2008; Zhou et al., 2014). These results demonstrated that anesthesia causes impaired social recognition memory formation and social discrimination ability in juvenile mice.

7. The Plausible Link between Cellular Neurotoxicity and Cognitive Impairments

If the neuronal death itself were directly linked to cognitive impairment, behavioral impairment would be expected immediately after anesthesia-mediated neuronal death. However, animal studies have shown that when anesthesia-induced cell death was observed, cognitive deficits did not occur until about 6 weeks after the exposure (Jevtovic-Todorovic et al., 2003; Satomoto et al., 2009; Stratmann et al., 2009b). Interestingly, these behavioral deficits, including social behavior and spatial learning and memory persist until at least 8 months of age (Fredriksson et al., 2004; Jevtovic-Todorovic et al., 2003; Satomoto et al., 2009; Stratmann et al., 2009a; Stratmann et al., 2009b). These findings might imply that there may be a disconnection between the anesthesia-induced neuronal death and the delayed neurocognitive dysfunction. Alternatively and perhaps more likely, the cell death-triggered consequent events are more directly associated with behavioral deficits. One emerging theory is that anesthesia may affect the stem cell pool and endogenous neurogenesis, which would create a late-onset and progressive neurocognitive decline. This may be why the primary cognitive deficit associated with neonatal anesthesia exposure is learning and memory. Children and adults require the constant supply of new neurons in the hippocampus to establish new memories (Schmidt-Hieber et al., 2004). These neurons come from the neural stem cell niche of the subgranular zone, the hippocampal dentate gyrus, and early anesthesia exposure may have long-term detrimental effects on this neurogenesis (Zhu et al., 2010).

Recent findings have provided some evidence that neuronal death may not be a prerequisite for delayed neurocognitive impairment (Stratmann et al., 2009a). They administered isoflurane in P7 rats for 1, 2, or 4 hr and then measured cell death in the brain 12 hr after anesthesia, and then assessed neurocognitive outcomes at 8 weeks after anesthesia. They found that a minimum of 2 hr of isoflurane exposure was required to induce cytotoxicity, but

this was not enough to produce a neurocognitive deficit. Only after 4 hr of isoflurane exposure was a neurocognitive deficit observed in spatial reference memory and spatial working memory tests. Others showed that prolonged isoflurane or midazolam exposure caused neuronal death but not spatial memory deficits (Fredriksson et al., 2004; Loepke et al., 2009). A 6-hr isoflurane exposure in P7 mice did not lead to decreased neuronal density 3 months after anesthesia although neonatal neuronal death occurred (Loepke et al., 2009). It is likely that the ultimate functional consequence of anesthetic exposure is largely dependent on the balance between neuronal cell death and neurogenesis. In the event that sufficient neurogenetic activities remain sufficient to replace dead cells, the long-term behavioral development may be corrected or not be affected.

Recent clinical studies have shown that anesthetic exposure before 4 years old could affect the development of reading, writing, and math skills, as well as increase the risk of learning disabilities (Wilder et al., 2009). These findings suggest that further investigations should be conducted to study the link between anesthesia-induced neurotoxicity and delayed functional abnormalities. However, a causal connection between brain cell death and delayed neurocognitive impairment remains unclear (Stratmann, 2011).

8. Potential Therapeutic Strategies and Implication in Clinical Stage

One of the benefits of animal models is that they help us to probe into the mechanisms of neurotoxicity, due to the wide array of cellular and molecular biological techniques available. This can improve our understanding of the pathophysiology of anesthesia-associated neurodevelopmental deficits and provide clinical targets for future treatments. For the translational purpose, however, the animal models do not have optimal face validity, due to the differences in the developmental timeline between rodents and primates/humans (Dobbing and Sands, 1978; Malkova et al., 2006; Petit et al., 1988). Furthermore, there are significant differences in anatomy, anatomical regionalization, synaptic density, spine density, and the tissue level neural networks; all of which are much more sophisticated and complex in humans.

Although general anesthesia has been clearly shown to induce a frequency- and duration-dependent neurotoxicity, whether or not this translates into cognitive impairments in humans is inconclusive. Recently there has been a surge of retrospective and preclinical studies into the effects of general anesthesia in neonates. However, by virtue of the majority of these studies being retrospective, the outcomes are not preemptively chosen by the investigator, and so the findings may not be as meaningful as blind, randomized, controlled studies. Furthermore, many of these studies employ different modes of cognitive assessments, from standardized tests, to academic achievements, to neuropsychological abnormalities, such as personality disorders. The lack of a consistent and universal assessment of neurodevelopment makes it difficult to interpret the results of the studies with respect to clinical application. Additionally, sometimes the results may be ambiguous. For example, one clinical study showed that anesthesia exposure prior to the age of three resulted in no differences in academic performance (Hansen et al., 2011). However, exposure does lead to an increased risk of deficit on a neuropsychological clinical outcome measure (Ing et al., 2014). There were other studies that resulted in negative findings between anesthesia

exposure and proper neurodevelopment, but that may because the studies implemented broad assessments of learning, such as group test of academic performance (Flick et al., 2011; Ing et al., 2014). One study did find that early anesthesia exposure lead to a significantly greater risk of learning disabilities. Another confounding factor in these studies, despite being paired or sibling-matched, is that the anesthesia exposure group always receives a surgery, such as hernia repair (Hansen et al., 2011). The effects of the surgery, both immediate and psychological, may play an important role in the developmental outcome of the child.

The inconsistencies of the results of animal studies vs. the retrospective studies calls for a need to conduct more controlled investigations into the effects of early anesthesia exposure in humans. There are several ongoing clinical trials that may provide more conclusive results towards corroborating the results of the animal studies, as well as for determining the course of action for future neonatal anesthesia projects. These studies include the Pediatric Anesthetic NeuroDevelopmental Assessment (PANDA), the Mayo Safety in Kids study (MASK), and the General Anesthesia and Spinal (GAS) study. These studies will provide the first set of neurocognitive and behavioral data that will be prospectively collected, as well as provide more information on the age ranges of greatest susceptibility and the importance of toxicity factors, such as frequency and duration. The results of these trials will be critical for developing appropriate guidelines for assessing developmental benchmarks and neurological deficits, as well as towards implementing appropriate protocols for anesthesia administration in neonates and infants.

9. Summary and Conclusion

The development of anesthetics has revolutionized modern medicine. Tens of millions of surgeries are performed under anesthesia every year in the US alone, approximately six million of which are on pediatric populations (DeFrances et al., 2007). Anesthetics can prolong the quality and longevity of human life by enabling increasingly complex surgeries and procedures. However, over recent years, the safety has come under contention after new evidence revealed that anesthesia exposure in immature animals can cause neuronal degeneration, long-lasting cognitive and behavioral deficiencies (Cao et al., 2012; Jevtovic-Todorovic et al., 2003). This has led to an urgent movement to try to resolve this question and develop new measures to handle neonatal anesthesia in clinics. Current data agree that general anesthetics can cause apoptotic neuronal cell death in the developing brain, while the association or the mediator between the cytotoxicity and the long-term, long-lasting cognitive and behavioral deficiencies is unclear. Finally, a few papers have demonstrated the detrimental effects of neonatal anesthesia on endogenous neurogenesis and stem cell survival, and this may provide a mechanism to fill the knowledge gap between the acute cytotoxicity of anesthesia and the delayed neurological impairments. The observations in animal models and its relevance to humans also remain unclear and require substantial investigative effort. The upcoming results from several clinical trials will help to improve the understanding of the effects of general anesthetics in young population and the improvement of clinical application of general anesthetic.

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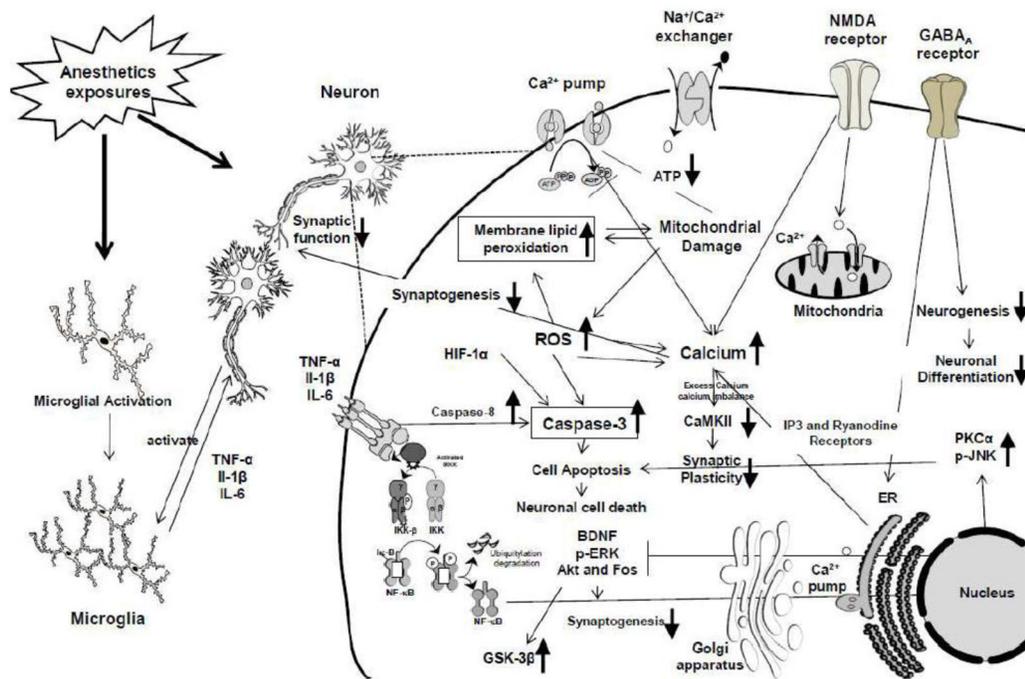
Figure 1. Schematic diagram on possible mechanisms in neonatal anesthesia toxicity
Abbreviation: ATP: Adenosine triphosphate; ROS: Reactive oxygen species; NMDA receptor: N-methyl-D-aspartate; GABA$_A$ receptor: Gamma-aminobutyric acid type A receptor; TNF-α: Tumor necrosis factor-α; IL-1β: Interleukin-1β; IL-6: Interleukin-6; IP3 receptor: Inositol triphosphate receptor; BDNF: Brain-derived; Akt: Phosphorylated serine/threonine-specific protein kinase; CaMK II: Calcium/calmodulin-dependent Protein Kinase II; ERK: extracellular signal-regulated protein kinase; PKC: Protein kinase C; p-JNK: Phosphorylated c-Jun N-terminal kinase; GSK-3β: Glycogen synthase kinase 3β; IKK: IκB kinase; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells); ER: endoplasmic reticulum; Ca^{2+}: Calcium ion. ↑: increase; ↓: decrease; ⊣ : block or inhibit.