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INTEGRATED STUDY MASTER'S THESIS

Consequences of General Anesthesia in Infancy on Behaviour and Brain Structures

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1. ABSTRACT

This literature review explores the effects of general anesthesia on infant brain development, emphasizing potential neurocognitive and structural consequences. Given the rapid and complex nature of brain maturation in early life, concerns have emerged regarding anesthesia-induced neurotoxicity, particularly in relation to synaptic development, neuronal apoptosis, and long-term cognitive function.

The developing brain is highly plastic and undergoes rapid changes, making it particularly susceptible to external influences, including anesthetic agents. General anesthetics primarily act on γ-aminobutyric acid (GABA) and N-methyl-D-aspartate (NMDA) receptors, disrupting normal synaptic activity and potentially leading to widespread neuronal apoptosis, synaptic dysfunction, and neuroinflammation. While animal studies have consistently demonstrated significant neurotoxic effects, including neuronal apoptosis, structural changes and major neurocognitive alterations, human studies present more variable and less pronounced findings.

Neurocognitive and behavioural outcomes following anesthesia exposure in infancy remain a topic of debate. Large-scale human studies such as the GAS, PANDA, and MASK trials indicate that a single, short exposure does not significantly impact intelligence quotient or broad cognitive function. However, repeated or prolonged exposures have been associated with deficits in memory, executive function, and behavioural regulation. Epidemiological data suggest an increased incidence of neurodevelopmental disorders, including attention deficit hyperactivity disorder (ADHD) and autism spectrum disorders, in children with multiple early-life anesthetic exposures. These findings highlight the need for further research into the precise mechanisms underlying anesthesia-induced neurotoxicity and its long-term consequences.

Given these concerns, potential mitigation strategies have been explored. Research into molecular pathways suggests that pharmacological interventions may offer neuroprotective benefits. Alternative anesthetic techniques, such as regional anesthesia, opioid adjuncts, and multimodal analgesia, may help reduce the reliance on high-dose general anesthetics in pediatric procedures. While current evidence does not support the routine postponement of necessary surgeries, careful risk-benefit assessment is crucial, particularly for elective procedures. Future research should focus on identifying at-risk populations, optimizing anesthetic protocols, and developing targeted neuroprotective interventions to ensure safer outcomes for infants undergoing anesthesia.

2. ABBREVIATIONS

Abbreviation | Definition

ADHD | *Attention Deficit Hyperactivity Disorder*
AMPK | *AMP-activated Protein Kinase*
BDNF | *Brain-Derived Neurotrophic Factor*
CNS | *Central Nervous System*
EDi | *Early Development Instrument*
EEG | *Electroencephalogram*
FSIQ | *Full-Scale Intelligence Quotient*
GABA | *Gamma-Aminobutyric Acid*
GFAP | *Glial Fibrillary Acidic Protein*
HIPK2 | *Homeodomain Interacting Protein Kinase 2*
IQ | *Intelligence Quotient*
JAK/STAT | *Janus Kinase / Signal Transducer and Activator of Transcription*
mTOR | *Mammalian Target of Rapamycin*
NHP | *Non-Human Primate*
NMDA | *N-Methyl-D-Aspartate*
PI3K | *Phosphoinositide 3-Kinase*
RNA | *Ribonucleic Acid*
WPPSI-III | *Wechsler Preschool and Primary Scale of Intelligence – Third Edition*

3. KEY WORDS

General anesthesia, neurotoxicity, apoptosis, neurocognitive outcomes, infancy

4. METHODOLOGY

Medical science is a field of cumulative endeavour where literature reviews can be seen as a good tool to keep up with growing knowledge. Understanding the basic aspects of methodology is essential for the outcome reliability and validity of a study (1). By integrating findings and perspectives from many empirical findings, literature review has ability to answer to the questions with a power that no single study can do (1).

Aim of this literature review was to gain understanding of existing timely research, present the knowledge and to summarize the most important findings from reliable studies. Aim was the strong baseline for the literature review and objectives formulated the key aspects, outlining a common thread during the process.

4.1 RESEARCH TYPE

Literature review is type of research which offers a way of synthesizing research findings and to show evidence on a meta-level on the areas where uncovering and more research is needed. Literature reviews are critical component of creating theoretical frameworks and building conceptual models (2).

As a common agreement with thesis supervisor, Eglė Kontrimavičiūtė, non-systematic approach for literature review was decided. For one author to review every single relevant article of the topic was simply not possible, non-systematic approach was justified.

Purpose of research was to detect themes, theoretical perspectives, common findings and issues within the topic (2).

This non-systematic literature review explored how general anesthesia in infancy affects to the developing brain structures and neurocognitive functions. It underlined most important studies from the resents years, providing the overview of the current knowledge and the questions yet to answer. Literature review follows guidelines of good quality, it is objective, reliable, with appropriate conduct, data collection and its analysis with logical interpretation (1).

4.2 RESEARCH STRATEGY

Comprehensive literature search was made using best known databases; PubMed, Cochrane, EMBASE, OVID Medline and Scopus. Relevant search terms were used to collect relevant studies for the literature review. Key words for the search were “general anesthesia” AND “children” OR “infant” OR “neonate” combined with one of the following;

1. AND “brain structures” OR “brain development”
2. AND “neurocognitive outcome” OR “neurotoxicity” OR “behaviour”
3. AND “molecular mechanisms”

Also additional literature search was made to describe brain development and molecular mechanisms more deeply and gain better insight of the topic itself.

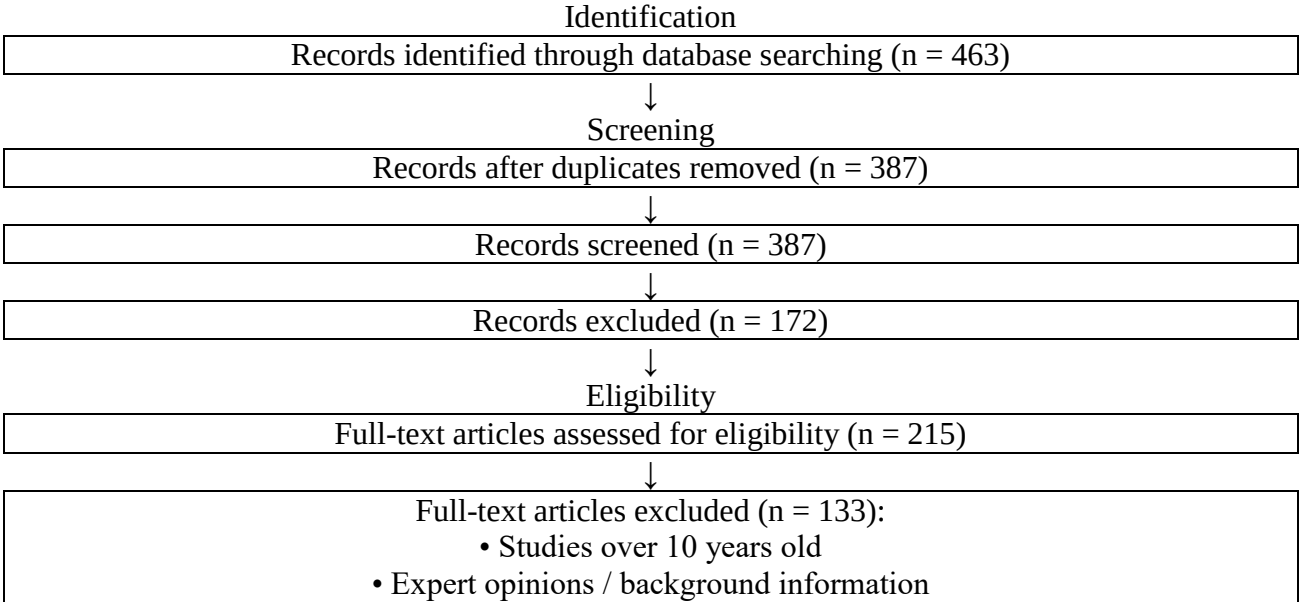
Careful study plan including all its components was created and designed before start of the research and writing process and the study protocol was strictly adhered during the conduct of the study (1).

Total of 463 studies were recorded throughout search in databases. Screening was done and duplicates removed which led to total of 387 records to be evaluated by their eligibility.

After the search of the studies appraisal was made with pre-defined literature inclusion and exclusion criteria. Pre-defined exclusion criteria included animal studies but after quality assessment was rejected from the final criterion. Many animal models (3–5) serves useful insight for better understanding, further studies and treatment options.

Final inclusion and exclusion criteria were decided by three categories; type of study, publish date and definitions/populations used. Inclusion criteria included studies under 10 years old, observational (case control, cohort) studies, intervention studies(clinical trials), meta-analyses/systematic reviews and children between 0-3 years. Exclusion criteria included studies over 10 years old, expert opinion, background information, case reports/case series and children over 3 years old. Flow chart of the literature search is visualized in the Figure 1.

Eligible full text articles included in thesis are timed, up to date studies from publishing date under 10 years. Couple of exceptions had to made to ensure quality of the study as more narrow time frame would have severely limited the number of eligible important studies like GAS, which were crucial to be included.



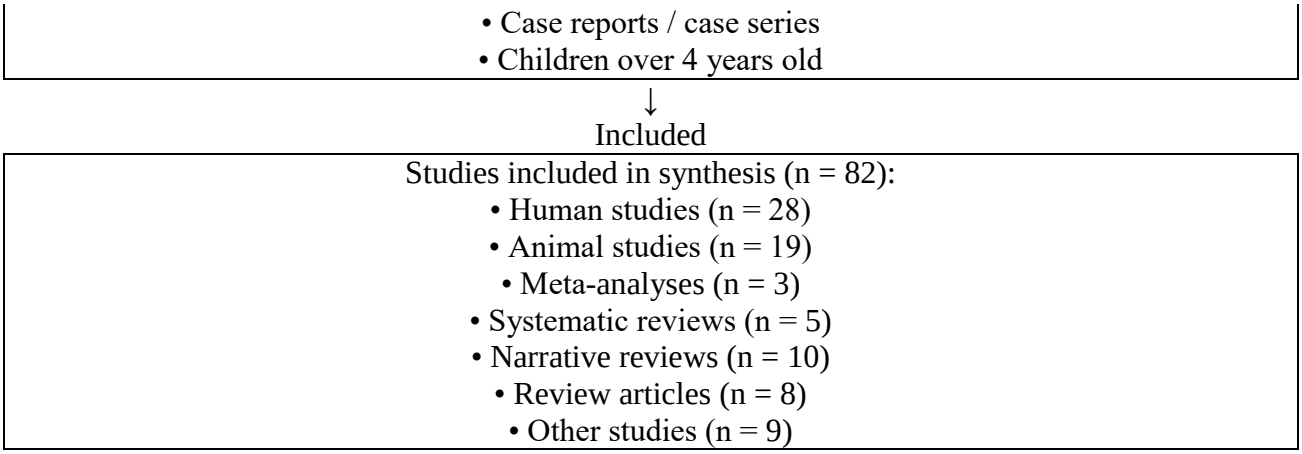


Figure 1. Prisma flow chart of the review process leading to final studies included.

5. INTRODUCTION

It was not until 1987 when first article about pain and its effects in the Human Neonates and Fetus was published by the Anand and colleagues (6) in The New England Journal of Medicine. Before this, pediatric surgeries were done without any proper anesthetic care. The important discussion about the surgery and anesthetic care of infants was declared. Expected end result was that humane considerations should be forcefully applied to the care of nonverbal neonates and infants as done with adult population. Conclusion marked the beginning of new practise, anesthesia to infants as we know it today.

Years later, many studies published several warnings about using general anestethics and sedation drugs in young children and pregnant woman. Clinical and non-clinical studies showed results of neurotoxicity and possible cognitive and structural changes to infant brain.

At the year of 2016, FDA reviewed these warnings suggesting that single relatively short exposure to general anesthetic and sedation drugs in infants is unlikely to have negative effects- thus further research should be made about longer duration of exposure or multiple anesthesia (7). Same time warnings of the use in infants and pregnant woman were added to labels of general anesthetics and sedation drugs.

Brain of an infant is constantly developing, growing and changing organ making it extremely vulnerable to experiential and environmental perturbations. At the early post-natal period three major changes are occurring, the formation of synapses, connection among neurons and myelination.

In the first three year of life apoptosis in brain is a normative process and it is estimated that 40-60% of all neurons born prenatally will eventually disappear. Despite this fact, field of Medicine has raised major concerns about neurotoxicity of general anesthesia to infant brain. Main concerns are possible changes in apoptotic processes leading to death of too many neurons and changes in the synapses and neuronal networks. In short there is a suggestion that anesthesia in infancy can lead to loss of neurons and loss of neuronal connections.

Infants are vulnerable population, yet crucially important group when it comes to our future. General anesthesia for them is not studied well enough and has been interesting topic during last few years, getting more attention. This thesis is non-systematic literature review, searching up to date articles and publications and collecting most important data we have.

Objectives of the thesis:

1. To evaluate what behavioural and cognitive outcomes can general anesthesia in infancy induce
2. Assess how general anesthesia in infancy affects the brain structures
3. Compare if younger age and/or longer anesthesia correlates with higher risk for neurological changes
4. To assess possible neuroprotective agents that can be used to minimize neurocognitive effect of general anesthesia in infancy

Aim of the thesis is to gain understanding of the neurotoxic effects of infant anesthesia by evaluating existing timely research, present the knowledge and to summarize the most important findings from reliable studies.

6. LITERATURE REVIEW

General anesthesia is a medically-induced loss of consciousness with concurrent loss of protective reflexes due to anesthetic agents (8). Parts of the central nervous system contributing to the state of consciousness are the brain stem, pons, thalamus, and cortex with their connecting neural pathways (9).

The action of anesthetics and sedative agents to the central nervous system (CNS) is via the interacting with neurotransmitters which resolves neuronal integration between different brain regions (10). These substances target the lateral temporo-parieto-occipital junction and the mesial

cortical core inducing the unconsciousness by disrupting cortical integration and cortical information capacity (11). As general anesthetic drugs are apolar they cross the blood-brain barrier and are able to interact with receptors increasing the inhibition or decreasing the excitation by causing neuronal hyperpolarisation (10).

Anesthetics used in today's clinical practise act by two major mechanisms; increasing inhibition through γ-aminobutyric acid (GABA) receptors (e.g., benzodiazepines, barbiturates, propofol, etomidate, isoflurane, enflurane, and halothane) and by decreasing the excitation via *N*-methyl-d-asparate (NMDA) receptors [e.g., ketamine, nitrous oxide (N2O), and xenon] (10).

Infants and young children are at critical stages of brain development suggesting them to be under significantly increased risk of postoperative neurotoxicity and cognitive impairment when using general anesthetics for surgical procedures (12).

Anesthetic agents can be divided in the two main groups, the volatile/inhalation anesthetics and the non-volatile/intravenous anesthetics. Propofol is most commonly used non-volatile anesthetic whereas the most common volatile agent is sevoflurane (6).

6.1 ANESTHESIA AND MOLECULAR MECHANISMS

Understanding of the molecular mechanisms behind anesthesia induced neurocognitive alterations and structural changes to brain, is the key to prevent them. The underlying mechanisms are complex and involve various related molecular signalling pathways, cell mediators, autophagy, and other pathological processes.

General anesthetics can be either inhalation and intravenous agents and they are divided into three main classes; barbiturates (eg.thiopental), halogenated (e.g. sevoflurane, desflurane), and miscellaneous (e.g. propofol, ketamine). Many of the previously used anestethics are not used in today's clinical practise because of the implicated major side effects. Overall propofol has become the most common general anestethic in use but in infant anesthesia volatile agents are more often used. Table 1 summarizes the findings by Wang&Liu (12), presenting how certain general anesthetics are shown to induce structural brain changes in molecular level.

General anesthetic	**Related molecular studies**
Halothane	Increased inflammatory reaction of brain

Nitrous oxide	Vitamin B12 deficiency
Sevoflurane	Iron overload, decreased number of excitatory synapses and protein levels
Isoflurane	Ferroptosis, neuronal cell cycle activation, migration of dentate gyrus granule cells
Desflurane	Decrease synaptic integrity, decreased NMDAR-mediated excitatory postsynaptic current
Ketamine	Iron overload, increased NMDAR at extrasynaptic sites
Propofol	Altered synaptic plasticity, mitochondrial damage in hippocampal neurons

Table 1. General anesthetics and related molecular mechanisms (12).

Research has been able to present many affecting signalling pathways (14,15) related to neurocognitive changes of general anesthesia exposure in infancy, Table 2. Presents most up to date knowledge.

Signalling pathway	Significance in general anesthesia
HIPK2/Akt/mTOR	Responsible for cell apoptosis, works as a negative feedback regulation; HIPK2 increases apoptosis where Akt/mTOR inhibits it. Neurotoxicity occurs by increased apoptosis, likely to occur in all commonly used anesthetics.
PI3K/Akt	Activation can *reduce* neuroinflammation and *inhibit* apoptosis
HIPK2/JNKs/c-Jun	Activation *induces* neuronal apoptosis leading to neurocognitive dysfunction, likely to occur in all commonly used anesthetics.
JAK/STAT	Sevoflurane *inactivates* JAK/STAT pathway, damage to astrocytes. Increased proinflammatory factors.

AMPK	Sevoflurane-induced neuronal apoptosis and hindered proliferation are related to the inactivation AMPK which has neuroprotective and anti-neuroinflammatory role

Table 2.General anesthetics and signalling pathways (12).

To conclude the findings, general anethesia changes the function of signalling pathways presented in Table 2. General anesthetics have known toxic effects on the brain, including apoptosis of nerve cells, neuroinflammation, cerebral ischemia and hypoxia. General anesthesia will either directly, or indirectly, activate these signalling pathways which reduces the toxicity. It has been shown that general anesthetics can cause neurotoxicity in the infant's brain (14) but interestingly body has a self-repairing function by the above presented signalling pathways (Table 2.). Studies have found that long-term exposure to anesthetics can seriously damage the development of the infant's brain but short time of exposure to general anesthetics will not cause significant damage (19,20).

Non-coding RNA has also significance in anesthesia induced neurocognitive consequences. MicroRNA, lncRN and circRNA are all regulators of neuronal cell expression while general anesthesia has shown to change the expression of these RNA's (14).

6.2 BRAIN DEVELOPMENT

Human brain development during gestation is complex and multiphased process. To understand the consequences of general anesthesia in infancy on behaviour and brain structures, it is essential to familiarize not only with molecular mechanisms, but also brain development itself.

Between the postconceptional days 20-28, the ontogeny of the human brain originates in neural tube formation. When the neurulation is completed, neuroepithelial cells (neural stem cells) start to proliferate rapidly, deriving precursor neurons and supporting glia cells (21).
At 8 postconceptional weeks neural circuits form when neurons start developing axons and dendrites. At the time of 15 weeks of gestation transient subplate forms and is responsible for early connectivity between brain regions being a crucial phase for the development of healthy brain. Later subplate is replaced by the cortex itself. Peak of neuronal migration is seen 3-5 months of gestation, in mid-fetal period, but does not end until third semester of pregnancy (21).

Early preterm period between 24-32 weeks includes many vitally important development steps; permanent thalamocortical circuit is formed, subplate reaches the peak size, later cortex development starts and subplate is gradually replaced input-dependent cortical activity (21,22). Oligodendrocyte lineage development is also involved in preterm period and myelination starting from 32 week of gestation continues until early adulthood. During third semester of gestation synaptogenesis, dendritic differentiation, axonal growth and rapid development of oligodendrocytes takes place (23).

Postnatal period is predominantly characterized by continuing synaptogenesis, elimination of exuberant connections, myelination, and overall brain growth. Neuronal architecture matures, glia cells including astrocytes and oligodendrocytes proliferate and short-range cortico-cortical association fibres continue to grow into their target sites. Also the subplate diminishes and transposes into a thin layer at the white and gray matter interface (21,23).

Referring to what's described previously, development of neocortex is rapid process, full of critical phases and vulnerable to many disruptions. When it comes to development of cerebellum, development is even faster (21).

The cerebellum forms elaborate circuits with various cortical regions and they are all crucial for he further development of the infant. These circuits includes sensorimotor, limbic, and association cortices. Cognitive impairment, language delay, and behavioural problems are all associated with postnatal cerebellar injury and the issues in framework connections. Research shows that anesthesia during infancy is linked to similar deficits (24). Figure 2. summarizes the timeline of brain development (21). For this literature review, the infancy and time after birth are the most interesting and meaningful periods. As presented in Figure 2. neural migration, synaptogenesis, axonal growth, myelination, pruning and cell death continues still after the birth has taken place.

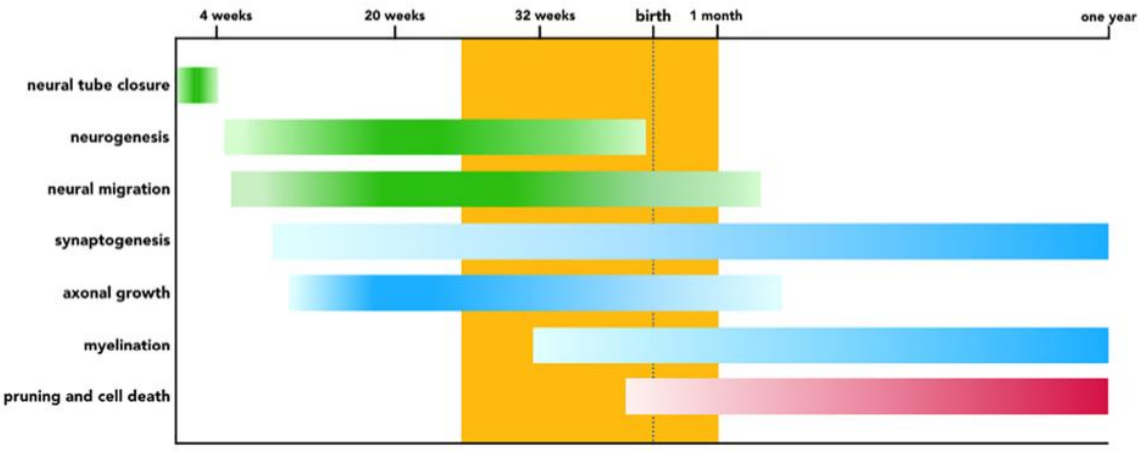


Figure 2. Timeline of brain development (21).

Grey matter forms early in development from the ectoderm. Specific cells are born from the continuing dividing of ectoderm and it continues until the entire central nervous system, both the brain and the spinal cord, has formed. Throughout development, the volume of grey matter increases until around the age of eight (25).
After the age of eight, the amount of grey matter in certain areas of the brain starts to decrease, but its density actually increases. This increase in density enhances processing abilities and supports further mental development (25).
Between 13 and 18 weeks post conception major white matter tracts including projection and commissural pathways emerge in the fetal brain between. Later during fetal period thalamocortical and association pathways develop All major white matter tracts are present by the end of normal gestation (37–42 weeks) (26,27).

6.3 EPIGENOME

Epigenetics are alterations in gene expression that are not a result of alteration of DNA sequence but are still heritable (28). Their biochemical processes influence the readability of the genome. General anesthesia during infancy not only alters the plasticity of neural circuits and changes the biochemistry of synaptic neurotransmission, but also leads to epigenetic dysregulation (29). General anesthesia triggers epigenetic changes which results in downregulation of GABAergic system by reduced synthesization of GABA-synthesizing enzymes (GAD65 and GAD67) and of the brain-derived neurotrophic factor (30).

Research have found that all commonly used general anesthetics; propofol, sevoflurane, ketamine and isoflurane, changes the expression of DNA and histone modifying enzymes. These changes lead alterations in epigenetic methylation, histone acetylation and histone methylation on inflammatory genes (e.g., TNF-alpha, IL-6 or IL1 beta) and genes which are responsible for neuronal development. (29,31) Figure 3. visualizes after mentioned.

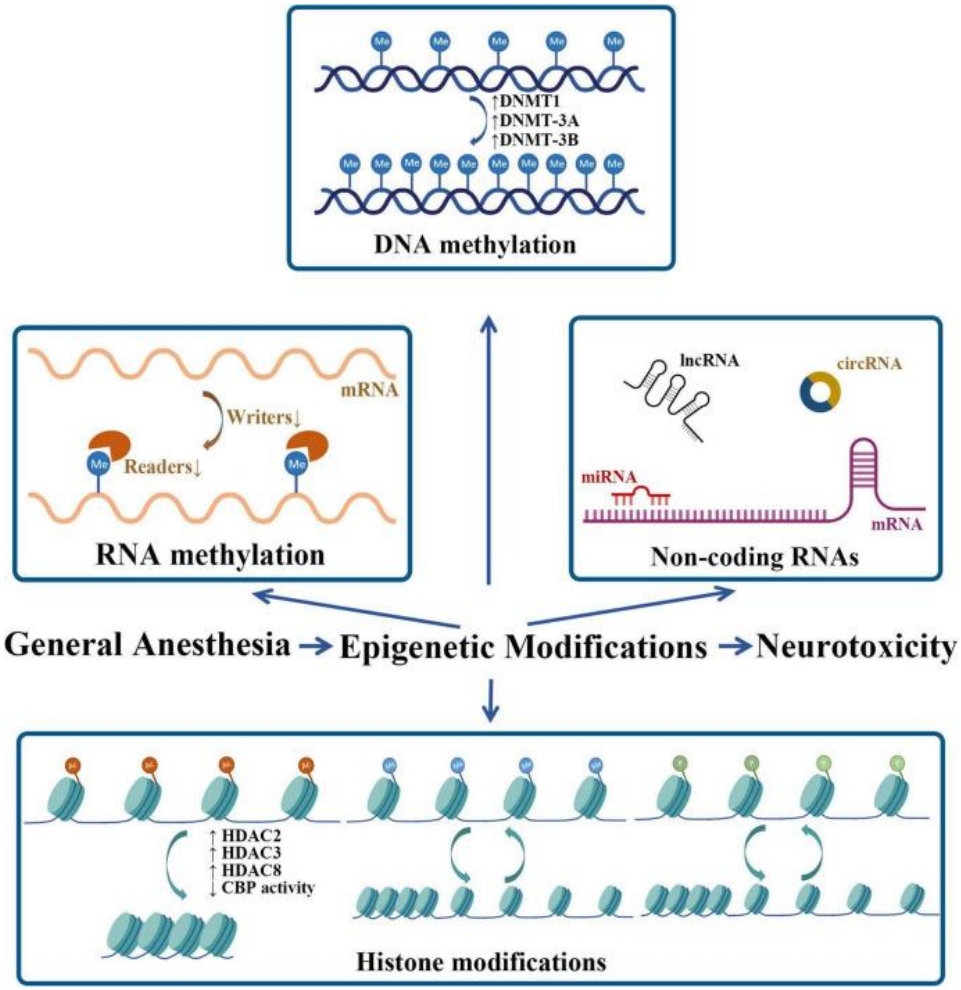


Figure 3. General anesthesia induced epigenetic modifications in infants (32).

General anesthesia triggers epigenetic changes which results in downregulation of GABAergic system by reduced synthesization of GABA-synthesizing enzymes (GAD65 and GAD67) and of the brain-derived neurotrophic factor (30).

DNA methylation and the histone modifications have been studied for longer period of time but instead the research about post-translational modifications of non-coding RNA and RNA methylation are only few so far. Hypothesized cognitive impairment induced by general anesthesia may be caused by the epigenetic alterations. In current studies post-translational modifications of the histones mainly focuses on histone acetylation but seldom on methylation. Other important modifications such as histone phosphorylation is not studied well enough yet (32).

6.4 ANESTHESIA IN NEONATAL AND INFANCY PERIOD

For over decade studies have presented alarming findings of general anesthetic exposure to non-human primates and how detrimental it can be to their brain. Data of the studies have revealed widespread apoptosis of neurons and oligodendrocytes as well as cognitive, motor, and behavioural problems (14,29,31).
Evidence have suggested that the exposure to commonly used gaseous and intravenous general anesthetics induces the biochemical and morphological changes in the immature neurons ultimately resulting in their demise. Long after the initial anesthesia exposure significant cognitive and behavioral impairments has also been observed (33).

Most commonly used general anesthesia inducing substances; propofol, isoflurane, sevoflurane and ketamine is found to have neurotoxic reactions.
Anesthesia in adults produces only minimal side effects and usually they are not long-term. This has led to false believe that brain of an infant is miniature of adult brain. Many important processes, such as synaptogenesis, neurogenesis, synaptic neurotransmission and myelination continues after birth during infancy, and they are responsible for healthy brain development and structure (14,29).

6.5 BRAIN STRUCTURES

The central nervous system is made up of grey matter and white matter, grey matter making up the outer most layer of the brain. Both are essential sections of the brain and spinal cord. Grey matter has it tone from high concentrations of neuronal cell bodies (34).

Both animal and human studies show structural changes to infant brain exposed to anesthesia. White matter and gray matter alterations all have been observed (3,20,35). Anesthesia in infancy can lead to synaptic changes and alterations in myelination process. Pathological neuroapoptosis in several critical brain regions may also take place (10,14,15,21).

White matter is important, highly evolving part of developing infant brain. It is responsible for cognitive abilities, language, and visual-spatial working memory in children at older ages (36). Gray matter is responsible of many important steps in infant development being responsible for cognition, learning, speech, voluntary movements, as well as sensation and perception.

The ability of white matter fiber tracts to control specific nodes depends not only on the connection strength within the structural connect, but also on the grey matter volume at the host nodes. Research shows that the interaction between connectivity strength and grey matter volume plays a key role in the brain's control functions (37).

6.5.1 GREY MATTER

Anesthesia induced neurodegeneration and changes in the gray matter in a variety of brain regions has been observed as well as changes in myelination process (20). Disproportionate cell death between excitatory and inhibitory cells induced by anesthesia exposure can lead to a long-term shift in the excitatory/inhibitory balance, which affects both learning-specific networks and sensory systems.

Backeljauw et al. (38) performed retrospective study, observing the decrease of overall volume of the gray matter with anesthesia in children aged less than 4 years. They used T1-weighted magnetic resonance imaging and two different neurocognitive assessment methods, Oral and Written Language Scales and the Wechsler Intelligence Scale for comparison.
Figure 4. presents brain regions of interest, superimposed with white color. Focus was on the thalamus and retrosplenial cortex.

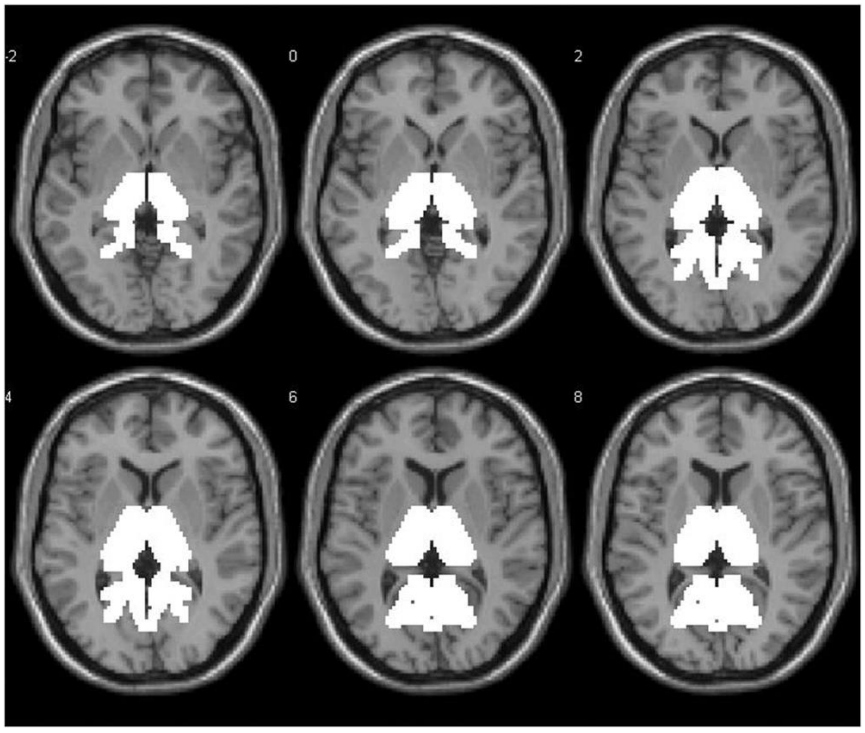


Figure 4. T1 weighted MRI image of grey matter decrease. Decrease of overall volume of the gray matter with anesthesia in children aged less than 4 years was observed (38).

Significant, widespread, apoptotic neurodegeneration of immature brain cells on rats and rodents have been observed in multiple studies (39,40). Especially apoptotic processes in the thalamus and retrosplenial cortex have been associated with neurocongnitive changes and learning difficulties. Significant grey matter volume reduction in these regions could have been expected to be detectable not only in rats and rodents but also in humans- yet it was not. Many factors could explain the difference; degrees of neuroplasticity, exposure times, and assessment methods and most importantly the specie difference.

T1-weighted image sections in Figure 5. presents decreased gray matter volume in the posterior brain regions in correlation with decreased performance IQ for children who underwent surgery with anesthesia before their fourth birthday when compared with matched, unexposed peers. Blue and red color demonstrates steeper correlations between decreased performance IQ and diminished regional gray matter volume. Areas were localized to the cerebellum, the occipital lobe/lingual gyrus, and the orbitofrontal cortex.

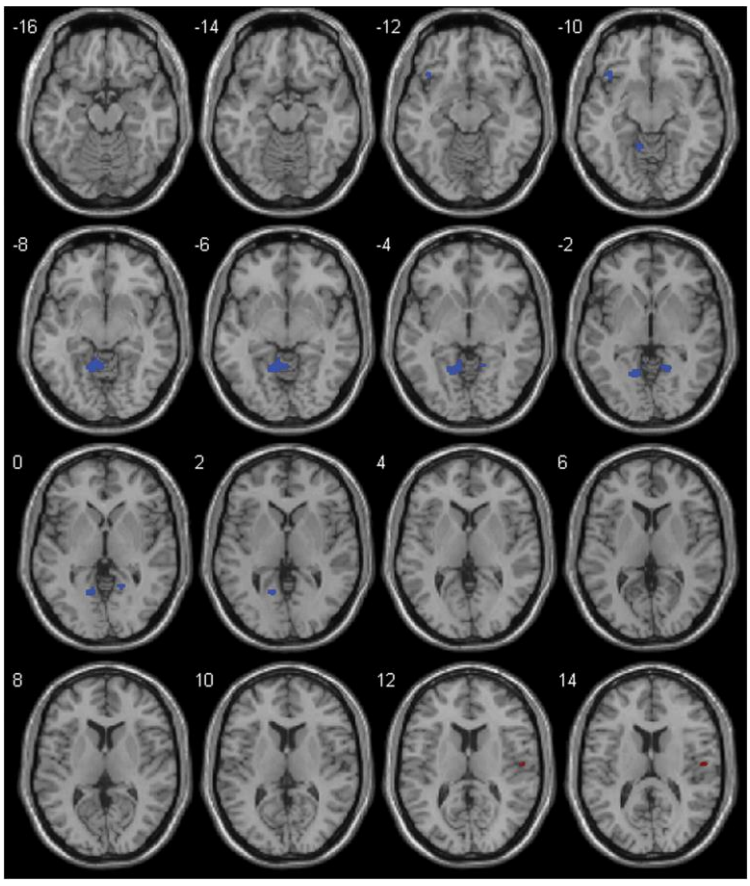


Figure 4. Grey matter volume decrease in correlation with IQ. Decreased gray matter volume in the posterior brain regions was observed to correlate with decreased performance IQ (38).

Study of Backeljauw et al. (38) concluded that surgical exposure did not result in noticeable neuronal loss in brain regions previously identified in animal models. However, children who were exposed showed reduced language comprehension and performance IQ, which were linked to decreased grey matter, mainly in the posterior brain regions. No significant associations were found between changes in white matter volume and neurocognitive performance scores in this study (38).

6.5.2 WHITE MATTER

White matter development is a complex process, commencing already in utero and extending into adulthood. Peak of the development happens around the 4th decade of life. Myelination of axons in white matter begins at 20th week of gestation and it persists throughout adolescence and adulthood, modifications can occur during whole lifespan (41).
White matter is vital part of neuronal communication and disruption is proven to cause neurodevelopmental disorders (42–44).
Myelination is provided by oligodendrocytes in the brain, their role is to preserve myelin, provide metabolic support to neurones and enhance viability, regulate homeostasis and trough plasticity of white matter also affect cognition and behaviour (41).

Non-human primate study conducted 2021 by Young et al (3) showed results of white matter integrity changes in the brain of rhesus monkeys after multiple exposures to general anesthetics. There was a significant correlation between total normalized exposure and the extent of impact on white matter microstructure and to overall reduction in the integrity of white matter tracts- but not significant correlation with age or sex.
Other animal study on infant rhesus monkeys by Creeley et al. 2014 (35) showed white matter alterations in many structural regions of the brain, being most prominent in caudate nucleus, putamen, nucleus accumbens, amygdala, and several divisions (frontal, parietal, temporal) of the neocortex- all of which are important part of the development. Both glial and neuronal response and apoptosis was seen.
In non-rodent animal study anesthesia induced neuroapoptosis in gray matter happened approximately in the same level compared to changes in white matter (35).

Disruptions in oligodendrocyte lineage cells and deficits in myelination have been observed in neuropathological studies as vital elements in anaesthesia-induced developmental white matter

alterations. With a help of advanced magnetic resonance imaging both macrostructural and microstructural cerebral changes associated with anaesthesia exposure has emerged (41).

Figure 6. presents a visualization of expected average effect of anesthesia exposure in non-human primate of two different study groups. Neuroimaging data on 28 subjects were obtained from the Wisconsin Neurodevelopment Rhesus Database, Harlow Primate Laboratory (upper row) and second data set of 15 subjects from Yerkes National Primate Research Center (middle row). The lowest from the rows is the comparison group (3).

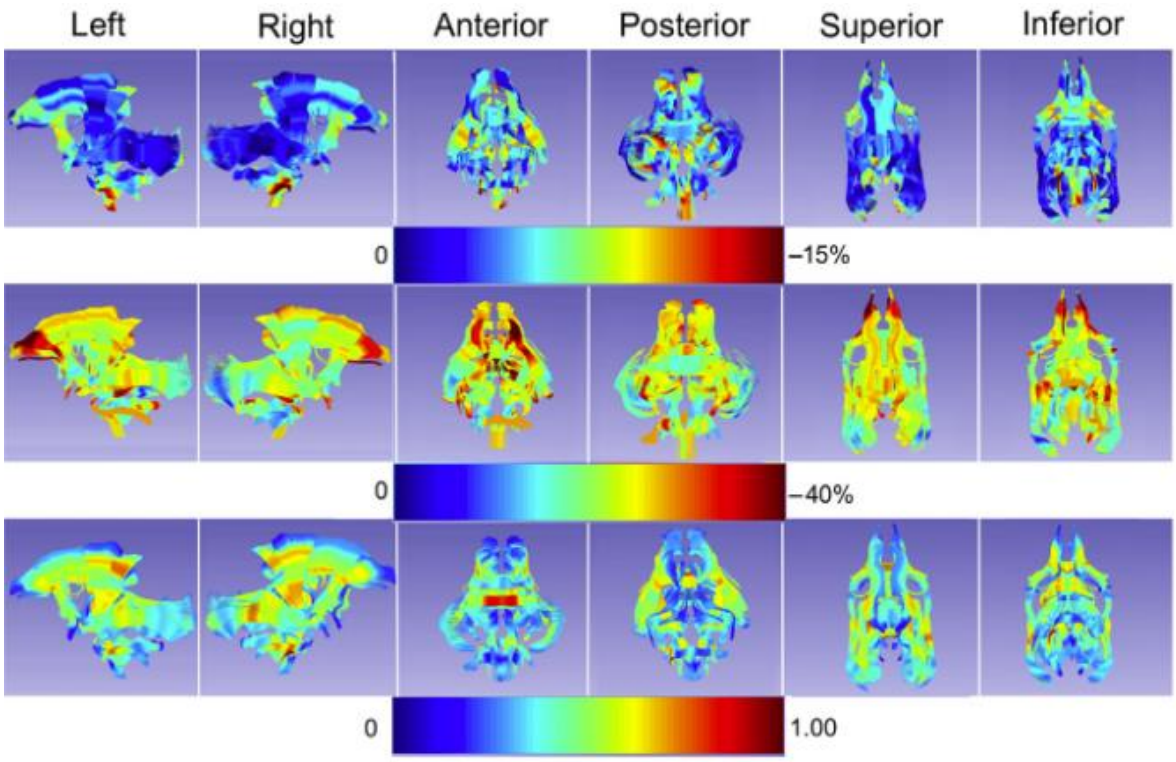


Figure 6. Expected average effect of anesthesia exposure in non-human primate of two different study groups (3).

Apoptotic cell death distribution of grey matter and white matter is visualized in figure 7. General anesthesia on non-human primate was induced with 5 hour isoflurane administration compared to control subject (35).
Red dots present neuronal profiles and white dots oligodendrocyte profiles. Computer plot display the location of activated caspase 3 in midrostrocaudal (A and B) or cerebellar/ brain stem (C and D) level of a fetal control brain (A and C).

The apoptotic effect was mostly seen in red and white dot dense areas, the frontal and temporal cortices and all folia of cerebellum. Vulnerable oligodendrocytes were distributed to the location of major axonal pathways in the white matter.

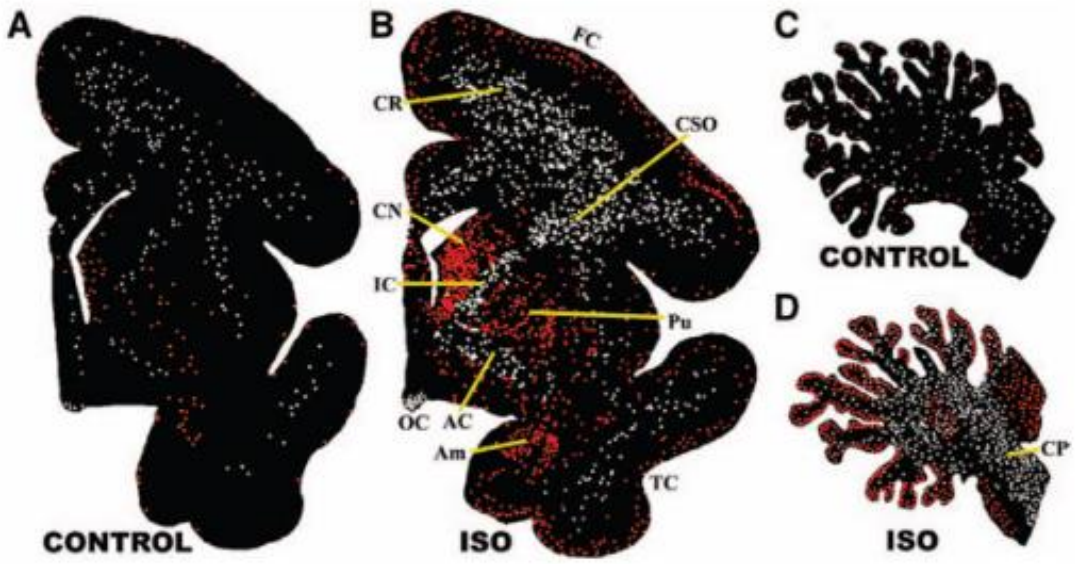


Figure 7. Neuroapoptosis after isoflurane administration in non-human primate. ISO=group of isolurane anesthesia , CONTROL=control group, FC=frontal cortical area TC= temporal cortical area, CN= caudate nucleus, Pu=putamen, Am=amygdala, CR=corona radiata, CSO=centrum ovale, IC=internal capsule, AC=anterior commissure, OC=optic chiasm, CP=cerebellar peduncle (35).

Anesthesia induced oligodendrocyte apoptosis in heavily myelinated pathways are shown in figure 8. Creeley et al. (35) used different methods to evaluate apoptotic processes, activated caspase-3, silver and myelin basic protein(MBP). All the methods detected cell death. Fluorescent with MBP revealed dying cells positive for fractin (marker of apoptotic cells), but there are were no MBP-positive cell bodies. Two additional markers of apoptosis, silver and caspase-3, confirmed the findings.

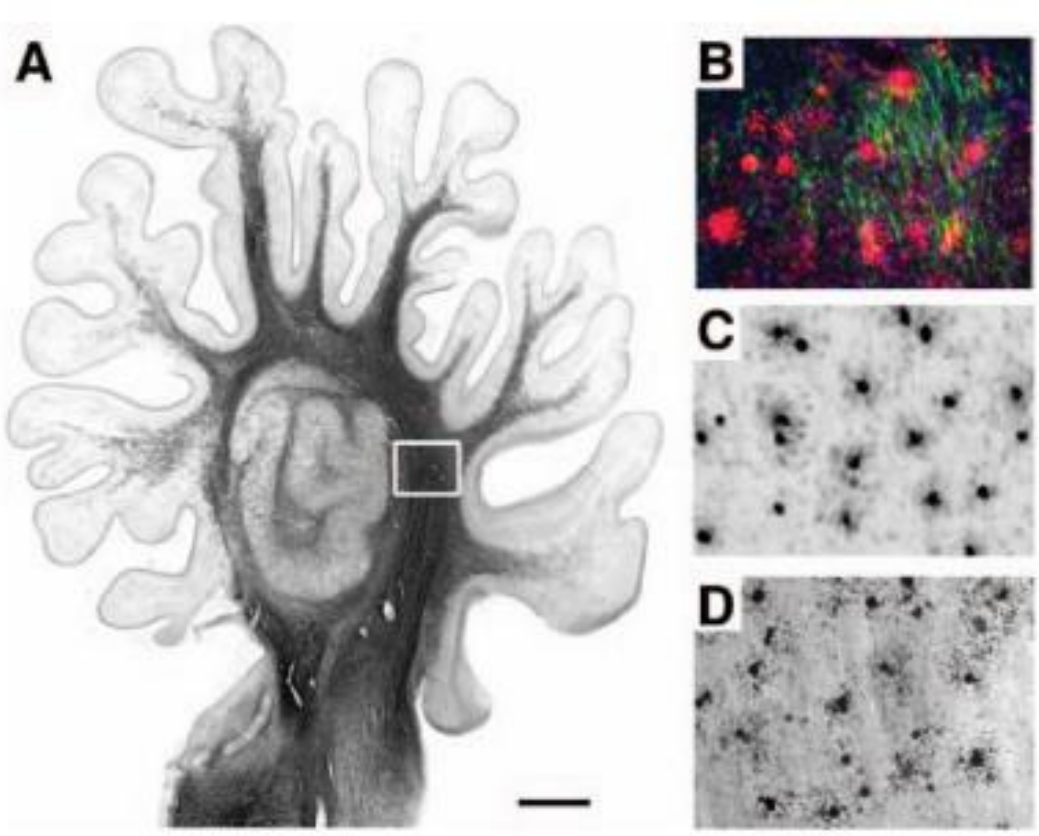


Figure 8. Oligodendrocyte apoptosis in the cerebellum of non-human primate A: cerebellum, bright field image, B: fluorescent with myelin basic protein (MBP) C: activated caspase-3 D: silver method (35).

Neuronal apoptosis in frontal cortex as well as apoptosis of glial cells was also observed in isoflurane anesthesia of non-human primates. Figure 9. And 10. visualizes apoptosis, Creeley et al. used activated caspase-3, silver, fractin, neuronal marker (NeuN) and glial fibrillary acidic protein (GFAP) to reveal cell death in microscopy.

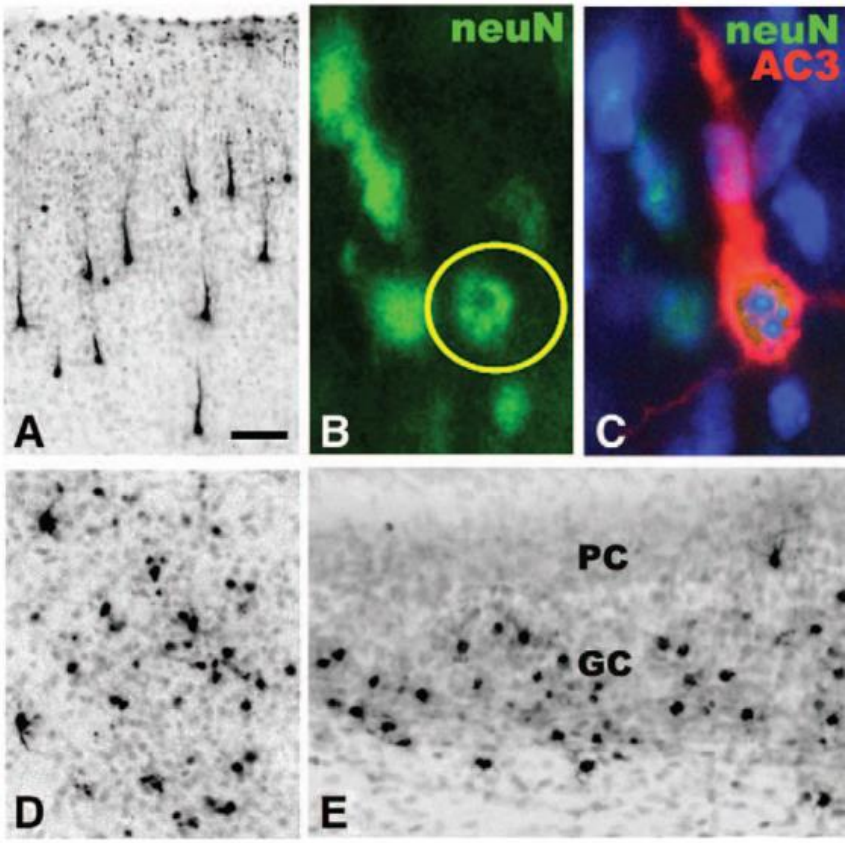


Figure 9. Frontal cortex neuronal apoptosis induced by isoflurane anesthesia (35).

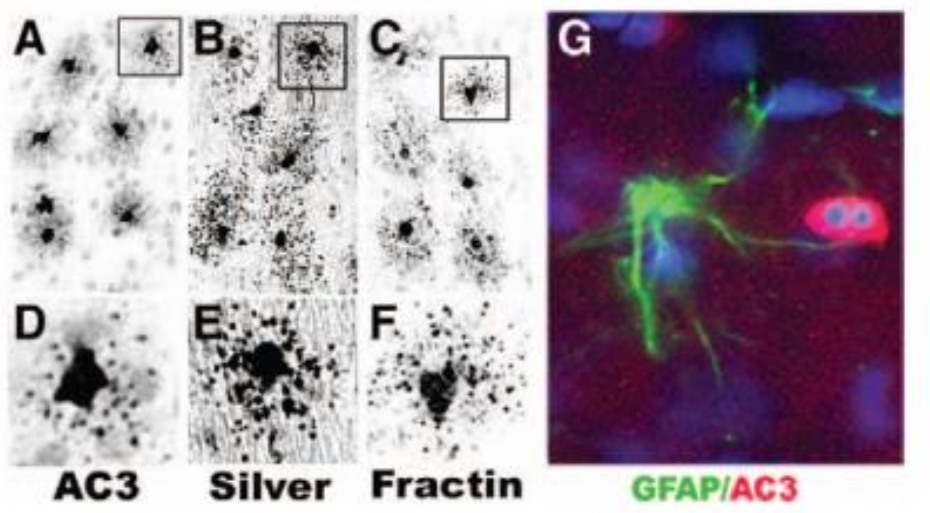


Figure 10. Frontal cortex glial cell apoptosis in white matter (35).

Brain maturation defects happens also in neonate rodents after sevoflurane anesthesia. (40) Histological images of the sevoflurane exposed rodents reveal same apoptotic processes as isoflurane exposed non-human primates (35,40). Figure 11. and figure 12. presents results from lateral area, saggital plane of the brain and thalamic area, where apoptosis is pointed with activated caspase-3, NeuN and Hoechst33342 (40). Increased cell death is clearly seen, especially in the multiform layers of the cerebral cortex and internal pyramidal

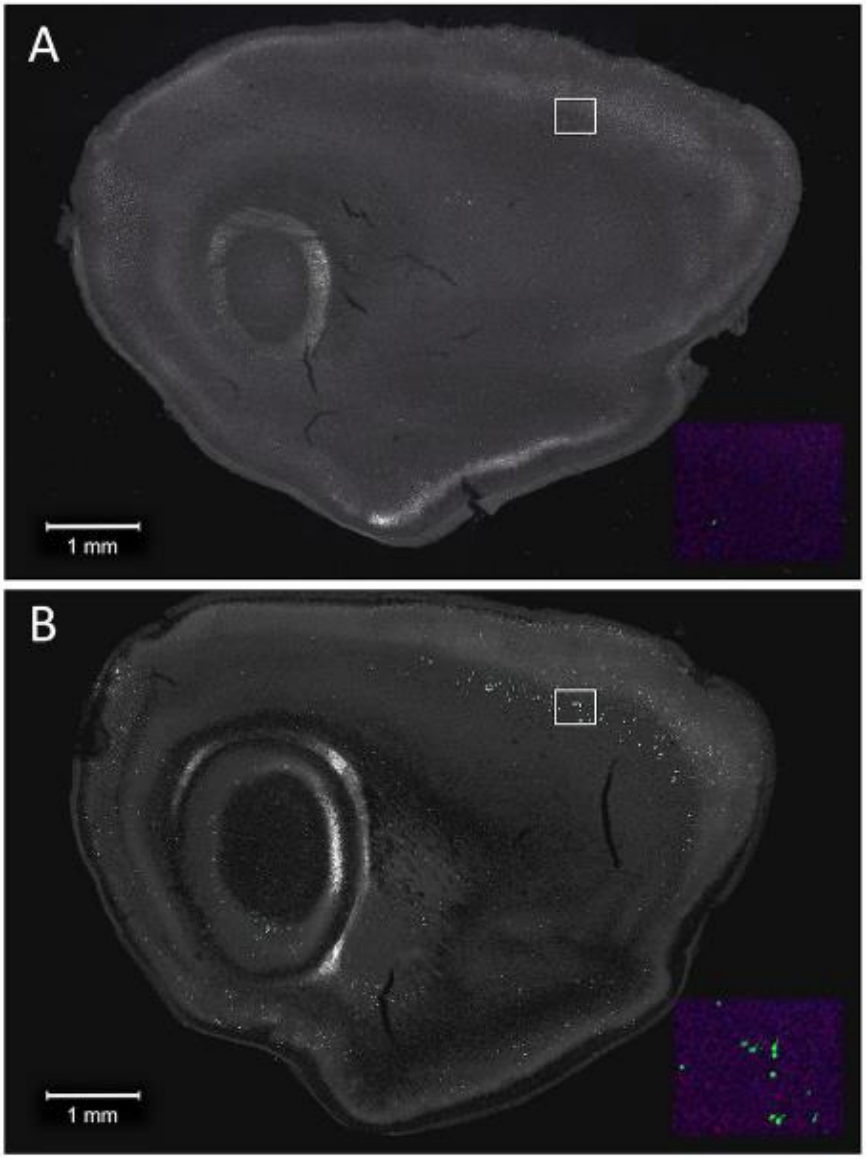


Figure 11. Thalamic neuroapoptosis of rat brain. Microscopy pictures of the lateral sections of rat brain. A presents unexposed control, and B presents rat after five hour sevoflurane exposure. Green color presents activated caspase 3, red color NeuN and blue color Hoechst33342 (40).

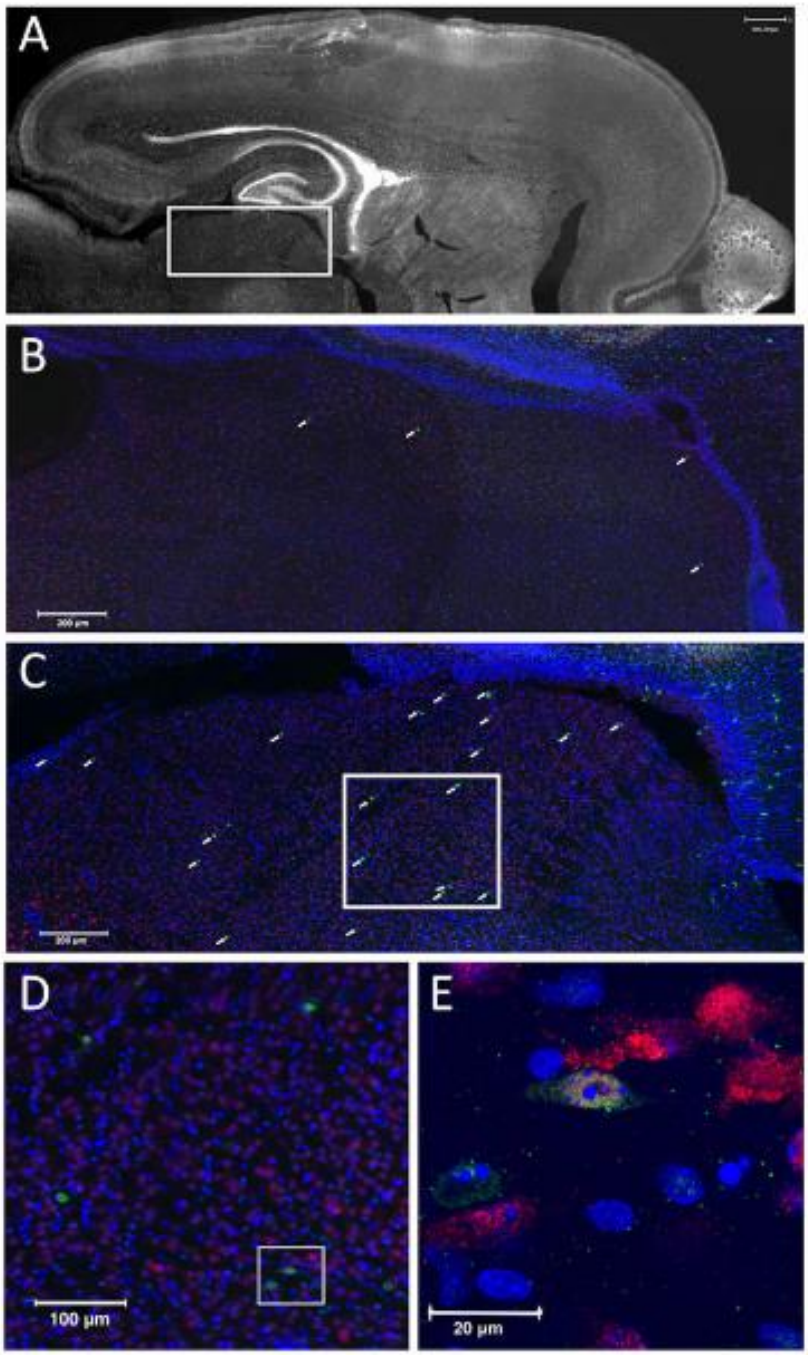


Figure 12. Thalamic neuroapoptosis of rat brain. Microscopy of the thalamus of the rat brain, located in the image A. Green color presents activated caspase 3, red color NeuN and blue color Hoechst33342. B image presents unexposed rat and C image five hour sevoflurane exposed rat. In the image D and E higher magnification is used to present apoptotic process more precisely (40).

Walsh et.al 2021 (45) described an association between structural alterations in brain and cognitive and motor deficits after exposure to general anesthesia of preterm born infants. General anesthetic used was flurane-based volatile gas, fentanyl was combined as an analgesis.

Infants were monitored at the age of two years with cognitive assessment (Bayley Scales of Infant and Toddler Development, 3rd Edition) and MRI with fractional anisotropy mapping. Infants with longer exposure to anesthetic drug, had the greatest relative changes in white and grey matter volumes, and lowest cognitive and motor scores during early childhood. Association with the brain tissue volumes was only significant when comparing relative volumes. Results were in line with other similar animal and human studies (27,31,33,39). Anesthesia lowers the volume of infant brain white and cortical gray matter. Figure 13 by Walsh et al. shows how white matter tracts were affected.

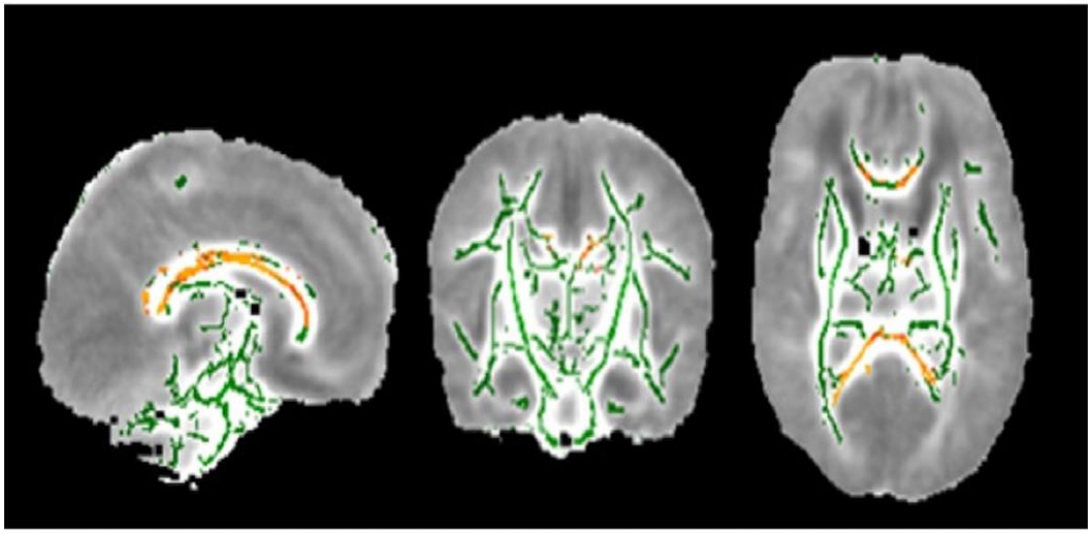


Figure 13. MRI imaging of infant brain, tract bases spatial statistics and fractional anisotropy mapping. Red and orange tracts indicate decreased fractional anisotropy and green colour indicates no difference in fractional anisotropy (45).

6.5.3 SYNAPSES AND APOPTOTIC PROCESSES

Synaptic plasticity is the fundamental mechanism by which neurons process, encode, and store activity-dependent information (31). Definition can be quite simple but synaptic plasticity itself has multidimensional complexity. Clinical studies in humans and laboratory investigations in animal models suggest that exposure to general anesthetic agents have harmful effects on brain development (17). Synaptogenesis requires precisely coordinated chemical, morphological, and genetic signals (12). Recent evidence suggests that many of the today's anesthetics might cause the disruption of the key events in synaptogenesis.

In the study of 2018, Xu et al. (17) found that general anesthetics disrupts the synaptogenesis in primary neuron culture via mTOR pathway. The research included the use of isoflurane, sevoflurane and propofol and the conclusion was that all them have the capacity to up-regulate

signalling in both branches of the mTOR pathway, mTOR1 and mTOR2, in neurons during synaptogenesis.

Previously mentioned epigenetic changes altering the plasticity of neural circuits and biochemical processes are not the only changes when it comes to synaptic transmission, studies also show structural changes. Anesthesia in infancy is a powerful modulator of dendritic spine density. In subcellular level study found neuropil to be vacuous and disorganized with dearth of presynaptic axon terminals. Many synapses were actively undergoing destruction weeks after anesthesia exposure. Not only mitochondria density is reduced at synaptic terminals, but also the mitochondria degeneration, swelling and damage to inner cristae has been observed (29).

Non-human primate study (35) examined the effects of five hours of general anesthesia exposure on the fetal brain of Rhesus macaques, using Isoflurane as the anesthetic. The results revealed a significant increase in apoptosis of both neurons and oligodendrocytes, particularly at a stage when oligodendrocytes were just beginning to myelinate axons. The cerebellum, caudate, putamen, amygdala, and several cerebrocortical regions exhibited the highest levels of neuroapoptosis. Overall, the apoptotic process was 4.1 times higher compared to the brains of drug-naive control subjects.

Also non-volatile anesthetics have been investigated. Research by Creeley et al. (47) used propofol as an anesthetic for non human primates to see if propofol would cause same pattern of apoptosis as volatile anesthetics.
As a result a significant increase in apoptosis of neurons and oligodendrocytes was seen, mostly in the same areas of the brain where apoptotic processes takes with volatile agents. Apoptotic neurons were mostly found in several layers of the frontal cortices and temporal cortices, the caudate nucleus, putamen, amygdala, and in cerebellum and inferior colliculus of caudal regions. Apoptotic oligodendrocytes were found diffusively distributed to white matter regions, including the centrum semi-ovale, corpus callosum and internal capsule. Figures 14. visualizes the findings.

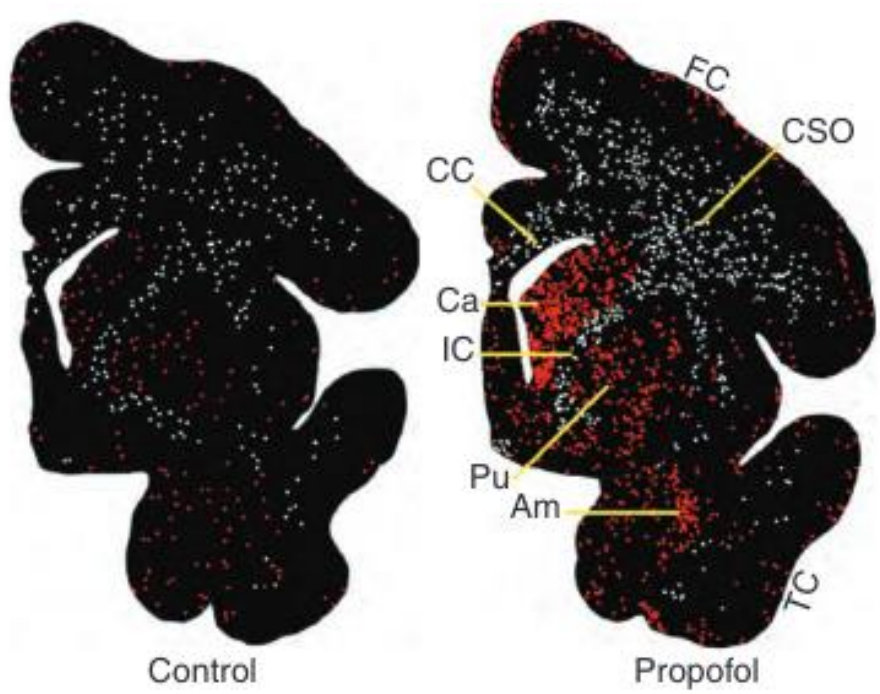


Figure 14. Apoptotic oligodendrocytes. Red dots present apoptotic neurons and white dots apoptotic oligodendrocytes. Ca= caudate nucleus, Pu=putamen, Am=amygdala, CSO= centrum semi-ovale, CC= corpus callosum, IC= internal capsule (47).

Magnitude comparison of isoflurane induced neuronal apoptosis and propofol induced apoptosis is presented in figure 15. Both reveal laminar pattern of apoptosis, isoflurane in the greater magnitude.

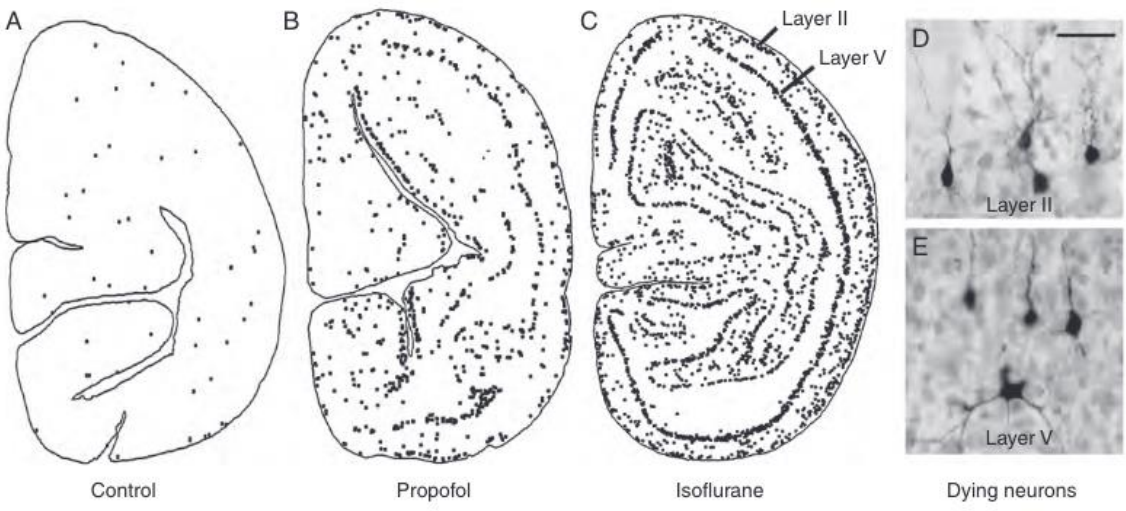


Figure 15. Propofol and Isoflurane induced apoptosis. Computer plots in the images A-C presents the location of apoptotic neurons in the section of primary visual cortex. Control group, propofol exposed group and isoflurane exposed group are all visualized (47).

For obvious ethical reasons, reliable histology including microbiological human studies from recent years are non-existing. Permanent neuronal apoptosis, cell death of glial cells and oligodendrocytes as well as disruptions of synaptogenesis have all confirmed in infant non-human primates and rodents. Substantial concern is raised if similar effects occur in children, yet most human studies suggest that not as devastating results occur in human infant compared to animals (38).
Even though not complete elimination of grey matter is proven to happen in human infants, the lower density of grey matter follows the anesthetic exposure (38,45,46).

7. NEURODEVELOPMENTAL OUTCOMES

What it comes to neurodevelopmental outcomes of anesthesia during infancy, studies have shown different conclusion ranging from no effect at all to detrimental effects. Human studies and animal studies both exist.
Many animal studies prove that general anesthesia has it's effects to structures of the brain and cognitive development (3,10,35,48,49) whereas human studies are still showing inconsistency in their findings (22,50–53).
Both human and animal research agree that worse consequences are in correlation with higher dose of anesthetic substance, duration of anesthesia and multiple repeated anesthesia.

To the year 2025 only one published randomized clinical trial of the effects of general anesthesia to human neurodevelopment exist, the GAS trial (22). All the other studies presented are observational and include their limitations.
Observational studies have the inability to determine the etiology or the reason behind the found effect. Other possible mechanisms to effect are example the surgical procedure itself, the psychological trauma or other underlying medical conditions.
Also, observational studies uses many different assessment methods to monitor the outcomes of general anesthesia making them hard to be compared or generalized. Many of them have unknown amount of general anestethic dosing and varying time of exposure. Monitoring the depth of anesthesia, example EEG, is not well presented or known by the studies.
Most of studies are limited to certain ethnic groups, social class or demographic area.

7.1 HUMAN STUDIES

This chapter presents the most important human studies from the recent years, comparing the consistency in their findings. Neuronal cell apoptosis, changes in synaptogenesis and volume decrease of white and grey matter could be hypothesized to have their consequences to cognition. Neurodevelopmental outcome at 5 years of age after general anaesthesia or awake-regional anaesthesia in infancy (GAS) trial, ongoing Pediatric Anesthesia & Neurodevelopment Assessment (PANDA) project and The Mayo Anesthesia Safety in Kids (MASK) study are discussed first as they can be considered the most important studies of the field. These are followed by other quality studies.

Randomized clinical trial, the GAS trial (22), could be considered to be one of the most important studies in the field. This research was made to measure the long-term effects of two commonly used modes of infant anesthesia; regional, spinal, anesthesia and general anesthesia using sevoflurane as an anesthethic substance. Goal was to determine if both methods result in equivalent neurodevelopment outcomes.
719 infants were included to research, and they were collected in multi-central manner where researchers from Children's Hospital Boston collaborated with researchers from nine other centres in the United States, as well as centres in Canada, Australia, Europe, and the United Kingdom.

Infants undergoing hernia surgery were randomly assigned to receive general or spinal anesthesia and were tested at the age of two and five years. Duration of general anesthesia was 54 minutes by average. At the age of 2, developmental testing was done and in the age of 5 neurodevelopmental and intelligence testing was done. Determination whether there were neurocognitive differences between the groups was done.
The method used to assess the children was the Wechsler Preschool and Primary Scale of Intelligence Third Edition Full Scale Intelligence Quotient (WPPSI-III FSIQ). The scoring analysis 79 or below: Below average IQ, 80 to 89: Low average IQ, 90 to 109: Average IQ, 110 to 119: High average IQ.
The mean FSIQ score was 99.1 in the awake regional anesthesia group and the same, 99, in the general anesthesia group, stating the average IQ. Mean difference was 0.2 (95% CI -2.6 to 3.1). The majority of secondary behavioural and neurocognitive outcomes were also similar between groups. Conclusion drawn was that single short exposure to general anesthesia with sevoflurane in the first year after birth showed no difference in intelligence quotient (IQ) at age five. It is important to note

that GAS trial did not evaluate behavioural problems and did not have follow up beyond the age of five.

The Pediatric Anesthesia Neurodevelopment Assessment (PANDA) project was developed and designed over ten year ago by the interdisciplinary team of professionals aiming to address the clinical relevance of anesthetic neurotoxicity in children. Animal and human studies differ in their results and the goal of PANDA study was to present more definite evidence and conclusion. Since establishment seven annual PANDA symposiums have been taken place. The last Symposium was held February 19th 2022.

The pilot study of PANDA project (53) used sibling matched comparison evaluating the effects of anesthesia to neuropsychological functions and behavior. Siblings were used in order to minimize the effect of genetic background, familial environment, parental education, and other indexes of socioeconomic status- which are all key factors affecting neurodevelopment. Age range of 0 to 36 months (anesthesia before age of three) was chosen as the peak of synaptogenesis occur in various human brain regions on that time (54).

PANDA study concluded that when comparing healthy children with a *single* anesthesia exposure before age 36 months to their healthy siblings with no anesthesia exposure, there were no statistically significant differences in IQ scores in later childhood.

The Mayo Anesthesia Safety in Kids (MASK) Study (52) observed adverse neurodevelopmental outcomes of *multiple* anesthesia exposures. Sample was collected from children with anamnesis of multiple anesthesia exposures before the age of three and the primary evaluation method was the Full-Scale intelligence quotient (IQ) standard score of the Wechsler Abbreviated Scale of Intelligence. Secondary method also included individual domains from a comprehensive neuropsychological assessment and parent reports.

In the primary outcome measured with Full Scale IQ score, multiple anesthesia exposures were not associated with significant differences in general cognitive ability relative to unexposed children. Regarding secondary outcomes, multiple, but not single, exposures were associated with decreases in reading and fine motor coordination skills in speed task related tests, but not on the other psychometric domains. The parents of multiply-exposed children reported more behavioral problems, problems with executive function and reading and the parents of singly-exposed children reported more problems related to executive function and reading but not the behavioral problems.

GAS trial, PANDA project and MASK study are in line with one another. GAS did not find neurocognitive alterations and also PANDA and MASK concluded the lack of evidence of neurocognitive changes in their primary results. However, major finding in secondary outcomes was found to be consistent with all of the three studies. Parent reports significant findings in the area of emotions, behaviour and executive function (cognitive abilities, guiding, directing, behavioural and emotional functioning). As a scientific proof, parent reports have their admittable limitations but in whole clinical context and evaluation they are essential component.
MASK study gave also additional interesting result as it examined the multiple anesthesia exposures which were associated with many neurocognitive alterations.

Swedish nationwide cohort study 2017 (50) compared 33 514 children exposed to general anesthesia (22 484 male and 11 030 female) and 159 619 unexposed children (105 812 male and 53 807 female) concluding that exposure to anesthesia and surgery before age 4 years has a small association with later academic performance or cognitive performance in adolescence on a population level.

Canadian retrospective cohort study 2016 (51) analyzed 3,850 children exposed to a single general anesthetic, 620 children exposed to two or more, and over 13,000 unexposed children. The study used the Early Development Instrument (EDi), a 104-item questionnaire assessing five developmental domains, completed in kindergarten, as the outcome measure. The findings showed no association between anesthesia exposure before the age of two and EDi scores. However, for children aged between two and four, both single and multiple anesthetic exposures were linked to lower EDi scores. Exposure to a single anesthetic before the age of four was associated with small but statistically significant neurodevelopmental deficits, particularly in the communication/general knowledge and language/cognitive domains.

A population based cohort study by O'Leary et al. 2019 (55) investigated the association between surgery done early life and child development at the time of primary school entry. Assessment method used was Early Development Instrument (EDi). Suggestion was drawn that children who undergo surgery before primary school age are at increased risk of early developmental vulnerability, but the magnitude of the difference between exposed and unexposed children is small. Findings are mostly consistent with Canadian retrospective study (49) using the same analysis method.

Human study by Backeljauw et al. (38) compared healthy participants from a language development study, aged 5 to 18, who had undergone surgery with anesthesia before the age of 4, to unexposed peers matched for age, gender, handedness, and socioeconomic status. Neurocognitive assessments included the Oral and Written Language Scales and the Wechsler Intelligence Scales (WAIS or WISC), depending on the participant's age. Structural brain comparisons were made using T1-weighted MRI scans, which revealed a decrease in grey matter volume, as detailed in chapter 2.5. Study found also that from cognitive view previously exposed children scored significantly lower in listening comprehension and performance IQ.
Research show inconsistency in findings as a study of Cognitive Performance in Children Aged 7–11 years after general anesthesia in the first 3 years of life (56) by Schüttler et al. 2021 indicated that general anesthesia in early childhood is not associated with markedly reduced intelligence in later years, although noninferiority could not be demonstrated.

General anesthesia is shown to increase the risk of attention and autism spectrum disorders. Pikwer et al. 2022 (57) searched the correlation between infancy anesthesia exposure and autism or autism spectrum disorders and found the risk to be higher in children gone through anesthesia in early childhood. In other follow-up cohort study conducted 2021 (55) concluded that exposure to anesthesia and surgery at a young age, including just a single exposure, is associated with an increased incidence of ADHD.

Comparative analysis in the year of 2014 (58) with children exposed to anesthesia before the age of three and between their unexposed piers was done by Ing et al. They assessed three types of outcomes at age of ten years; neuropsychological testing, International Classification of Diseases 9th Revision Clinical Modification-coded clinical disorders, and academic achievement.

In assessing the individual outcomes in the restricted cohort of 781 children, the incidence of deficit in each outcome was found to be range from 5.1 to 7.8% in the neuropsychological tests, 14.6 to 29.5% in the ICD-9–coded clinical outcomes, and 4.2 to 11.8% in the academic achievement tests. Important finding was that result of neurocognitive function assessment may be depended on the analysis method itself.

7.2 ANIMAL STUDIES

In recent years many studies using animal models have focused to pathophysiological, cellular and histological level. They offer valuable knowledge from mechanistic and molecular processes which

would not otherwise be available from human studies. Animal studies offer something like manipulated and controlled experimental studies, histological analysis of tissues after anesthesia as well as standardization of methods. In the part of infant general anesthesia effects to brain structures, many important findings were presented.

What it comes to neurocognitive consequences of infant anesthesia, human studies could considered to be most relevant and animal studies mostly offer useful information from brain structural point of view. Nevertheless, animal studies should not be undervalued because they offer possibilities for clinical research studies which would be markedly harder and more expensive to organize with human population.

A variety of animal models have been utilized to investigate the developmental neurotoxicity linked to both short-term and prolonged exposure to common general anesthetics at clinically relevant concentrations. Pediatric anesthesia models involving nonhuman primates (NHPs) may provide a more accurate representation of the human condition due to their close phylogenetic similarities to humans in terms of reproduction, development, neuroanatomy, and cognition. (59).

Prolonged exposure to commonly used general anesthetics have confirmed to have neurotoxic effect to immature brain. Many animal studies with non-human primates (60–62), rodents (19,40,63) and other animals (64,65) have been conducted to reveal the neurocognitive effect of neurotoxic anesthetics. Animal studies reveal the same consequences to the neurodevelopment as human infant studies but most of them as greater extent.
Interestingly, literature and rodent studies shows that environment and time after exposure has also role on recovery. Infants who have buffering relationships tend to recover much better than those who do not have (3,13).

According to animal studies learning and memory functions are affected by the neurotoxicity of general anesthetics. Both reward- and aversion-based learning paradigms have shown to be changed (31). Poor learning and memory capacity appears to extend to nonspatial memory as well, being a replicable and stable outcome of infant anesthesia.
Non-human primate study suggests the long-term consequences of infant non-human primate general anesthesia affects both, motor, and socio-emotional aspects of behavior. Effects are revealed to be dose-dependent, with multiple exposures resulting in more profound changes. Socioemotional effects is particularly seen as long-term heightened emotionality (60).

Multiple studies report the same outcomes and come up with alike conclusions. Infant anesthesia is associated with increased risk of learning and language disability, impaired executive function, and deficits in internalizing behaviour (29,31). Also anxiety and increased reactivity to stressors are found to increase in infant animals exposed to repeated anesthesia (60,66).

2023 Zuo et al. (4) conducted study with infant mice, hypothesizing that anesthesia induced neurocognitive changes may be caused by dysfunction in iron metabolism. Sevoflurane was used as an anesthetic substance. Study found that sevoflurane inhibited the oligodendrocyte proliferation and myelogenesis causing significant cognitive deficiency. What is more, it also induced iron deficiency.

8. RESEARCH RESULTS AND DISCUSSION

Brain of an infant is remarkably malleable and plastic organ. A single short exposure of neurotoxic event during infancy is unlikely to have any long-lasting consequences even if it initially led to alterations in the number of brain cells or the number of connections simply because brain has extraordinary capability of rebounding. To give example- if there are 20 neurons in a circuit and five of them dies during general anesthesia lasting one hour, other neurons will step up and replace those five. Yet, anesthesia in infancy is a serious concern and during last few years gotten more needed attention, many new studies and amount of research is rising.

Most studies agree with the fact that unwanted consequences of anesthesia to infant neurocognitive development and brain structures are in correlation with higher dose of anesthetic, longer duration of anesthesia and multiple exposures. Also, the state of neuron development is crucial as immature neurons are more vulnerable to effects of anesthetics.

Many external factors are shown to affect the recovery from anesthesia, infant should have safe, development enhancing and supportive relationships with their caregivers. Human and animal studies have suggested that wellbeing buffering environment could compensate the possible harm of anesthetics.

8.1 ALTERNATIVES TO GENERAL ANESTHESIA

To avoid possible neurocognitive changes of general anesthesia alternative methods to mitigate potential of adverse effects should be applied.

Firstly the use of regional anesthesia with local anesthetics combined with lighter planes of general anesthesia is an option to everyday practise. As the research shows correlation with neurocognitive changes and dose of anesthetic substance, combined regional anesthesia offers the possibility to use general anesthetic much less, while sleep state is still induced to infant. Ultrasound guided peripheral nerve blocks and other appropriate infiltrations like are the future in the field of anesthesia. Example of these peripheral nerve blocks are something like paravertebral blocks to major surgeries of chest wall or brachial plexus block to surgeries of the upper parts of the body. Central neuroaxial blocks-epidural, spinal and combined- should also be considered. Some operations could maybe be done without general anesthesia at all, example hernia surgeries done under spinal anesthesia in infancy.

As general anesthesia consists of three main essential components: amnesia/unconsciousness/hypnosis, analgesia and muscle relaxation the fine balance between these three should be found (Figure 16.). The use of opioids during anesthesia will lead to much less need of general anesthetic itself. Opioids blunt hormonal and metabolic autonomic responses and stress responses, when neurons are firing during surgically induced pain. Increasing the opioid use during anesthesia might be less harmful to infant compared the use of higher doses of general anesthetic but more research is still needed. In future opioids combined only with regional/local anesthesia would eliminate the need for general anesthetic substance entirely in some surgeries.

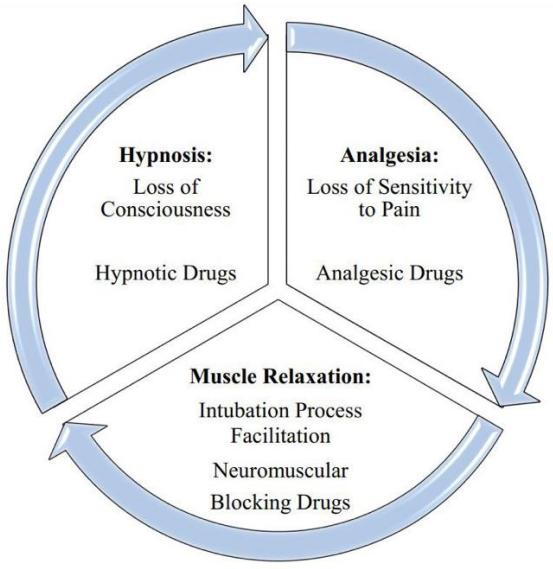


Figure. 16 Three essential components of anesthesia (67)

8.2 OTHER ANESTHESIA RELATED FACTORS

General anesthesia lowers the blood pressure, predispose to ischemic injury and ventilation problems. Only well-trained pediatric anesthesiologist together with multidisciplinary team should perform anesthesia to infant. Today we have many ways to monitor the vital functions and follow the child under anesthesia, constant blood pressure monitoring, pulse oximetry, capnometry, EEG, TOFF, depth of the anesthesia, just a mention most important ones.

8.3 TREATMENT

Several treatment options have been suggested for anesthesia related neurocognitive toxicity and promising results has also been found. To come up with treatment options the knowledge of the pathophysiology and molecular mechanism of anesthetic substances must be strong and meaningful. Fortunately, medical science has reached many major steps and in recent years our knowledge has been increasing greatly as the topic has gain lot of interest among experts and doctors in everyday practise.

To start with molecular mechanisms, we can go back to the very beginning, in the theoretical part of literature review under the topic 1.1. anesthesia and molecular mechanisms. The following considerations are all based on the effect of anesthetic substances to children and the molecular mechanisms behind the effect. To understand possible treatment modalities, one should be familiar with the theoretical background itself.

8.3.1 PI3K/Akt/mTOR, HIPK2/JNK/c-Jun, JAK/STAT AND AMPK

To remind, PI3K/Akt activation can reduce neuroinflammation and inhibit apoptosis (12). Panax Notoginseng Saponins and Hemin can inhibit neuronal apoptosis by activating the Akt signalling pathway, which in turn attenuates the neurotoxicity and cognitive impairment caused by general anesthetics (68,69). To be fair, this has only been tested with animal models and further research is still needed. In addition, Atractylenolide III has also been shown to produce the same benefits by activating the PI3K/Akt/mTOR signalling pathway, which can inhibit neuronal apoptosis (70).

Other promising signalling pathway that can be affected is the inhibition of the HIPK2/JNK/c-Jun. It may propose significance for treating neonatal brain neurotoxicity and cognitive impairment (15). Animal studies have shown that HIPK2/JNK/c-Jun pathway is activated when using isoflurane in neonatal rats, leading to neuronal apoptosis (17,71). Further research should be made to find out whatever other mechanisms are also involved.

As already mentioned, JAK/STAT signalling pathway can effectively inhibit the occurrence of inflammation in the nervous system, so by promoting the expression / inhibiting inactivation of this molecular pathway we could be able to prevent the neurotoxicity (15). Again, animal models with rats concluded that sevoflurane exposure led to increase in proinflammatory markers leading to suppression of JAK/STAT signalling pathway and finally the neurotoxicity of this anesthetic substance (72).

To discuss our final main molecular pathway, AMPK signaling and remembering that anesthetic substances induce toxic traits by inhibition/inactivation of this pathway, hypothesis exist that activation could potentially reduce toxic consequences. In fact, some studies already suggest this hypothesis to be true.

To summarize, the activation of all these above mentioned molecular pathways- PI3K/Akt/mTOR, HIPK2/JNK/c-Jun, JAK/STAT and AMPK, shows promising results by preventing anesthesia induced neurocognitive changes in children.

8.3.2 NON-CODING RNA AND EPIGENOME

Targeting the non-coding RNA has also suggested to be promising treatment target for future drug development. Preliminary studies show that by upregulating or downregulating certain microRNA strands neurocognitive toxicity of anesthesia could be reduced and also protective functions established (73–76) Many microRNAs participate in regulation of protein synthesis, signaling and neuronal proliferation.
The function of non-coding RNAs is not fully understood and with more studies focusing on the potential function in the future, their role in general anesthesia neurotoxicity will be better understood.
RNA methylation research in this field have witnessed an outbreaking increase in the past 10 years, the reversible methylation of microRNA is emphasized as one of important epigenetic modifications to prevent the unwanted effects of general anesthesia (32).

Epigenome is also timely topic of medical research offering interesting possibilities to treat and reduce neurotoxicity of general anesthesia. It has been suggested that at least some deleterious functional changes caused by infant anesthesia exposure can be reversed pharmacologically by targeting the epigenome (31). The therapeutic window may be large enough to intervene at timepoints before, during, and after the developing brain is subjected to the neurotoxic effects of anesthetics.

8.3.3 INSULIN

Insulin shows to have many important functions in the brain, including neurotrophic and neuroprotective activities, regulation of neural development and plasticity, and a role in learning and memory (77,78). Insulin administration has its perks, direct administration to brain is obviously challenging and peripheral administration could have serious hypoglycaemia causing consequences. This was problem until researchers reported that insulin can be administered intranasally with much less side effects (30). As a conclusion, successful administration restores insulin signalling, increases the levels of synaptic proteins, and reduces Aβ level and microglia activation, preventing anesthesia-induced cognitive impairment and chronic neurobehavioral changes (5,79).

8.3.4 IRON

Earlier sevoflurane induced iron deficiency was discussed which led to idea consider iron supplementation as a possible treatment/preventative measure to minimize neurocognitive effects. In the same study (4) conducted with laboratory mice also tested whatever supplementation had any benefits. Iron supplementation was given before anesthesia of mice and interestingly, results showed that damaging effects of sevoflurane could be significantly eliminated by iron supplementation before anesthesia- at least in this animal model.

8.3.5 OTHER

Other notable molecular proteins for further research are klotho, DJ-1, histone deacetylase 2, apolipoprotein, tau protein, GABA, BDNF and intracellular calcium- all of which are studied to play part in neurocognitive effects of general anesthesia to nervous system (56,76,78,63,79,80) . Rapamycin is suggested to prevent the inhibition of synaptogenesis, which is mTOR inhibitor (17).

8.4 LIMITATIONS OF THE RESEARCH

Non-systematic literature reviews have their pros and cons. It is unbeatable research method to analyze and synthesizes gathered studies from abundant material but may to lead to biased results. Carefully planned inclusion and exclusion criteria minimized the risk of under- and overestimations, but limitations should still be admitted. No recommendations or guidelines should entirely be based to non-systematic type of review.

Thesis includes observational (case control, cohort) studies, intervention studies (clinical trials), meta-analyses and systematic reviews. Carefully formulated study criteria aimed to retain quality, yet all studies have their limitations.

Observational studies included to thesis have difficulty to differentiate between the effects of potential confounding factors and the presence of residual confounding from unmeasured covariates. Observational studies are not good at inferring causality.

Many observational retrospective studies have made during the 70s and 80s when practise of anesthetic care was fully different compared general anesthesia we know today. Exclusion criteria marked off these studies but certainly they have their effect to current research as well.

Different studies have variety between assessment methods they use which may propose difference in conclusions. To minimize this limitation thesis includes only studies which used validated methods (eg.Baley-III). Also follow-up times varied greatly and more reliable results are seen with longer follow up periods and multiple assessments.

Thesis includes both, human studies, and animal studies. Human studies can be considered be most reliable and animal studies should be faced with criticism. Human population cannot be straightly compared to non-human primates let alone rodents or other animals. Most importantly the morphology of animal and human brain is dissimilar and neurotoxic effects leading to neurocognitive changes may vary. However, the affected regions under general anesthesia remain the same.

Criticism towards most important studies of the field, GAS, PANDA and MASK has also taken place. Critic mainly concern follow-up duration, sample size and assessment methods

9. CONCLUSION AND PRACTICAL RECOMMENDATIONS

This non-systematic literature review critically examined the neurotoxic potential of general anesthesia during infancy, focusing on its structural and neurocognitive effects on the developing brain. While human studies offer somewhat reassuring evidence—particularly the GAS, PANDA, and MASK trials (22,52,53) - indicating that single, short exposures to general anesthesia are unlikely to result in significant long-term cognitive deficits, consistent concerns remain around prolonged or repeated exposures.

Anesthesia during infancy is suggested to cause neuronal apoptosis, triggering widespread programmed cell death, particularly in developing neurons (10,14,15,21). Decreased volume of grey matter in critical brain regions such as the thalamus and retrosplenial cortex appears to affect cognition and sensory processing (20,35,38–40) while disruptions in white matter myelination and integrity may contribute to neurodevelopmental disorders (38,41,42,44). Disruption of synaptogenesis pose reduced synaptic density, mitochondrial damage in synaptic terminals, and structural disorganization in neuronal circuits (10,17,21,29).

Immature neurons are more sensitive to anesthetic agents, making infants particularly susceptible to neurotoxicity. Multiple and prolonged anesthetic events correlate with more significant neurocognitive deficits and structural brain changes while higher doses of anesthetic substances increase the likelihood of adverse effects (22,52,53).

Animal studies, particularly involving non-human primates and rodents, consistently demonstrate that general anesthetics can disrupt critical processes like synaptogenesis, neuronal survival and promote unwanted neuronal apoptosis. These effects manifest structurally as decreased gray and white matter volume and functionally as learning, memory, behavioral, and emotional deficits (10,14,15,21). Although animal findings cannot be directly extrapolated to humans due to physiological and developmental differences, they underscore plausible mechanisms that may also apply to human infants.

Different pharmacological and non-pharmacological treatment methods to reduce anesthesia induced neurotoxicity have been proposed (68,69,72,73,77,79). Anesthetic technique modifications are practical and rather simple ways to reduce need of general anesthetics, regional anesthesia is getting deserved attention. To come back with more practical everyday work, also the exposure duration of anesthesia, exposure times, concentrations of anesthetic substances, pain relief and other co-administered medications as well as caregiving after the anesthesia are at least of equal value when compared with possible future drug treatments.

The big question exist, should surgeries under general anesthesia in infancy be postponed? There is no definitive answer and much more studies are needed to be able to answer this question conclusively. Now risk of anesthesia is indefinite and non-specific and there are situations putting infants' health to much greater risk than theoretical risk of general anesthesia itself.

Most critically, there is currently not enough high-quality, long-term human research to draw conclusive connections or to form universal guidelines on the use of general anesthesia in infancy.

The heterogeneity of existing studies, variability in assessment tools, and lack of long-term follow-up make it clear that future research is essential.

Based on the aim, objectives and conclusions of this non-systematic literature review, following recommendations for future research are made:

- Neuroprotection during general anesthesia in infants, clinical approach
- The mechanism of association between surgery/anesthesia; neurotoxicity, inflammation, hypotension, hypoperfusion, ischemia, stress
- Neuroprotectivity of anesthesia
- Anesthesia induced dysregulation the genes and epigenome

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