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Title

Preventing the long-term effects of general anesthesia on the developing brain: how translational research can contribute.

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Abstract

In 2017, the Food and Drug Administration published a safety recommendation to limit the exposure to general anesthesia as much as possible below the age of three. Indeed, several preclinical and clinical studies have questioned the possible toxicity of general anesthesia on the developing brain. Since then, recent clinical studies tried to mitigate this alarming issue. What is true, what is false? Contrary to some perceptions, the debate is not over yet. Only stronger translational research will allow scientists to provide concrete answers to this public health issue. In this review, we will provide and discuss the more recent data in this field, including the point of view of preclinical researchers, neuropsychologists and pediatric anesthesiologists. Through translational research, preclinical researchers have more than ever a role to play to better understand and identify long-term effects of general anesthesia for pediatric surgery on brain development in order to minimize it.

Keywords

Children, developing brain, general anesthesia, translational research, neurotoxicity.

Introduction.

Each year, millions of children require surgery. One out of seven children is subjected to general anesthesia before three years old in western countries (Shi et al., 2018). General anesthesia for pediatric surgery is a safe procedure with a low risk of immediate complications (Habre et al., 2017). However, preclinical studies have shown that exposure to general anesthesia during the early stages of the developing brain- *in utero* or in the early infancy- could lead to structural and functional brain abnormalities, as well as cognitive and behavioral disorders (Jevtovic-Todorovic, 2018). From a neurochemical aspect, cerebral consequences are conceptually possible, as most anesthetic agents exert their hypnotic effect through their agonist activity on GABA receptors or their antagonist activity on NMDA receptors, which are critical in neurodevelopmental processes. These preclinical considerations rightfully opened the debate on the possible neurotoxicity of general anesthesia in children and have been the driving force behind several retrospective clinical studies. In this context, in 2017 the Food And Drug Administration (FDA) issued a warning that repeated or prolonged general anesthesia in children younger than 3 years old should be performed with caution (Andropoulos and Greene, 2017). Following this recommendation, recent clinical studies have provided reassuring evidence of the safety of anesthesia in children. Yet, the debate is not over.

After pointing out the limits of first preclinical and retrospective clinical studies, we will discuss the most recent data of the literature, including the points of views of preclinical researchers, neuropsychologists and anesthesiologists. While the latest major clinical studies on this topic seem to minimize the impact of general anesthesia on the developing brain, and thus convey a reassuring message to practitioners and the population, their data should be considered with caution. It is likely that the answer to the enigmatic question of long-term effects of general anesthesia on the developing brain lies in an improved translational research in which preclinical researchers will play a key role. At the end of this literature review, we will therefore propose the outline of an original translational study in mice and human which could strongly contribute to providing answers to this major issue.

General anesthesia for childhood surgery: a recurring phenomenon.

Recent epidemiological data have shown a high incidence of general anesthesia in childhood (Shi et al., 2018). No previous study directly measured the incidence of receiving procedures

requiring general anesthesia in a population of children. Thus, among a 20,922 american children cohort, 3,120 (14.9%) underwent at least 1 general anesthesia before age 3. There is no gender difference in the incidence of general anesthesia in childhood. Seven hundred and twenty-three children (3.5%) had at least 1 subsequent procedure. The first procedure occurred in the first year of life in 1,208 (39.0%) children. The most common classification of first procedure received was otorhinolaryngologic surgery (44.9%). Estimated gestational age <32 weeks and low birth weight were independently associated with receiving repeated anesthesia. Eight hundred and twenty children (3.9%) had a single prolonged exposure above 3 hours, multiple exposures prior to age 3, or both. This means that approximately 1 in 4 children who received general anesthesia fall within the high-risk category as defined by the recent FDA warning whose aim was to define the high-risk categories of neurotoxicity of general anesthesia during childhood established on the basis of the underlying described literature.

Preclinical and retrospective clinical studies have opened the debate on the long-term effects of general anesthesia on the developing brain.

Preclinical studies

During the last 20 years, most of the commonly used anesthetic drugs, either injected intravenously or inhaled, have been reported to interfere with the developing brain (Marchesini and Disma, 2019). Preclinical research on the potential toxicity of general anesthesia on the developing brain started at the end of the 20th century with Ikonomidou study (Ikonomidou et al., 1999). After administering an NMDA antagonist (MK801) to young rats (postnatal days 3, 7, 14, 21) and to pregnant rats (embryonic stage 17, 19, 21), examination of the brain at 24 hours by TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling) revealed a large apoptotic degeneration in the developing brain, both after those prenatal or postnatal blockades. This supported the hypothesis that anesthetic agents – some of which act on NMDA receptors – could possibly impair the development of the brain. That's why, in 2003, Todorovic and colleagues administered a combination of anesthetic agents (midazolam, nitrous oxide and isoflurane) for a 6-hour anesthesia in 7-d-old young rats (Jevtovic-Todorovic et al., 2003). Immuno-histological analysis for activate caspase-3, highlighted extensive apoptotic neurodegeneration in the developing brain. But these findings were all the more worrying that it was also noted –

thanks to behavioral analysis at subsequent ages – an impairment of spatial reference memory on postnatal days 32 and 131 and, moreover, a spatial working memory impairment at postnatal day 53. This was one of the seminal studies that suggested that in addition to a structural impact on the developing brain, general anesthesia could lead to long-term behavioral changes (Mintz et al., 2013).

These results in rodents have been confirmed in non-human primate studies. Within a population of 24 socially reared rhesus macaques, was examined how exposure to either single or multiple early postnatal isoflurane affected a set of behavioral tasks (Coleman et al., 2017). Young animals were exposed to 5h of isoflurane 0.7 to 1.5 vol% once (postnatal day 5) or three times (postnatal days 6, 9 and 12). Reflex development and anxiety were evaluated using standardized tests over a time period of one year. Macaques were weaned and housed indoors with other subjects. Animals which has been exposed to repeated isoflurane anesthesia appeared to have significantly lower basic motor reflex after 1 month and demonstrated an exacerbated anxiety and affiliative/appeasement behavior at 1 year as compared to those exposed only once to isoflurane. In fact, no significant behavioral alteration was observed following a single exposure to isoflurane. Behavioral tasks of Rhesus monkeys after general anesthesia were also evaluated in other studies. Animals of both sexes underwent either 3 times repeated 4h exposures to anesthesia with sevoflurane or brief repeated maternal separations (controls) at 6, 14 and 28 days postnatal (Raper et al., 2015). Depth of anesthesia was adjusted based on reaction to calibrated pressure stimulus. Throughout the experiment, monkeys remained close to their mother, forming large social groups, excepted for overnight observations after each anesthetic and control procedure. When reaching 6 months of age, each monkey was measured on the human intruder test, a testing paradigm for emotional reactivity in nonhuman primates. Anxiety behaviors were significantly more frequent in monkeys that had been exposed to anesthetics than in controls animals.

The possibility that exposure to general anesthesia induces neuroinflammation in the developing brain has also been explored. Repeated exposure of young mice to sevoflurane anesthesia for 2 hours on 3 consecutive days induced a marked increase in interleukine-6 and tumor necrosis factor (TNF) protein levels in the brain, and also led to decreased activity of the AKT-glycogen synthase kinase 3 β (GSK 3 β) signaling pathway in these animals (Shen et al., 2013; Zhang et al., 2014). Altogether, these preclinical investigations suggest a wide range of deleterious impacts of general anesthesia on the developing brain, including the commonly used anesthetic drugs and possible structural and morphologic changes and

functional or behavioral impairments (Jevtovic-Todorovic, 2018; Vutskits and Xie, 2016). However, although these preclinical studies have raised the major issue of the neurotoxicity of general anesthesia on developing brain, their conclusions cannot be directly transposed to clinical practice. Indeed, discrepancies between species is a major limitation including correspondences of the different stages of cerebral development. Furthermore, preclinical studies consist in exposition to anesthetic agents without any surgical procedure or control of systemic blood pressure and oxygenation. Yet, the preclinical literature justifies the need to investigate whether changes in behavior or social development also occur in the clinical setting.

Retrospective clinical studies

Most of the clinical studies regarding the effects of general anesthesia on developing brain have a retrospective design. Although some studies suggested an association between surgery during childhood and either a lower academic performance or increased risks of behavioral disorders, other have not identified such associations. In a cohort of 188,557 children (28,366 children who underwent surgery before 5-6 years and 55,910 unexposed children), O'Leary et al. showed an association between surgery in early life and children performances at entry to primary school measured by the Early Development Index (EDI) (O'Leary et al., 2016). Early developmental vulnerability was higher in the exposed group (25.6%) when compared to the unexposed control group (25.0%). However, the overall magnitude of the risk was small (adjusted odds ratio, 1.05; 95% CI 1.01 to 1.08). In another population-based cohort (116 multiply exposed, 457 singly exposed and 463 unexposed children) multiple – but not single – exposures to anesthesia before 3 years of age resulted in higher frequency of learning disabilities and attention-deficit/ hyperactivity disorder up to age 18 years (hazard ratio for learning disabilities: 2.17; 95% CI 1.32 to 3.59) (Hu et al., 2017). Single exposure resulted in only a slight decline in reading and language achievement but not in cognitive functions. Thus, even if several clinical studies are in favor of a repercussion of general anesthesia for pediatric surgery on brain development, others are not. Indeed, in a retrospective matched-cohort study (Ko et al., 2015) including 114,435 children among whom 5,197 were exposed to general anesthesia and surgery before the age of 2 years, there appeared to be no difference in the frequency of autistic disorder between the exposed group (0.96%) and the unexposed control group (0.89%) ($p=0.62$). The hazard ratio of exposure to general anesthesia and surgery was 0.93 (95% CI 0.57 to 1.53) post adjustment for potential cofounders. Remarkably, age at the time of the first exposure appeared to have no influence on the risk of autistic disorder.

What could explain such discrepancies between clinical studies? Among other, these retrospective clinical studies differ in the number and duration of exposures, the delay between exposures to anesthesia and neurodevelopmental evaluations, the mode of data retrieval (diagnostic codes in database (Ko et al., 2015) or parental interrogations (Bong et al., 2013)), the age of anesthesia (Flick et al., 2011), and the regimen of anesthetic agents administered (halothane versus sevoflurane etc.). In addition, there are three different categories of outcomes. Some studies focused on academic performances evaluated by national educational achievement test (results and nonattainment scores) and teachers rating of cognitive problems (Hansen et al., 2011) whereas other investigated clinical outcomes such as learning disabilities (Sprung et al., 2009), behavior disorders (Hu et al., 2017) or neuropsychological tests (de Heer et al., 2017). Discrepancies could also be explained by the presence of confounding factors, including the personal medical history of patients (prematurity (DiMaggio et al., 2009), birth weight, visual or hearing impairment) or the socioeconomic and educational contexts (parental age, lone-parent family, parental income). Despite solid clinical confirmations, preclinical and retrospective clinical studies have brought enough elements leading the Food and Drug Administration (FDA) to issue a warning in 2016 that repeated or prolonged use of general anesthesia in children younger than 3 years old should be performed with caution. These label changes were applied to some of the most commonly used anesthetic and sedative drugs from 2017 (Research, 2019). Thus, an exhaustive list of general anesthetic and sedation drugs concerned has been established by the FDA including anesthetic and sedation drugs that block N-methyl-D-aspartate receptors and/or potentiate gamma-aminobutyric acid activity. No specific medication has been shown to be safer than any other. A disturbing fact is that approximately 1 in 7 children in the United States is exposed to anesthetic agents before age 3 years, and 1 in 4 of these children is considered to be at highest risk of anesthesia-related neurotoxicity by the FDA (Shi et al., 2018). This recommendation was likely written as a matter of urgency, to provide an answer to the public on the major public health issue which is the toxicity of general anesthesia on the developing brain. Indeed, only clinical studies with a higher level of evidence could settle this debate. This is what recent clinical studies have tried to achieve.

Even if recent clinical studies with a higher level of evidence have provided a reassuring message for practitioners and the public, the debate is not over.

Although recent clinical studies provide reassuring evidence for practitioners and the general population (Warner et al., 2018; Sun et al., 2016; McCann et al., 2019), their conclusions should be considered with caution. In particular, behavioral assessment used in these studies presented several limitations for interpreting the results and for concluding that exposure to procedures requiring general anesthesia before 3 years of age is not associated with cognitive consequences. Indeed, these studies proposed traditional measures of intelligence (e.g., IQ measures such as the Wechsler Abbreviated, or Preschool and Primary, Scale of Intelligence), provide information regarding other cognitive abilities such as attention and executive functions (using the Behavior Rating Inventory of Executive Function, BRIEF), and found a lack of impact of anesthesia on both IQ measures and BRIEF scores. Nevertheless, it should be noted that BRIEF scores are extracted from parents' responses to a questionnaire: BRIEF scores did not reflect children cognitive abilities *per se*. A more fine-grained analysis from experimental tasks, performed by children themselves, will thus be necessary in future work to confirm that anesthesia had actually no impact on cognitive abilities. In particular, precise estimations of executive functions assessment seem essential, according to developmental psychology evidence suggesting that executive functions and self-control played a key role in childhood, for predicting physical health (Moffitt et al., 2011) and future academic performances (Duckworth and Seligman, 2005). As a matter of fact, IQ measures and BRIEF questionnaires are thus probably not sufficient to assess the potential detrimental impact of anesthesia on cognitive abilities in children. This is in agreement with statements from children's parents who reported increased problems related to executive function, behavior or even reading abilities (Warner et al., 2018). Moreover, even if it has been suggested that children IQ seemed predictive of later school abilities (Batty et al., 2006), recent work evidenced that fundamental school abilities are also strongly linked to executive functions (Viarouge et al., 2019). One could argue that executive functions were already assessed in previous studies that aimed at investigating the impact of anesthesia in children, thanks for instance to the Trail Making or the Wisconsin card sort tests (Warner et al., 2018). Unfortunately, these executive tests mainly evaluated cognitive flexibility process and did not provide indication regarding other executive functions, such as inhibitory control (see Diamond, 2013, for a recent discussion about executive functions (Diamond, 2013)). Given

the importance of inhibitory control in daily life situations (Borst et al., 2015) and for school performances (Diamond et al., 2007), future work will be necessary to further investigate the consequences of anesthesia on this essential cognitive function, using for instance Stroop task that precisely allow its assessment (Poirel et al., 2012). Finally, another issue concerns the lack of brain imaging data associated with behavioral measures collected in children. Indeed, outcomes from recent studies have to be interpreted cautiously because findings cannot directly demonstrate causality between brain morphometry (or brain functioning) variations due to anesthesia and behavioral assessment. Given that no behavioral differences were found between groups in these studies (Warner et al., 2018; Sun et al., 2016; McCann et al., 2019) one could hypothesize that long-term anesthesia consequences should be associated with more subtle and specific pattern of injury between brain modifications and cognitive outcomes. Consequently, multimodal exploration each child, with both behavioral and brain imaging data, seems a necessary area of future study.

PANDA, MASK and GAS studies all have delivered a reassuring message about the effects of general anesthesia on the developing brain. A history of single or repeated exposure to general anesthesia before the age of 3 years would not alter the primary outcomes of these studies measuring general intelligence (Vutskits and Culley, 2019). As a consequence, the FDA recommendation has not been approved by several European societies, in particular the European Society of Anesthesiology and the European Society For Pediatric Anesthesiology. However, these studies do not definitively close the question of the cerebral toxicity of anesthesia in young children, particularly because their neurodevelopmental evaluation is insufficient. Thus, one could say it is only the end of the beginning (Warner et al., 2018). The time has come to turn again to preclinical research.

Preclinical research will always be a major actor in research on the toxicity of general anesthesia.

We can go further in research about anesthesia neurotoxicity. Indeed, if expert opinion is divided over the applicability of first preclinical studies to humans, the interpretation of how recent clinical studies should influence clinical practice is still unclear (Hansen, 2017). Further high-quality multidisciplinary research is required to clarify and, if necessary, mitigate risks of anesthesia neurotoxicity (Disma et al., 2018). It will necessarily involve preclinical research.

Role of basic science

Many issues regarding the long-term effects of general anesthesia on the developing brain are not yet resolved. Difficulty lies in the fact that the molecular, cellular and systemic events implicated in the maintenance of anesthetic states overlap with the mechanisms that lead to the sustained effect of anesthetic agents on neuronal structure and function. Only preclinical research could identify which mechanisms are implicated in neurotoxicity so as to open up avenues for neuroprotective strategies (Marchesini and Disma, 2019). Moreover, knowledge of how structural changes induced by anesthetic agents causes behavioral impairment is unclear. Otherwise, we do not understand why the increased vulnerability of central nervous system structures to anesthetic agents depend or not to region-specific time windows during brain development. Finally, mechanisms underlying the physiological context-dependent effect of anesthetic agents on morphologic and functional changes in the brain also need to be explored (Jevtovic-Todorovic, 2018). In vitro and in vivo models could elucidate such issues.

The major role of translational research

Clinicians have often criticized the difficulty in translating preclinical conclusions into clinical practice (Todd, 2004). A strong effort should be made in this area. An effort that will open up new fields of preclinical research such as defining original neuroimaging and biological markers of anesthesia neurotoxicity. One of the flaws in preclinical research is the inadequate monitoring or physiological parameters in laboratory animals when compared with human-anesthesia management. Indeed, because of their size, appropriate hemodynamic monitoring in rodents is a challenging tasks especially in early postnatal period (Vutskits and Patel, 2014). This is a major issue because disturbance of systemic homeostasis contributes to anesthesia-induced neurotoxicity both in the young and in the old brain (Planel et al., 2007). This is why the concept of SAFETOTS recently emerged in pediatric anesthesia clinical practice (Weiss et al., 2015). The SAFETOTS initiative has enabled to list perioperative causes for cerebral morbidity in young children during the perioperative period. The concept of the 10-N quality pediatric anesthesia takes the form of a quality checklist of factors that anesthesiologists must know and control during the perioperative period: No fear, Normovolemia, Normotension, Normocardia, Normoxemia, Normocapnia, Normonatremia, Normoglycemia, Normothermia, No pain. SAFETOTS are all factors whose preclinical research can allow us to determine the importance in the supposed toxicity of general anesthesia. Thus, future experimental studies will require improvements in quality and reproducibility adhering to the appropriate conduct and reporting guidelines (Kilkenny et al., 2010) and tend to use as much as possible physiological monitoring during general anesthesia.

Another flaw identified in preclinical studies relates to the drug-exposure times in rodent pups when compared with those in human context. That is why studies using non-human primates are interesting in the experimental field of research about toxicity of general anesthesia. Indeed, they more closely resemble human physiology and pathophysiology, especially during early development (Wang and Slikker, 2008). Behavioral studies have also shown that well-trained non-human primates can perform certain tasks with comparable accuracy to children, supporting their extrapolation to humans (Paule et al., 2011). Another difficulty is that most experiments were performed in the absence of surgery or other painful stimuli, a situation rarely encountered in humans. This is an important issue because painful stimuli themselves can trigger pathological morpho-functional plasticity in the neonatal brain (Koch and Fitzgerald, 2013). Neurodevelopmental assessment after a surgery carried out under exclusive locoregional anesthesia (Baldi et al., 2007) in animals could make it possible to measure the extent to which surgical stimulation contributes (or not) to brain impairment after surgery under general anesthesia during childhood. This type of study could also position locoregional anesthesia as a major tool in a multimodal strategy to reduce the neurodevelopmental impact of general anesthesia. Moreover, surgery-related inflammation and painful stimuli may both impair central nervous system function. Future preclinical studies will have to study the impact of the pain of surgery on neurodevelopment while proposing the most translational models possible. If when it comes to the long-term effects of general anesthesia during the infancy, it is necessary to consider as much about the impact of the surgical stimulus as the effects of the anesthetic agent used, it is also necessary to take into account the structural impact of environmental stimuli on brain. For example, a multimodal neuroimaging study assessed the consequences of psychological trauma due to World Trade Center attacks (Ganzel et al., 2008). More than 3 years after September 11th, the adults most exposed to the attack presented a reduced grey matter volume versus a control group notably in the amygdala, hippocampus and insula. When elucidating the mechanism underlying long-term effects of anesthetic exposures on neurodevelopment, it will also be necessary to causally link structural changes observed in immature brains immediately following exposure with long-term cognitive consequences. This will require the identification of biological or neuroimaging biomarkers so as to detect biological or imaging signature of general anesthesia neurotoxicity (**Figure**). The question is not whether general anesthesia should be used or not in younger patients, but the challenge is more to offer safer children care during surgery which we think will be the least neurotoxic possible. One of the most important issues is to determine whether all anesthetic agents currently used have comparable risks for toxicity

(Vutskits and Xie, 2016). Experimental data are controversial regarding this issue. These works will be of great translational importance because they could guide future human trials so as to find the safest drug combinations in clinical practice.

An original translational study to contribute to the anesthesia neurotoxicity debate.

Future translational studies will be necessary to further examine if a general anesthesia exposure is associated (or not) with impaired neurocognitive development and abnormal behavior in later childhood. Here we propose an original translational study which would take place in two phases: a preclinical phase and a clinical phase. Initially, mice - animals whose brain development is substantially shorter than human (weeks as opposed to years) and well documented in the literature (Dobbing and Sands, 1979) - could be repeatedly exposed to general anesthesia with isoflurane in the postnatal period. Indeed, halogenated anesthetic agents are the most commonly used class of hypnotic agents in pediatric anesthesia. Due to the specific metabolism of rodents, a repeatedly exposed mice to general anesthesia to get a greater chance to reveal any effects. These mice would be compared to a control group not exposed to postnatal general anesthesia at an age corresponding to adolescence (4 weeks of life). This evaluation should include both behavioral testing and in high-definition neuroimaging (for example, Magnetic Resonance Imaging) in order to develop a mechanistic approach to possible exacerbated behavioral disorder. The behavioral approach would ideally be multimodal and would allow us to explore several domains such as locomotor activity, anxiety, contextual emotional memory and working memory. In a second stage, a mirroring study should be carried out in humans. Thus, two groups of children would be compared during adolescence in terms of behavior (experimental tasks performed by children themselves and hetero-evaluations) and neuroimaging according to their antecedents or not of general anesthesia in childhood. Behavioral testing would focus not on measures of general intelligence but on cognitive domains considered to be disturbed in mice anesthetized during the preclinical phase of the study. It should be noted that attention will have to be paid to biases in the clinical phase of the study, such as the socio-economic educational context (Farah, 2017) but also co-morbidities which could influence neurodevelopment, notably a history of prematurity (Ream and Lehwald, 2018). Particular attention should also be paid to intra-operative anesthetic data, as intraoperative management through the maintenance of

physiological homeostasis (Weiss et al., 2015) which is a key factor in long-term neurological outcome (Marchesini and Disma, 2019). The application of this kind of experimental design will investigate if a direct causality between long term anesthesia, developing brain and cognitive abilities in children is present or not.

If recent clinical studies have put the risk of cerebral toxicity of general anesthesia in young children into perspective and take a step back from the FDA recommendation that advises against anesthesia before the age of 3, debate about general anesthesia on developing brain is not over. The potential for adverse neurodevelopmental effects of general anesthesia clearly remains a complex issue. Preclinical research offers key opportunities in removing these grey areas in this field of research. It has to go through improving preclinical models so as to strengthen link between laboratory and clinical practice. Indeed, it is now only through stronger translational research that safer anesthetic techniques will be identified for a better care of our children who will have to be operated.

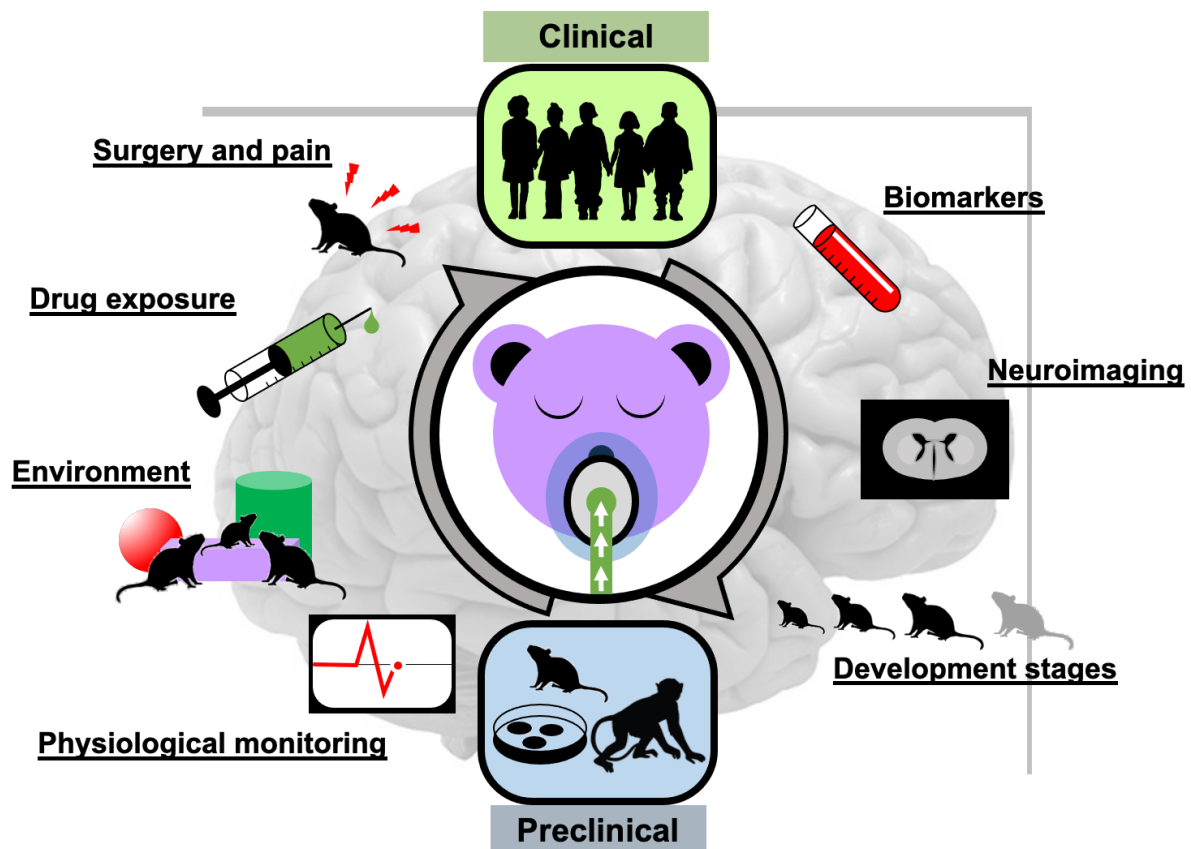


Figure: The future of research about the long-term effects of anaesthesia on the developing brain will necessarily involve translational findings. The development of tools to highlight the impact of general anaesthesia for paediatric surgery will be possible thanks to preclinical research. This must require the control of a number of factors to reinforce the translational nature of the results.

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