



# Commentary: Early-in-life Isoflurane Exposure Alters Resting-State Functional Connectivity in Juvenile Non-human Primates - a Role for Neuroinflammation?

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## ABSTRACT

The concern about anesthesia-induced developmental neurotoxicity (AIDN) in infants and young children arises from animal studies indicating potential long-term neurobehavioral impairments following early-in-life anesthesia exposure. While initial clinical studies provided ambiguous results, recent prospective assessments in children indicate associations between early-in-life anesthesia exposure and later behavioral alterations. Ethical constraints and confounding factors in clinical studies pose challenges in establishing a direct causal link and in investigating its mechanisms. This commentary on a recent study in non-human primates (NHPs) focuses on exploring the role of neuroinflammation and alterations in brain functional connectivity in the behavioral impairments following early-in-life anesthesia exposure. In juvenile NHPs, chronic astrogliosis in the amygdala correlates with alterations in functional connectivity between this area with other regions of the brain and with the behavioral impairments, suggesting a potential mechanism for AIDN. Despite acknowledging the study's limitations, these findings emphasize the need for further research with larger cohorts to confirm these associations and to establish a causal link between the neuroinflammation and the behavioral alterations associated with early-in-life anesthesia exposure.

## Anesthesia-induced developmental neurotoxicity (AIDN)

There is a concern that anesthesia exposure early in life may be neurotoxic to infants and young children and cause long-term impairments in their neurobehavioral development. This concern originated from preclinical studies in rodents, which found that early-in-life exposure to anesthetics commonly used in pediatric anesthesia caused acute increase of neuroapoptosis and long-term alterations in cognition and behaviors<sup>1</sup>. Most of these findings also have been observed in non-human primates (NHPs)<sup>2-8</sup>. In contrast, early clinical studies with retrospective assessments provided ambiguous results, with some suggesting that anesthesia exposure affected primarily cognitive abilities,<sup>9-13</sup> while others found no associations. However, most recent clinical studies with prospective assessments have provided some valuable outcomes that indicate an association between early-in-life anesthesia exposure and alterations in behavior later in life,<sup>14-16</sup> which have been highlighted in a recent meta-analysis<sup>17</sup>. Ethical limitations and the presence of numerous confounders in clinical studies make it a challenge to establish a direct causal link between anesthesia exposure and neurodevelopmental deficits in children, as well as to investigate potential mechanisms. Therefore, we designed a study in NHPs that were anesthetized during infancy under conditions that resemble those in the pediatric

operating room, to investigate alterations in behaviors and accompanying long-term structural and functional changes in the primate brain. Results from these studies have been published in separate papers<sup>18-21</sup>. In this commentary on our latest report<sup>21</sup> we focus on 1) the potential role of neuroinflammation in the behavioral outcomes associated with early-in-life anesthesia exposure and 2) the alterations in brain functional connectivity, assessed with magnetic resonance imaging (MRI), as a mechanistic link between neuroinflammation and those behavioral alterations.

### Role of neuroinflammation in AIDN

The mechanisms underlying AIDN are not fully understood, but among them anesthesia-induced neuroinflammation has been proposed as a potential contributor to AIDN. Neuroinflammation is a common hallmark of many neurodegenerative and developmental diseases such as Alzheimer's disease (AD), dementia, and autism spectrum disorders (ASD)<sup>22, 23</sup>. Here neuroinflammation plays a role in neuronal dysfunctions including cognitive impairments in neurodegenerative diseases and behavioral alterations in ASD. The neuroinflammatory response involves activation of microglia and astrocytes and commonly elevated cytokines in the brain include interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF $\alpha$ ), among others. Several reports documented acute or chronic neuroinflammation after early-in-life anesthesia exposure over the last decade, including studies in rodents and piglets showing acute neuroinflammation with increased microglia activation as well as elevated IL-6, TNF $\alpha$ , and IL-1 $\beta$  in brain tissue after single or repeated exposure to volatile anesthetics early in life<sup>24-33</sup>. Studies have also reported neuroinflammation present at an adult age in rats after early-in-life exposure to sevoflurane<sup>34, 35</sup>. In addition, maternal exposure during late pregnancy also causes neuroinflammation in the murine fetal brains and impairments in the offspring's neurodevelopment<sup>36, 37</sup>. Consequently, studies applying known anti-inflammatory substances such as inhibitors of the NF $\kappa$ B pathway during early-in-life exposure reported reduced acute neuroinflammation and ameliorated long-term neurodevelopmental impairments<sup>24-30, 37</sup>. Based on the beneficial effects of pharmacologically reducing the acute inflammatory response of the brain, it's reasonable to conclude that neuroinflammation plays a significant role in mediating the neurodevelopmental impairments of early-in-life anesthesia exposure in rodents. In NHPs, microglial activation has been detected in infants after exposure to isoflurane using positron emission tomography (PET)<sup>38</sup>. This inflammatory response lasted for up to 7 days, suggesting that neuroinflammation in the primate brain may play a significant role in the neurodevelopmental impairments that develop later in life. We provided first evidence that neuroinflammation is present in brains of

juvenile (2-year-old) NHPs that were exposed to isoflurane early in life (on post-natal day 6). This was revealed as increased GFAP (Glial Fibrillary Acidic Protein) staining of specific brain areas, indicating increased astrocyte activation at this age<sup>20</sup>. This long-term astrogliosis was found in brain areas known to play an important role for the behavioral alterations the animals displayed at this juvenile age<sup>19</sup>, suggesting a role for chronic neuroinflammation in mediating the behavioral impairments in NHPs. However, the mechanisms linking neuroinflammation with neurodevelopmental impairments after early-in-life anesthesia exposure were not investigated either in NHPs or in other animals.

### Brain functional connectivity: a link between neuroinflammation and AIDN

Neuronal dysfunction can be reflected as alterations in brain functional connectivity that can be measured by resting-state functional connectivity magnetic resonance imaging (rs-fcMRI). In clinical studies with ASD, AD, and dementia patients, abnormal functional connectivity has been found to be associated with worse cognitive deficits or behavioral impairments and higher levels of neuroinflammation (microglia activation)<sup>39-41</sup>. This suggests that altered functional connectivity not only mediates cognitive deficits and impairments in behaviors, but that it is also linked to neuroinflammation. In ASD patients, extended microglial activation was suggested to be linked with a loss of connections between different brain areas or to result in functional underconnectivity<sup>42</sup>. Astrocytes also have been implicated in supporting functional connectivity in the healthy brain while driving abnormal connectivities in neuropsychiatric diseases with chronic neuronal dysfunctions<sup>43, 44</sup>. To investigate a possible mechanism linking our findings of behavioral alterations and neuroinflammation in the juvenile NHPs that were exposed to anesthesia during infancy, we followed a seed-based approach of rs-fcMRI analysis and found altered brain functional connectivity between the amygdala with other brain areas [the primary auditory cortex, the premotor cortex, and the posterior cingulate cortex (PCC)]<sup>21</sup>. We chose the amygdala as a seed area because it showed robust astrogliosis in the NHPs at this age<sup>20</sup>. In addition, it is an important hub for the processing of social behaviors which appeared impaired after early-in-life anesthesia exposure as evidenced by the decreased close-social behavior of the NHPs at the same age<sup>19</sup>. This increased functional connectivity of the amygdala was positively correlated with the increased astrogliosis, suggesting that chronic activation of astrocytes may have contributed to the alterations in functional connectivity of the amygdala and to the behavioral impairments in the juveniles exposed to anesthesia during infancy. Thus, our neuroimaging study in the same cohort of juvenile NHPs

provides novel insights to better understand the underlying mechanisms of AIDN by linking neuroinflammation with alterations in brain functional connectivity and behavioral impairments. While, at this point, we do not have direct evidence that the histopathological and neuroimaging changes in NHPs after early-in-life anesthesia exposure lead to the behavioral impairments, studies in rodents provide some evidence that altered astrocytes activity has a causal role in modulating behaviors (recently reviewed in<sup>45-47</sup>). Depending on the specific brain area, alterations in astrocyte activity have been reported to modulate specific behaviors. For example, optogenetic activation of hippocampal astrocytes altered anxious behavior in mice exposed to an anxiogenic environment<sup>48</sup>. Another rodent study showed that selective activation of astrocytes in the striatum resulted in behavioral changes that resemble the symptoms of attention-deficit hyperactivity disorder (ADHD)<sup>49</sup>. In a rodent model of depression, inhibition of astrocyte activation ameliorated the depression-like behaviors<sup>50</sup>. In addition, a recent study using an induced pluripotent stem cell (iPSC) approach demonstrated that ASD patient-derived astrocytes when transplanted into the healthy mouse brain induce some of the behavioral impairments associated with ASD, thereby providing evidence for the astrocyte involvement in the etiology of those behaviors<sup>51</sup>. The amygdala also plays an important role in anxiety, which also is known to be affected by early-in-life anesthesia exposure<sup>6, 7, 18</sup>. Increased anxiety was found in our cohort of NHPs at the age of 1 year<sup>18</sup>. However, a relationship between anxiety and alterations in brain functional connectivity (and neuroinflammation) could not be established as the animals were not scanned at this age. Our rs-fcMRI analysis in the 2-year-old NHPs showed altered functional connectivity between the amygdala and the PCC, which itself is involved in social behavior. Therefore, additional analysis using the PCC as seed unsurprisingly detected altered functional connectivities with three other brain areas, and these alterations were correlated with the decreased close-social behavior. Unfortunately, the PCC was not available for our histopathological assessments, so the link between neuroinflammation and functional connectivity alterations in this area needs further investigation.

## Conclusion

While we acknowledge that our NHP cohort had a small number of animals per group and we want to be cautious about overinterpreting results, our findings suggest that chronic astroglia activation (neuroinflammation) in the amygdala (and possibly other brain areas) is associated with early-in-life anesthesia exposure and may play a role in the behavioral alterations of juvenile NHPs. In addition, alterations in brain functional connectivity of the amygdala (and possible other brain areas) provide a mechanistic

link between neuroinflammation and behavioral alterations. Further studies with larger numbers of NHPs are encouraged to confirm these findings, as well as new studies using neuroinflammation modulating interventions (e.g., using anti-inflammatory drugs) to establish the causality between the neuroinflammation and the behavioral alterations associated with early-in-life anesthesia exposure.

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## Declaration of Conflicting Interests

The authors declare that there is no conflict of interest.

## Authorship Statement

VN, JFP-Z and AMB conceptualized the MS. VN and JFP-Z wrote the first MS draft. All authors revised and edited the final MS.

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