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***In vivo* Monitoring of Sevoflurane-induced Adverse Effects in Neonatal Nonhuman Primates using Small-animal Positron Emission Tomography**

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It is now undisputed that anesthetic agents with effects on N-methyl-D-Aspartate (NMDA) and/or γ -aminobutyric acid (GABA) A receptors (e.g. isoflurane, sevoflurane, ketamine or midazolam) can induce cellular damage in the rodent or non-human primate brain when administered during a window of developmental vulnerability, coinciding with high rates of synaptogenesis, maturation of neurons, and neurogenesis^{1,2}. For rodents this period spans from postnatal day (PND) 7–14³, while in non-human primates PND 6 has been highlighted⁴. Anesthesia-induced neurotoxicity in the developing animal brain is associated with learning disabilities as well as abnormal behavior including anxiety^{1,5}.

Disturbingly, the question of whether or not anesthesia neurotoxicity occurs in the young human brain remains unanswered. Retrospective, observational human studies linking anesthesia exposures in young children to cognitive and behavioral deficits later on in life have yielded conflicting and somewhat inconclusive results^{6,7}. This has in part been attributed to “lack of an obvious human phenotype for anesthesia neurotoxicity”⁸. Prospective clinical studies are currently underway (clinicaltrials.gov), with some very recent data raising doubts as to the clinical relevance of this effect⁹. Given the uncertainty, there has also been a strong effort to search for new approaches to explore and validate the possibility of neonatal anesthesia neurotoxicity in the human brain.

Molecular imaging approaches can play an important role in shedding light on the mechanisms involved in neonatal anesthesia-induced neurotoxicity. Such methodology has been extremely informative in understanding neurochemical and metabolic changes in disease states such as depression¹⁰, addiction to substances of abuse^{11,12}, multiple sclerosis¹³, and Parkinson’s disease¹⁴, leading to discovery of new diagnostic and therapeutic targets. In this issue of *Anesthesiology*, *Zhang et al.* take an innovative approach to this problem with a translational tool by using Positron Emission Tomography (PET) in combination with a radiotracer, ¹⁸F-labeled fluoroethoxybenzyl-N-(4-phenoxy pyridin-3-yl) acetamide (¹⁸F-FEPPA), to explore the state of ‘inflammation’ in the brain of neonatal non-

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human primate (NHP) rhesus monkeys after exposure to 2.5% sevoflurane for 8 hours. The authors report that ^{18}F -FEPPA binding (measured quantitatively as 'standard uptake values', SUV) is significantly increased in the frontal and temporal lobes 24 hrs after sevoflurane exposure compared to non-exposed neonatal controls; and remains elevated up to 1 week after exposure. The authors conclude that increased ^{18}F -FEPPA uptake in the neonatal NHP brain is representative of glial cell activation (inflammation) and associated sevoflurane-induced neurotoxicity; the latter confirmed histologically in a separate series of animals. In addition, it is reported that acetyl-L-carnitine (ALC) effectively blocks the inflammation as defined by increased ^{18}F -FEPPA SUVs and it is suggested that ALC may be protective in the setting of neurotoxicity from sevoflurane exposure in the neonate.

What is the evidence for the presence of neuroinflammation in the setting of an increase in ^{18}F -FEPPA binding in the brain? The ^{18}F -labeled FEPPA ligand binds with high affinity to the translocator protein 18kDa site (TSPO),^{15,16} which in the central nervous system is expressed mainly in glial cells (microglia as well as astrocytes). Specifically, TSPO is located on the outer membrane of mitochondria in glial cells and is part of a larger complex comprising a voltage-dependent anion channel and the adenine nucleotide carrier¹⁵. There are several other radiolabeled tracers available for the TSPO site, but ^{18}F -labeled FEPPA is advantageous due to its high affinity for the TSPO site, its favorable kinetic and metabolic profile, excellent penetration into the brain, and further that ^{18}F -FEPPA binding can be quantified¹⁷. One disadvantage pertaining to the second generation TSPO PET tracers in the human brain including ^{18}F -FEPPA is that a polymorphism (rs6971) in exon 4 of the TSPO gene influences binding which necessitates genotyping in all study participants¹⁸. This polymorphism is relatively high in humans of European ancestry (allelic frequency of about 30%), but does not appear to be present in NHP.

The observed *increase* in ^{18}F -FEPPA binding in the neonatal NHP brain exposed to sevoflurane compared to non-exposed controls as shown by *Zhang et al.*, is interpreted as a sign of glia cell activation – presumably microglia cell activation. Under normal conditions, microglia cells comprise 10% of the cellular population in the brain and are capable of changing from a surveilling, ramifying state to an amoebic, phagocytic reactive state in the setting of injury or infection¹⁹. Activated microglia cells secrete a wide range of cytokines including interleukin1, interleukin-6 and tumor necrosis factor alpha, and can also attract and interact with T cells. The microglial responses to various types of injury in the brain varies dependent on the challenge, for example when removing apoptotic cells microglia release anti-inflammatory factors²⁰.

It is important to know that although increased ^{18}F -FEPPA binding in the brain is highly suggestive of reactive microglia cells (and/or astrocytes) it may also signify an increase in the mitochondrial population in each of the glia cells. The exact function of TSPO in activated microglia is still unclear. For example, in mice deficient of TSPO (C57BL/6-Tspot^{m1GuWu}(GuwiyangWurra)-knockout mice), activation of microglia after neuronal injury appears to be unimpaired; suggesting lack of a specific 'phenotype' of TSPO in the setting of inflammation²¹. Nevertheless, post-mortem studies have documented the presence of activated microglia in several disease states including Alzheimer's disease, multiple sclerosis, or Parkinson's disease, with paralleled findings using *in vivo* PET and ^{18}F -FEPPA.

For example, in a recent quantitative PET study, ^{18}F -FEPPA was used to compare TSPO binding in patients with AD and in cognitive intact age matched controls; and areas of increased binding was observed in the internal capsule and cingulum bundle²².

What is the potential clinical significance of increased ^{18}F -FEPPA binding in the neonatal NHP brain? Based on *in vivo* and *in vitro* studies using the TSPO radioligands it is clear that an increased binding signifies the presence of activated glial cells and neuroinflammation. However, as is well known that the microglia reactive response is very variable dependent on the ‘stimulus’ and it could be that the increased response in the setting of sevoflurane exposure is merely reflecting the necessary phagocytosis of apoptotic neurons. In this context therefore, the effect of ALC documented by *Zhang et al.*, would simply mean a reduction in neuroinflammation and microglial function. It is also intriguing that recent reports highlight the beneficial effect of an acute immune response in the brain to overcome injury²³. Further investigations are needed to clarify if ALC is neuroprotective after sevoflurane exposure, and whether or not it might reduce cognitive dysfunction later on in life. Nevertheless, it is clear that long-duration sevoflurane induces a glial cell response in the neonatal NHP brain, and this study has taken us one step closer towards a translational approach to exploring this problem. Furthermore, the study by *Zhang et al.*, is one of the first studies to look at multiple points within the same animal group, introducing the possibility of studying recovery from anesthesia induced neurotoxicity in the developing brain. Although radioactive exposure is not recommended in this age group, this response might in the near future be validated by this or other non-invasive methods in the young human brain.

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