



# ARTICLE OF THE MONTH

## Developmental Effects of Neonatal Isoflurane and Sevoflurane Exposure in Rats

Christoph N. Seubert, MD, PhD, Wanting Zhu, BS, Christopher Pavlinec, BS,  
Nikolaus Gravenstein, MD, Anatoly E. Martynyuk, PhD, DSc  
*Anesthesiology*. 2013; 119(2): 358-64.

*The neurodevelopmental effects of the inhaled volatile anesthetics, as well as intravenous anesthetics, are a matter of substantial interest in the current anesthetic, neurologic, and pediatric literature. Indeed, many healthcare groups and initiatives, such as the SmartTots program ([www.smarttots.org](http://www.smarttots.org)), are placing substantial research time, money, and efforts in hopes of gaining a better understanding of the possibly harmful effects of anesthetics administered on the developing central nervous system. In this month's first installment of the **SNACC Article of the Month**, we feature an important research article by Seubert et al. relating to this topic which appeared in *Anesthesiology*, accompanied by an expert editorial of the paper by Ansgar Brambrink, M.D., Ph.D. of Oregon Health & Science University. Dr. Brambrink is a recognized and well-published authority in the field of neurogenesis and plasticity in the infant non-human primate (NHP) brain and following experimental stroke, and is also a former President of SNACC. We hope this will stimulate thought, and comments, regarding this important and still "young" topic. We invite SNACC members to continued discussion on this paper with the [SNACC LinkedIn Group](#).*

~John F. Bebawy, MD

### Expert Opinion

Dr. Ansgar Brambrink MD, PhD

In this paper, *Seubert and coworkers*, studied the effects of isoflurane (ISO) and sevoflurane (SEVO) anesthesia in 4-6 days old rats. They explored effects on cortical electroencephalogram (EEG) during the anesthesia, on expression of apoptosis marker 'activated caspase 3' (AC3) in the cortex after 24 hrs, and on behavioral presentation of exposed rats at 24 days of life (response to a new environment; sensorimotor gating function). ISO was administered for 6 hrs at 1.2 Vol% and SEVO at 2.1 Vol% which is an equivalent MAC of 0.6 in rats. The authors found fewer electrographic seizures in rats anesthetized with ISO versus SEVO anesthesia. Also noted were increased levels of AC3 at 24hrs after ISO. The subjects were noted to spend more time in the 'immobile state' when exposed to a new environment at age 24 days after SEVO but not after ISO. Subjects displayed impaired sensorimotor gating after both ISO and SEVO (the latter data was published in a previous paper already). Pretreatment with the Na-K-2Cl 1 co-transporter (NKCC1) inhibitor bumetanide ameliorated seizure-like activity during SEVO (but not ISO), reduced brain tissue levels of AC3 at 24hrs after ISO, and lessened behavioral abnormalities in adolescent rats ('immobile state' after SEVO and impaired sensorimotor gating after SEVO and ISO). The authors concluded from their data that both ISO and SEVO are associated with

an increase in neuronal activity during anesthesia (seizure-like episodes) and that this may explain the short-term and long-term effects on the developing brain of rats. In addition they concluded that both drugs may also trigger respective effects through different mechanisms (for example some that are sensitive to bumetanide pretreatment and others are not) and that these findings suggest differences in the safety profile of the two drugs when used for anesthesia in the neonate.

This paper addresses an important topic of current debate: the potential of anesthetics to induce neurotoxic effects in the developing brain and whether that can result in long-term neurobehavioral deficits. The paper adds an interesting new component to the discussion in that the authors suggest electrographic patterns during anesthesia, which look like brief cortical seizures, may represent a pathway of injury. During both ISO and SEVO anesthesia such episodes were observed approximately 2 to 4 times per hour and with total lengths of 20-50 sec per 1 hr of anesthesia. The frequency of these episodes during SEVO anesthesia (but not those during ISO) were reduced when animals were pretreated with bumetanide, an inhibitor of a GABA<sub>A</sub>-receptor co-transporter that is expressed in the brain predominantly during development. Additional cohorts of animals were followed after ISO or SEVO exposures, which were either pretreated with bumetanide or not. Some animals were maintained alive for 1 day to determine the level of AC3 in total cortex, and others for ~24 days in order to test two different behavioral read-outs (response to a new environment and pre-pulse inhibition of acoustic startle response; see the publication's method section for details regarding these measurements).

The central limitation of this study is that it does not provide any direct link between the observed seizure-like cortical activity during ISO or SEVO exposure and the subsequent observations of increased cortical AC3 (at 24hrs) or neurobehavioral changes at ~24 days. Firstly several subjects did not exhibit seizure like activity during ISO anesthesia. Second bumetanide reduced the incidence only during SEVO, but not during ISO anesthesia. Third, bumetanide did not abolish seizure like episodes completely, and finally, co-treatment with a classical antiepileptic drug was not tested. Nevertheless, bumetanide pretreatment was able to ameliorate AC3 levels one day after ISO exposure and was able to normalize some, but not all neurobehavioral abnormalities that were measured at ~24 days. The authors do not explain why bumetanide, while being unable to suppress the brief seizure episodes, was able to mitigate some medium, and long-term sequelae after ISO and SEVO anesthesia in this neonatal rat model. This finding leaves their primary hypothesis unresolved as to whether differences in electrical activity during SEVO versus ISO anesthesia may be the cause of subsequent injury in developing rat brain. Another important limitation is that despite the author's concluding remarks, the presented results cannot guide the field in regards to a possible safety difference between ISO and SEVO in clinical medicine.

While studying this paper further, a few additional considerations are interesting to note:

First, bumetanide blocks NKCC1 that is predominantly expressed in the brain during early development. Activation of NKCC1 further increases intracellular chloride concentrations during GABA<sub>A</sub>-receptor activation, and thereby increases the tendency for cell depolarization (excitation). Thus, by pretreatment with bumetanide, excitatory effects of GABA activation should be ameliorated. It remains unclear why pretreatment with bumetanide reduced the incidence of seizure-like episodes during SEVO anesthesia but not during ISO anesthesia. Moreover, it is important to note, that bumetanide did not completely eliminate seizure-like episodes in either of the experimental groups.

Second, it remains unclear what the mechanism is that explains the authors' observations of brief seizure like episodes during SEVO and ISO anesthesia. The phenomenon may suggest a direct effect on cortical neurons or it might be an indirect effect, e.g. by silencing inhibitory interneurons. In addition, it remains unclear from the presented data whether this phenomenon can explain the increased presence of the apoptosis marker AC3 in the cortex at 24hrs after the exposure, or the observed long-term neurobehavioral deficits in adolescent rats. Further experiments are needed to determine a potential causal relationship.

Third, it would have been interesting to analyze the brains for the distribution of apoptosis and the cell types involved. Also, the choice of measuring AC3 at 24hrs after the initiation of anesthesia is different from previous publications, where apoptosis was assessed by AC3 levels only a few hours after the exposure. It is possible that using AC3 at this rather late time point may have underestimated the total extent of ISO-induced apoptosis.

Last, the brain development of the newborn rat approximates that of a human fetus toward the end of the second and the beginning of the third trimester, or that of a very premature infant, respectively. It is thought that GABA<sub>A</sub>-receptor activation, in contrast to the adult brain, in the developing brain predominantly results in depolarization/excitation. This is explained with the relative higher concentration of the NKCC1 co-transporter

compared to that of the chloride-extruding  $K^+Cl^-$  co-transporter KCC2 in immature brain cells. In rats it is not before the end of the first postnatal week that the expression of the co-transporter KCC2 starts increasing further, while at the same time that of the NKCC1 co-transporter begins to decrease. Together, the two processes are considered the reason why, at about two weeks of age in the rat, GABA<sub>A</sub>-receptor activation then results in hyperpolarization/inhibition, thereby having changed to the adult receptor phenotype ('developmental switch'). For the human brain, the exact timing of the 'developmental switch' of GABA<sub>A</sub> receptor responses has not been determined yet, although it is currently suggested to occur before or immediately after birth based on the developmental patterns of the relative expression of NKCC1 and KCC2. As an approximation and with awareness of the underlying simplification, the brain development stage (e.g. dynamics of synaptic growth etc.) of a human term newborn is about similar to that of a 12-14 days old rat. It remains therefore unclear, whether the presented results from 5-day old rats can predict the effects of ISO or SEVO in human infants, and whether any one of the two drugs would be safer than the other when used in pediatric anesthesia practice.

In summary, this publication adds interesting layers to the current discussion in that it points out (1) that ISO and SEVO induce some brief seizure-like episodes in the developing brain of rats, (2) that those produced during SEVO anesthesia can be ameliorated by bumetanide pretreatment, and (3) that bumetanide pretreatment is associated with an improved long-term behavioral outcome after neonatal ISO and SEVO anesthesia in rats.