

Introduction

Converging evidence from both human and animal studies has shown that neonatal exposure to volatile anesthetics increase the risk of learning and memory related behavior deficits during adolescence. However, perturbation of neuronal function during a critical developmental time window is associated with numerous neuropsychological disorders in addition to learning and memory deficits. Whether anesthetic insults on the early developing brain impose similar risks and the mechanisms underlying the deleterious effects of volatile anesthetics on the developing brain remain unclear. A previous *in vitro* study from our lab has implicated that mechanisms associated with the signaling pathway kinase mTOR are involved in sevoflurane (sevo) mediated downstream effects. We hypothesized that exposure to volatile anesthetics such as sevo during an early postnatal period would result in long lasting changes in mTOR activity and its downstream signaling pathway such as PKM ζ , resulting in behavioral changes.

Method

Male C57BL6 mice were used throughout the study. Mice were exposed to 2% sevo for 2 hours at postnatal day 7 (P7) (which resembles human 3rd trimester gestation) and returned to their home cage to be reared and weaned under standard conditions. Starting at P27 (which resembles human adolescent age) and continued until 2-3 months old (which resembles human adult age), untreated control and sevo treated mice underwent a battery of behavioral tests such as active place avoidance (APA) for learning and memory and social interaction, repetitive behavior and olfactory tests for neuropsychiatric-like behaviors. Analysis of gene expression changes were based on western blot hybridization results from two time points: (1) Whole brain tissues were taken immediately after sevo treatment at P7, (2) Hippocampal tissues were taken after the completion of behavior tests during adulthood. All plotted values are the Mean \pm S.E.M.

Learning and memory

Active place avoidance

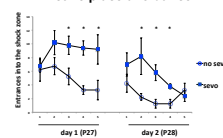


Fig 1. Neonatal sevo treated mice showed significantly more entrances into the shock zone compared to mice with no sevo treatment.

Locomotion and anxiety-like behaviors

Open field

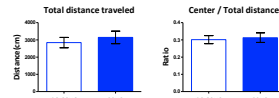


Fig 2. No differences was observed between the two groups (no sevo vs. sevo) on total distance traveled and the ratio of center/total distance traveled.

Neuropsychiatric-like behaviors

Reciprocal social interaction

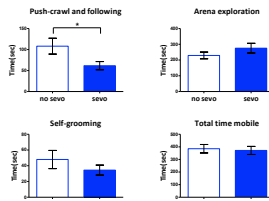


Fig 3. Sevo treated mice showed a significant reduction in push-crawl and following compared to no sevo group.

Olfactory habituation/dishabituation

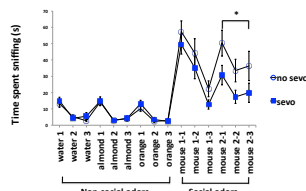


Fig 4. Both groups were able to habituate to the same scent and dishabituate when presented with a new scent. However, sevo treated group showed reduced interest when presented with a series of odors.

Three chamber social-interaction

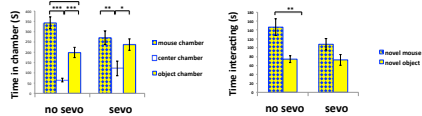
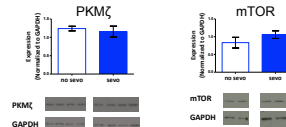


Fig 5. The no sevo group spent significant more time in the mouse chamber and had more interaction with the novel mouse, while the sevo group showed no preference between mouse and object.

Gene expression after sevo treatment

P7-day whole brain



Adult hippocampus

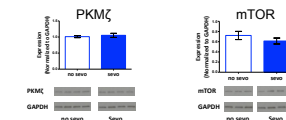
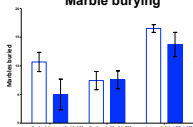


Fig 7. No difference in expression was observed for both PKM ζ and mTOR between the two groups.

Conclusion

- We confirmed that early life anesthetic exposure impacts cognitive behavior later on in life.
- Neonatal sevoflurane exposure reduces social interactions as demonstrated in three different behavioral paradigms, reciprocal social interaction, olfactory habituation/dishabituation and three chamber social interaction.
- Impairment in social interaction is often observed in mouse models of neuropsychological disorders, such as Autism Spectrum disorder
- The observed changes in behavior were not influenced by changes in general behavior since no difference was observed in locomotion and anxiety-like activity.
- Gene expression of PKM ζ and mTOR throughout development were not influenced by neonatal sevo exposure.

Marble burying



self-grooming

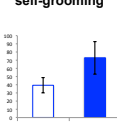


Fig 6. The two groups showed no difference in behavior paradigms: marble burying and self-grooming.