Neuroscience in Anesthesiology and Perioperative Medicine - Neonatally sevoflurane-exposed and unexposed male rat cagemates affect each other's neurodevelopmental phenotypes

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Introduction: Human twin studies found similar neurocognitive abnormalities in twins discordant for early life exposure to anesthesia and concluded that preexisting conditions, not anesthesia exposure, caused those neurocognitive abnormalities. We tested an alternative explanation, namely, that rats discordant for neonatal exposure to sevoflurane (SEVO), who are reared together in the same litter/cage affect each other's brain development. Male rats were chosen as a model because we have shown that sevoflurane induces more robust neurodevelopmental abnormalities in males than females.

Methods: All animal procedures were approved by IACUC. Figure 1 shows an overview of the study design. Postnatal day 5 (P5) male Sprague-Dawley rats from different litters were mixed together and assigned to one of three new litters, each of which received a newly-assigned foster dam. The three litters were as follows: 1) only pups that underwent exposure to 2.1% SEVO for 6 h (SEVO) on P5; 2) only pups that were not exposed to SEVO and remained with their foster dams (Control); and 3) equal numbers of SEVO-unexposed pups that remained with their foster dams and pups that underwent exposure to 2.1% SEVO for 6 h on P5 (MIXED). After weaning on P21, rats from Control litters were housed two per cage (Control, n = 16), as were rats from SEVO litters (SEVO, n = 16). The rats from MIXED litters were also housed two per cage after weaning, so that each cage contained one SEVO-unexposed and one SEVO-exposed rat. Based on exposure status, rats from MIXED litters/cages formed two separate study groups: the MIXED-unexposed group (n = 16) and the MIXED-exposed group (n = 16). Rats from all four experimental conditions were sequentially evaluated in behavioral and neuroendocrine tests prior to collection of tissue samples for mechanistic studies.

Results: As anticipated based on the results of prior studies, rats in the SEVO group, when compared to rats in the Control group, differed in gene transcription, stress responsivity and behavior. To our surprise, rats in the MIXED litters did not differ by sevoflurane-exposure status, with the exception of expression of two hippocampal and one hypothalamic genes. Compared to SEVO rats, MIXED-exposed rats showed less anxiety-like behavior in the elevated plus maze, had similarly-impaired sensorimotor gating, showed similarly-heightened endocrine responses to stress, but not significantly impaired spatial memory in the Morris water maze test. Similarly increased serum corticosterone levels after the prepulse inhibition of acoustic startle test and similar anxiety-like behavior during the elevated plus maze test in MIXED-unexposed and MIXED-exposed rats were accompanied by similar up- and down-regulation, respectively, of expression of hypothalamic corticotropin-releasing hormone (*Crh)* and hippocampal glucocorticoid receptor (*Gr*) genes, which are central in regulation of corticosterone responses to stress. To gain insight into whether cohabitation may affect epigenetic processes, we measured hippocampal and hypothalamic mRNA levels of DNA methyltransferase [*Dnmt*(s)] and methyl CpG binding protein (*Mecp2*). In the hippocampus, there were differences in expression of the *Dnmt1, Dnmt3a, Dnmt3b* and *Mecp2* genes between SEVO and Control rats, while only *Dnmt3a* mRNA levels differed between MIXED-exposed and MIXED-unexposed rats.

Conclusions: Our findings suggest that through cohabitating, rats can affect each other's brain development, ameliorating some SEVO-induced deficits in exposed rats, and inducing some deficits in unexposed rats at gene expression, neuroendocrine, and behavioral levels. These findings suggest that in twin studies of neurodevelopmental abnormalities in general, and anesthesia-induced neurodevelopmental abnormalities in particular, inter-sibling interactions should be considered a potentially important determinant of outcomes, in addition to environmental and genetic factors. These findings also suggest a new explanation of why neurodevelopmental neuropsychiatric disorders are more likely to occur among siblings.

References: 1. Bartels M, Althoff RR, Boomsma DI. Anesthesia and cognitive performance in children: no evidence for a causal relationship. Twin Res Hum Genet. 2009 Jun;12(3):246-53.

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