EPIGENETIC MECHANISMS MEDIATE MULTIGENERATIONAL EFFECTS OF EARLY LIFE EXPOSURE TO SEVOFLURANE IN RATS

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Background Clinical studies report learning disabilities and attention-deficit/hyperactivity disorders (ADHD) in those who had anesthesia early in life. We have found that rats, primarily males, neonatally exposed to GABAergic anesthetics exhibit behavioral abnormalities, exacerbated responses to stress and reduction in expression of hypothalamic K^+-$2Cl^-$ (*Kcc2*) Cl^- exporter.[1-7] The latter is implicated in development of psychiatric disorders, including male predominant comorbid with ADHD autism spectrum disorders. We tested whether parental early life exposure to sevoflurane, the most frequently used anesthetic in pediatrics, affects the next generation of unexposed rats.

Methods Sprague-Dawley rats (F0), unexposed or exposed to 2.1% sevoflurane for 6 h on postnatal day (P) 5, were used to produce 4 groups of offspring (F1) [control father/control mother (con-M*con-F), exposed father/control mother (sevo-M*con-F), control father/exposed mother (con-M*sevo-F) and exposed father/exposed mother (sevo-M*sevo-F)].

Results Male, but not female, progeny of sevoflurane-exposed parents were affected.[1] F1 males of both exposed parents exhibited impaired spatial memory (Fig. 1K-M) and decreased expression of the hippocampal and hypothalamic *Kcc2* (Fig. 2H). Offspring of only exposed sires had abnormalities in elevated plus maze and prepulse inhibition of startle (Fig. 1E-I), but normal spatial memory, and decreased expression of the hypothalamic, but not hippocampal, *Kcc2* (Fig. 2B,H). In contrast to exposed F0, their progeny exhibited normal corticosterone responses to stress (Fig. 1A,B). Bisulfite sequencing revealed increased CpG site methylation in the *Kcc2* promoter in F0 sperm (Fig. 3A) and F1 male hypothalamus and hippocampus (Fig. 3C,D) that was concordant with the changes in *Kcc2* expression in specific F1 groups.

Conclusions Our findings provide the first experimental evidence that neonatal exposure to sevoflurane may also affect the next generation of males through epigenetic modification of *Kcc2* expression, while F1 females may be at a diminished risk.

Illustration of the study design

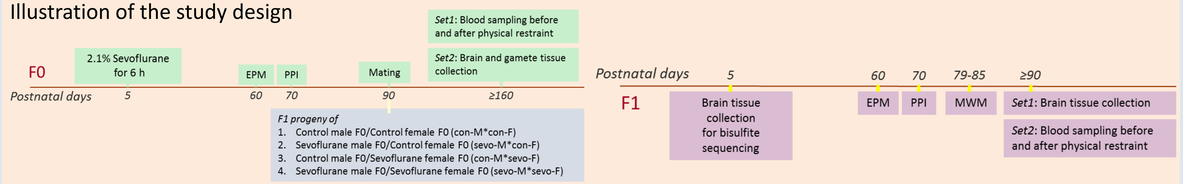


Figure 1. F1 male, but not female, offspring of parents (F0) exposed to sevoflurane on postnatal day 5, exhibit behavioral abnormalities, while both F1 females and F1 males had normal corticosterone responses to stress.

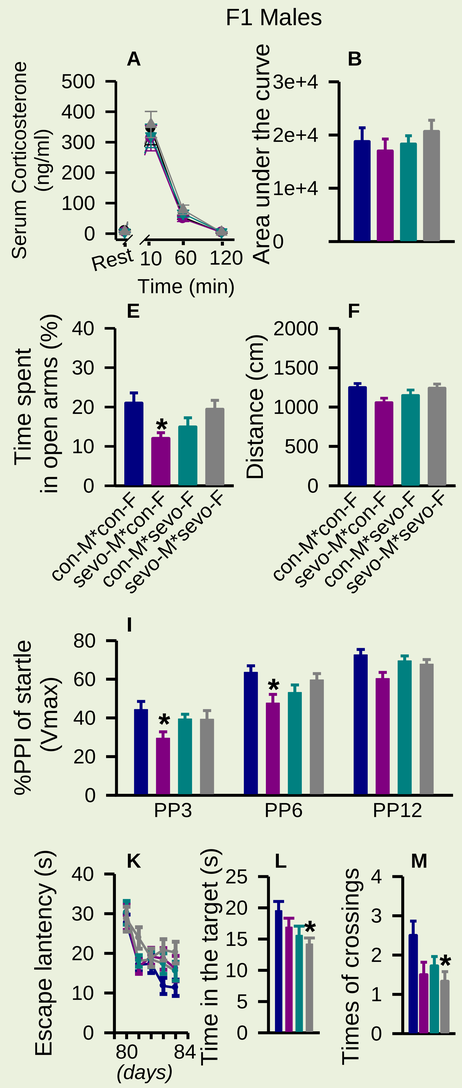


Figure 2. Gene expression for Na^+-K^+-$2Cl^-$ (*Nkcc1*), K^+-$2Cl^-$ (*Kcc2*) and glucocorticoid receptors (*Gr*) in the paraventricular nucleus (PVN) of the hypothalamus and hippocampus of F1 male rats (*see text for details*).

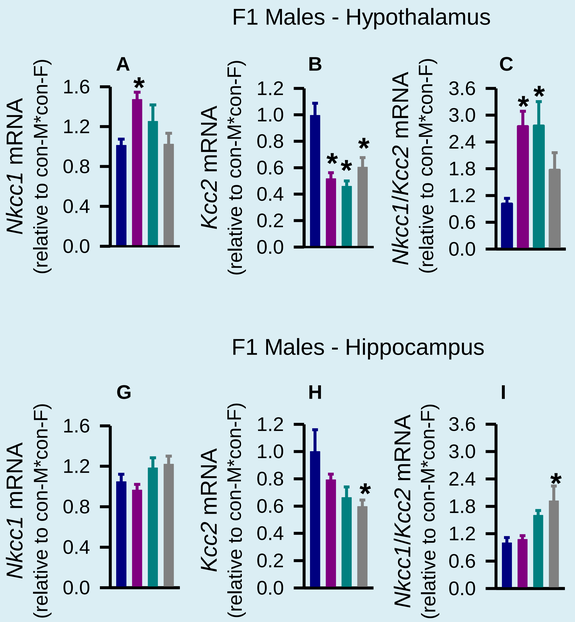
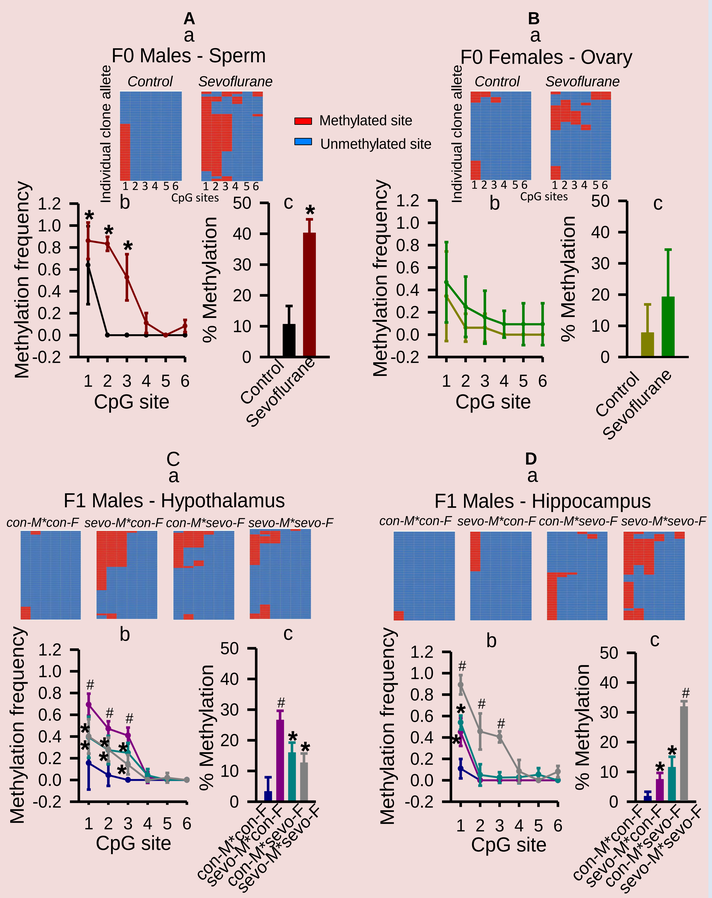


Figure 3. Bisulfite sequencing revealed increased CpG site methylation in the *Kcc2* promoter in F0 sperm (A) and F1 male hippocampus (C) and hypothalamus (D).



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