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Exposure to sevoflurane anesthesia during development does not impair aspects of attention during adulthood in rats

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ABSTRACT

Exposure to general anesthetic agents during development has been associated with neurotoxicity and long-term behavioral impairments in rodents and non-human primates. The phenotype of anesthetic-induced cognitive impairment has a robust learning and memory component, however less is known about other psychological domains. Data from retrospective human patient studies suggest that children undergoing multiple procedures requiring general anesthesia are at increased risk of attention deficit hyperactivity disorder. We therefore assessed whether single or repeated exposures of neonatal rats to general anesthesia caused long-term attentional impairments. Female or male Long-Evans pups were exposed to 2.5% sevoflurane for 2 h on postnatal day (P) 7, or for 2 h each on P7, P10 and P13. Rats were behaviorally tested in late adolescence on the sustained attention task and on the attentional set shifting task. There was no compelling evidence for anesthetic-induced impairment in attentional processing in adult rats exposed to general anesthesia as neonates. These results suggest that, at least at the developmental stage tested here, the phenotype of anesthetic-induced cognitive impairment does not involve disruptions to attentional processing.

1. Introduction

Human infants undergoing procedures requiring general anesthesia have been shown to be at increased risk for the development of attention-deficit hyperactivity disorder (ADHD) (Sprung et al., 2012). In a retrospective, population-based birth cohort study, the cumulative incidence of ADHD in children who underwent multiple procedures before the age of 2 years was found to be 17.9%, as compared to 7.3% for those who did not undergo procedures. The extent to which anesthesia contributes to these findings is unknown, as patient studies cannot exclude important factors such as the need for surgery and the surgical procedure itself.

The behavioral phenotype of ADHD is variable but characteristics fall broadly into two categories - inattention, and impulsivity plus hyperactivity (DSM-V, 2013); with the prevalence for the inattentive subtype potentially being as high as 5% (Wolraich et al., 1996) during childhood and 1.3% during adulthood (Murphy and Barkley, 1996). ADHD is a highly heritable disorder (Faraone and Doyle, 2000; Thapar et al., 2005) but a number of environmental factors have been proposed as contributors to the disorder, including early exposure to nicotine and alcohol (Biederman et al., 1995a; Biederman et al., 1995b).

Developmental exposure to general anesthetic agents has been shown to trigger apoptotic neurodegeneration in animal models (Ikonomidou et al., 2000; Jevtovic-Todorovic et al., 2003), and this has been proposed as a mechanism for resulting behavioral impairments. Anesthetic-induced neurodegeneration occurs throughout cortical and subcortical areas, including anatomical regions that are involved in attentional processing (Jevtovic-Todorovic et al., 2003). However, although evidence from animal experiments for anesthetic-induced impairments in learning and memory is compelling (Jevtovic-Todorovic et al., 2013), there is currently no evidence regarding the presence or absence of post-anesthetic attentional impairments.

In order to investigate whether anesthetic exposure during development would lead to post-anesthetic impairments in attentional processing, the effect of exposure to sevoflurane was tested in two behavioral tasks that are commonly used to assess attention in rodents. Rodent studies have associated sevoflurane during development with alterations in socio-emotional behavior (Satomoto et al., 2009), impairments in spatial reference memory and neuroapoptosis (Istaphanous et al., 2011; Zheng et al., 2013) as well as dose-related damage to hippocampal neuronal ultrastructure (Amrock et al., 2015). The behavioral tasks chosen for this study were: (1) The sustained attention task (SAT), developed from an auditory stimulus task devised by Bushnell and

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colleagues in 1994 (Bushnell et al., 1994). A version of the task was validated by (McGaughy and Sarter, 1995) and requires the subject to attend to, and indicate the presence or absence of a visual stimulus. (2) The intra-dimensional extra-dimensional set shift task (IDED), which is based on the Wisconsin Card Sorting task (WCST) used to assess attentional set shifting, or 'flexibility of thinking' in humans (Berg, 1948; Milner, 1963). The task requires the subject to dig for food reward, whose location is associated with a specific exemplar of a particular stimulus dimension (e.g., odor within the digging pot) of a compound stimulus consisting of three dimensions (the odor, digging media and texture covering the digging pot). Subjects are required to both maintain attention to a particular stimulus dimension when the specific exemplar varies, and shift attention to a different stimulus dimension during different phases of the task.

The effect of single versus repeated anesthetic exposure during development, in male and female rats, was investigated. It was hypothesized that exposure to anesthesia during development would lead to impairments in attention in adulthood, and that the impairments would be greater in rats that had been repeatedly exposed to anesthesia.

2. Materials and methods

2.1. Ethical approval

Experimental procedures were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Protocols were approved by the Institutional Animal Care and Use Committee of the Icahn School of Medicine at Mount Sinai (anesthesia) and the University of New Hampshire (behavioral testing).

2.2. Subjects

Post natal day (P) seven male or female Long Evans rat pups, from twelve natural litters, born in house, were allocated to one of three experimental groups, matched primarily for dam and sex and then, as far as possible, bodyweight. Rats received either a single 2-h exposure to anesthesia (group 1A: 5 males, 5 females) at P7; three 2-h exposures to anesthesia (group 3A: 5 males, 5 females) at P7, P10 and P13; or three 2-h exposures to the control condition (group C: 5 males, 5 females). Power analysis revealed that ten animals per group would give 80% power to detect an effect size of Cohen's d = 1.325, in a two tailed *t*-test. This would correspond to a difference of 12% in accuracy on the SAT task, and represents a much smaller difference in SAT accuracy (more subtle impact) than seen in rats with neurotransmitter specific lesions of the prefrontal cortex, where effect sizes of Cohen's d ≈ 3 were reported for different distracter testing sessions (Newman and McGaughy, 2008). At P21 rats were weaned, subsequently pair housed and kept in individually ventilated cages. At approximately P75 rats were transferred to a second Institution, for behavioral testing, where they were pair housed, in conventional caging, with automatically regulated lighting (12/12 light/dark cycle with lights off at 19:00 h).

2.3. Anesthesia protocol

Episodes of anesthesia consisted of 2 h of 2.5% sevoflurane (SEVOFLO, Abbott Animal Health, USA) delivered in 30% oxygen. Two hour exposures to volatile anesthesia, during pregnancy (Zheng et al., 2013) or infancy (Murphy and Baxter, 2013) can induce neurotoxicity and spatial memory impairment, and was therefore chosen for this study. Rats in the control condition received 30% oxygen in identical environmental conditions to rats receiving anesthesia. Equipment set up, monitoring and physiological support were as per previous work (Murphy and Baxter, 2013). Anesthetized rats were recovered in 30% oxygen for 20 min and returned to the dam with rats from the control condition.

In a separate experiment, rats that had received 2 h of 2.5% sevoflurane in 30% oxygen on (i) P7 (Group P7: $n = 4$), (ii) P7, P10 and P13 (Group P13: $n = 4$) or, 30% oxygen without anesthesia on (iii) P7, P10 and P13 (Group C: $n = 4$) were used for mixed arterial/venous blood gas analysis. Samples were collected at the end of the period of anesthesia (before recovery) and the pups immediately euthanized. Rats were removed from the anesthetic chamber and a trans-cardial blood sample was immediately taken and analyzed (Radiometer ABL80, Cleveland, USA) for pH, pCO_2 and pO_2. Control rats in group C were exposed to 6% sevoflurane in 30% oxygen until loss of righting reflex (approximately 20 s) just prior to blood sampling, in order to prevent distress.

2.4. Behavioral testing

At approximately P60, rats were food restricted and maintained at not <90% of age matched ad libitum levels. All rats underwent the sustained attention task testing at approximately P91, followed by attentional set shifting testing at approximately P120. Rats were trained and tested daily, in the same order, between 9:00 h and 14:00 h, by an experimenter unaware of anesthesia group allocation.

2.4.1. Sustained attention task

Sustained attention performance was tested in operant chambers, where rats were food rewarded for attending to, and distinguishing between, signal (visual stimulus) and non-signal (absence of visual stimulus) trials; by pressing the appropriate one of two levers extended after presentation (or lack of presentation) of the stimulus.

Apparatus and materials, behavioral training and task shaping were as per previous work (Newman and McGaughy, 2008). Briefly, rats were trained in operant chambers (Med Associates, VT, USA), equipped with two retractable levers located either side of a food dispenser. Rats were initially rewarded for pressing either lever, before being shaped to press a particular lever depending on whether a visual stimulus was or was not presented. Correct lever presses were defined as 'hits' when they occurred on a signal trial and 'correct rejections' on a non-signal trial. Incorrect lever presses were defined as 'misses' on a signal trial and 'false alarms' on a non-signal trial. Rats performed one session per day and were trained to a criterion of >75% hits to 500 ms signals and >75% correct rejections to non-signal trials for a maximum of 162 consecutive trials or 40 min, for at least two consecutive sessions before testing began. Performance on the second of these two sessions was recorded as baseline testing performance.

Baseline testing was carried out on a version of the task with varied signal duration, such that either 500, 100 or 25 ms signals were presented in a pseudo-random order. Each session consisted of 27 trials of each signal length and 81 non-signal trials, counter balanced into 3 blocks of 54 trials with each signal length presented 9 times plus 27 non-signal trials. Twenty-four hours after baseline testing, rats were tested on the first of 3 task variants (single session per task variant) designed to alter the attentional demands of the task. Between task variants rats were retrained to baseline performance on the non-manipulated task. The order in which rats carried out the task variations was counterbalanced, as far as possible, for anesthesia group and sex. The three task variants were as follows:

2.4.1.1. Short duration stimulus. Presented stimuli for signal trials were all 25 ms duration. In a session that contains only 25 ms signals, rats demonstrate *improved* performance to detect signals (as well as a small improvement in performance to reject signals) than when the 25 ms signals are presented embedded within a dynamic stimulus range (pseudo randomly presented variable signal duration), and this is thought to be because the decrease in uncertainty (of signal duration) leads to a decrease in attentional demand (Newman and McGaughy,