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Neurobehavioural abnormalities induced by repeated exposure of neonatal rats to sevoflurane can be aggravated by social isolation and enrichment deprivation initiated after exposure to the anaesthetic

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Abstract

Background: We tested the hypothesis that developmental effects of repeated neonatal exposure to sevoflurane in rats are exacerbated by stressful experiences received later in life.

Methods: Sprague-Dawley male rats received sequential exposures to 3% sevoflurane for two h on postnatal days (P) six, seven, and eight. After weaning at P21, rats were housed either in pairs in an enriched environment (EE) or singly in an enrichment-deprived environment (an adverse environment, AE). The hippocampal concentrations of brain-derived neurotrophic factor (BDNF), and synaptic markers were assessed at P8 and P53. The dentate gyrus neural progenitor proliferation was evaluated at P11 and P53 after administration of bromodeoyuridine (BrdU) at P8 to P10 and at P22 to P27, respectively. Neurobehavioural evaluations were performed at P49 to P53.

Results: Repeated sevoflurane exposure acutely reduced concentrations of BDNF, synaptic markers and neural progenitor proliferation. The sevoflurane group housed in the AE conditions (sevoflurane+AE) had decreased concentrations of BDNF and synaptic markers, and survival of new granule cells and impaired cognitive function compared with the control+AE, control+EE, and sevoflurane+EE groups. The neurobehavioural parameters in the sevoflurane+EE and control+EE groups were similar.

Conclusions: Neurocognitive abnormalities induced by repeated neonatal exposure to sevoflurane can be aggravated by stressful conditions such as social isolation and enrichment deprivation.

Key words: brain-derived neurotrophic factor; cognition; environment; rats; sevoflurane; social isolation

The majority of human retrospective epidemiological evaluations of children who underwent surgical procedures with general anaesthesia at an early postnatal age, report impairments in cognition and behavior [for a review, see Sanders and colleagues[1]]. Although human studies performed thus far do not provide a definitive answer to the question of whether neonatal

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Editor's key points

- Repeated anaesthesia in the neonatal period is linked to cognitive and behavioural deficits later in childhood
- Effects of early anaesthesia may be compounded by environmental factors
- This study assessed the effect of an enriched or deprived environment on neurobehavioural effects of early anaesthesia in rat pups
- Social isolation and lack of an enriched environment worsened the anaesthesia effects

anaesthesia itself affects brain development, similar findings in healthy animals exposed to anaesthesia in the absence of surgery strongly support this possibility. Furthermore, findings in affected children indicate that the duration of anaesthesia and repeated exposures to anesthetics may be among the aggravating factors.[2–4] The latter is in agreement with the results of well-controlled laboratory studies.[5 6] Such parallels between the findings of human and animal studies suggest that understanding the underlying mechanisms of the developmental effects of neonatal anaesthesia in animal models may facilitate an understanding of the developmental effects of neonatal exposure to anaesthesia in humans. Unfortunately, molecular targets and cellular pathways involved in the mediation of neonatal anaesthesia-induced abnormalities are incompletely understood, even in animal models.

It was recently reported by several laboratories that the adverse developmental effects of neonatal anaesthesia in rodents may be alleviated, not only by pharmacological interventions before exposure to anaesthesia, but also by post-weaning housing of the exposed animals in an enriched environment.[6–8] This brings us to the possibility that the functional consequences of the exposure of neonates to general anaesthetics may result from a combination of the acute effects of anaesthetics at the time of anaesthesia and the effects of 'post-anaesthesia' environmental factors, such as a healthy and fulfilled life or disease, hunger, pain, maternal deprivation, etc. Consequently, the effects of such environmental factors may be alleviating or exacerbating. In other words, two subjects exposed to the same anaesthesia protocol may have different long-term neurobehavioural outcomes based on post-anaesthesia life experiences. We tested this hypothesis by comparing the developmental effects of repeated exposures to sevoflurane in rats that were housed either in an enriched environment or in social isolation, deprived of an enriched environment.

Methods

Animals

The present study was approved by the Ethics Committee of Jinling Hospital, Nanjing University, China, and was performed in accordance with the relevant aspects of ARRIVE guidelines and the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health (Bethesda, MD, USA). Pregnant Sprague-Dawley rats were purchased from the Animal Center of Jinling Hospital, Nanjing, China, and were housed individually in standard conditions with a 12-h light/dark cycle (light from 07:00–19:00) at 24±1°C and *ad libitum* access to food and water.

Anaesthesia

The P6 to P8 male rat pups were randomly assigned to the sevoflurane (SEV) or control (CON, not exposed to sevoflurane) groups. Rats in the SEV group received 3% sevoflurane in O_2/N_2 (fraction of inspired oxygen 50%, or Fi_{O_2} 50%) for two h daily on three consecutive days, in a thermostated chamber set to 37±1°C. The total gas flow was two Litre min^{-1}. The rats breathed spontaneously, and concentrations of anaesthetic and oxygen were measured continuously using a calibrated Datex side stream analyser that sampled from the interior of the chamber. After anaesthesia, the rat pups were allowed to recover and were returned to the mothers on gaining the righting reflex. Rat pups in the non-exposed group were separated from the dams for the same duration of time in identical conditions (37 ± 1°C), Fi_{O_2} 50%), except for exposure to the anaesthetic. Arterial blood for gas analysis was obtained from separate groups of sevoflurane-exposed (n=5) and non-exposed (n=5) rat pups by thoracotomy and orthoptic puncture of the left ventricle at the end of the last sevoflurane exposure (i.e. third exposure), while the animals were still anaesthetized. The maternally separated non-exposed pups received pentobarbital (50 mg kg^{-1}, intraperitoneally, i.p.) before the arterial blood sampling at the end of the third maternal separation. Upon completion of the bleeding procedures and after all other procedures that required euthanasia, the animals received pentobarbital (100 mg kg^{-1}, i.p.) followed by decapitation. An adequate depth of anaesthesia before decapitation was ensured by lack of response to a nociceptive stimulus. The mean (SD) blood gas values in the non-exposed and exposed animals were: pH: 7.36 (0.039) and 7.30 (0.041); PC_{O_2}: 45.8 (3.4) and 40.2 (3.1) mm Hg; PO_2: 163.4 (7.3) and 154.2 (5.7) mm Hg. These findings are in agreement with the results of previous studies showing that anaesthesia with 3% sevoflurane for two h does not significantly change blood gas values.[6]

Experimental design of the study

The experimental design of the study is illustrated in Fig. 1. In the first set of experiments (Fig. 1A) the acute effects of sevoflurane were studied by assessing apoptotic neuronal profiles, concentrations of brain derived neurotrophic factor (BDNF) and synaptic markers in the hippocampus of the sevoflurane-exposed and control rats six h after the last sevoflurane exposure (n=6) or last maternal separation only (n=6), respectively. To determine the effect of sevoflurane on neural progenitor proliferation in the hippocampus, the sevoflurane-exposed (n=6) and control (n=6) rat pups received the thymidine analog, bromodeoxyuridine (BrdU, 75 mg kg^{-1}, i.p.), over three consecutive days from P8 to P10. The BrdU+ cells were determined at P11 using immunohistochemistry.

In the second set of experiments (Fig. 1B), the effects of the adverse and enriched environments (AE and EE, respectively) on sevoflurane-induced changes in brain biochemical markers and neurocognitive function were studied. To create the EE, large Plexiglas cages (55×40×30 cm) were equipped with a series of novel objects representing different types of stimuli: a voluntary running wheel for physical exercise, environmental complexity for social interaction, and environmental novelty (Fig. 1C). Every three days the objects were cleaned, disinfected, and rearranged to ensure novelty. Plexiglas cages (32×22×17 cm) lacking any enrichment objects represented the AE (Fig. 1C). The rat pups were weaned at P21 and housed one per cage in a cage deprived of enrichment objects (AE), or two per large cage filled with enrichment objects (EE). The rats were divided into four treatment groups: sevoflurane-exposed rats housed in the AE (SEV+AE); sevoflurane-exposed rats housed in the EE (SEV+EE); control rats housed in the AE (CON+AE); and control rats housed in the EE (CON+EE). A subset of animals (n=6) from each treatment group received BrdU (75 mg kg^{-1}, i.p.) for six consecutive