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Learning, memory and synaptic plasticity in hippocampus in rats exposed to sevoflurane

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ABSTRACT

Purpose: Developmental exposure to volatile anesthetics has been associated with cognitive deficits at adulthood. Rodent studies have revealed impairments in performance in learning tasks involving the hippocampus. However, how the duration of anesthesia exposure impact on hippocampal synaptic plasticity, learning, and memory is as yet not fully elucidated.
Methods: On postnatal day 7(P7), rat pups were divided into 3 groups: control group ($n = 30$), 3% sevoflurane treatment for 1 h (Sev 1 h group, $n = 30$) and 3% sevoflurane treatment for 6 h (Sev 6 h group, $n = 28$). Following anesthesia, synaptic vesicle-associated proteins and dendrite spine density and synapse ultrastructure were measured using western blotting, Golgi staining, and transmission electron microscopy (TEM) on P21. In addition, the effects of sevoflurane treatment on long-term potentiation (LTP) and long-term depression (LTD), two molecular correlates of memory, were studied in CA1 subfields of the hippocampus, using electrophysiological recordings of field potentials in hippocampal slices on P35-42. Rats' neurocognitive performance was assessed at 2 months of age, using the Morris water maze and novel-object recognition tasks.
Results: Our results showed that neonatal exposure to 3% sevoflurane for 6 h results in reduced spine density of apical dendrites along with elevated expression of synaptic vesicle-associated proteins (SNAP-25 and syntaxin), and synaptic ultrastructure damage in the hippocampus. The electrophysiological evidence indicated that hippocampal LTP, but not LTD, was inhibited and that learning and memory performance were impaired in two behavioral tasks in the Sev 6 h group. In contrast, lesser structural and functional damage in the hippocampus was observed in the Sev 1 h group.
Conclusion: Our data showed that 6-h exposure of the developing brain to 3% sevoflurane could result in synaptic plasticity impairment in the hippocampus and spatial and nonspatial hippocampal-dependent learning and memory deficits. In contrast, shorter-duration exposure (1 h) results in less damage. These results provide further evidences that duration of anesthesia exposure could have differential effects on neuronal plasticity and neurocognitive performance.

1. Introduction

Sevoflurane is a commonly used volatile anesthetic, particularly to induce anesthesia in the clinical pediatric context. The molecular mechanisms underlying its anesthetic effects remain unclear. However, it is known to activate the γ-aminobutyric acid type A ($GABA_A$) receptors and/or inhibit *N*-Methyl-D-aspartate (NMDA) receptors – thereby depressing synaptic transmission – and may lead to an anesthetic state associated with, for example, amnesia, unconsciousness, and analgesia (Mashour et al., 2005). In addition, previous studies suggested that potentiation of the $GABA_A$ receptor could also suppress neuronal plasticity and cause cognitive impairment (Möhler, 2007). Much experimental evidence has demonstrated that when the developing brain is exposed to sevoflurane, it could result in widespread neurodegeneration and neurocognitive dysfunction in rodents and non-human primates (Sinner et al., 2014; Jevtovic-Todorovic, 2012). This raises concerns about the effects of general anesthesia in children undergoing surgery, although a causal relationship between anesthetic exposure and developmental outcome in humans remains speculative (Olsen and Brambrink, 2013; Mellon et al., 2007; Loepke and Soriano, 2008; Nasr and Davis, 2015).

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Significant effort has been directed at identifying the mechanisms of sevoflurane-induced neurotoxicity at the cellular level, and particularly the role of neuroapoptosis and damaged neurogenesis in mediating learning and memory impairment (Stratmann et al., 2010). Some researchers have gone further recently by suggesting that persistent alternation of synaptic development and neuronal plasticity in cognition-related brain regions underlie the deficits in neurobehavioral performance, especially in hippocampus-dependent tasks (Vutskits, 2012). The hippocampus, part of a brain system responsible for learning and memory, has been shown to be an important target of general anesthetics. It has been suggested that neuronal plasticity impairment could underlie the cognitive abnormalities. However, the effects of sevoflurane exposure on synaptic plasticity in the developing brain are not completely understood.

Therefore, in the present study, we investigated the role of synaptic plasticity in anesthetic-induced hippocampal-dependent impairments in learning and memory. We used neonatal rats as a model of the developing brain, and exposed them to 3% sevoflurane for 6 h. We thus explored the anesthetic's effects on molecular, structural, and functional aspects of synaptic plasticity in the hippocampal area and on behavioral development, as manifested in performance later in adulthood. In particular, we examined the effects of a shorter sevoflurane exposure (1 h) on hippocampal synaptic plasticity, in order to compare exposure duration effects.

2. Materials and methods

2.1. Animals

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the Fudan University. At P6, litters of 8–10 rat pups were obtained from each of 10 Sprague-Dawley rat dams obtained from the Animal Care Center of Fudan University. On P7, all animals from each litter were randomly assigned to either a control (Group Control) or to 2 treatment groups receiving exposure to sevoflurane: (1) for 1 h (Group Sev 1 h) or (2) for 6 h (Group Sev 6 h). For each group, subjects at P21 were either killed for molecular (western blot: $n = 6$) and histological (Golgi staining: $n = 5$ and transmission electron microscope: $n = 3$) assessments or were weaned and further bred until electrophysiological ($n = 5$) and neurobehavioral ($n = 9$) tests (i.e., Morris water maze, novel-object recognition) were conducted.

2.2. Anesthesia procedure

The neonatal rats were separately anesthetized as we have previously described (Lei et al., 2013). Briefly, P7 rats were placed in a sealed box ventilated with 3.0% sevoflurane in 100% oxygen and treated for 6 h or 1 h at a flow rate of approximately 1 L/min. The box was adjusted to maintain a specific concentration of sevoflurane and oxygen at constant levels. Gases within the anesthetic box were monitored continuously through a gas sample line by using a monitor (Datex Ohmeda S/5, Helsinki, Finland). The temperature in the sealed box was maintained at $30 \pm 1\,^{\circ}C$ with a heating pad. The total survival percentage of P7 rats for 6 h anesthesia was 80% (7 deaths among the 35 pups) and at 1 h was 100% ($n = 30$). The control group ($n = 30$) received 100% oxygen but without anesthetic exposure under identical conditions as experienced by the anesthetized animals. Following anesthesia, the pups were returned to their dams for lactation. They were housed under the same standard lab housing with a 12-h light/dark cycle and a regulated temperature (20–25 °C) and humidity (45–65%). After weaning, the rats were housed 3 per cage and had free access to food and water. At P21 or P35, the brain samples were prepared according to experimental requirements, which are described in experimental methods, respectively. All behavioral experiments were performed during the light phase between 7:00 AM and 7:00 PM.

2.3. Protein extraction from hippocampal tissue and western blot analysis

The rats on P21 were then sacrificed by rapid decapitation, and bilateral hippocampus areas were harvested and stored at −80 °C until used in western blot analysis. Western blot was performed as described previously (Lei et al., 2013; Liu et al., 2015). Hippocampal tissues were homogenized by brief sonication in RIPA buffer (Millipore, Temecula, CA, USA) containing a complete protease inhibitor cocktail and 2 mM phenylmethylsulfonyl fluoride (PMSF) solution. The homogenate was centrifuged at 12,000 rpm for 15 min at 4 °C. After the protein samples were quantified using a BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL, USA), 60 μg of each sample was electrophoresed through a 10% sodium dodecyl sulfate-polyacrylamide gel and wet electrotransferred to nitrocellulose membranes (Millipore). The blots were blocked with 5% skim milk in Tris-buffered saline (150 mM NaCl, 0.1% Tween 20, 20 mM Tris, pH 7.4) for 1 h, then incubated overnight at 4 °C with anti-syntaxin (1:200, sc-13994, Santa Cruz Biotechnology, CA, USA), anti-SNAP25 (1:4000 dilution, BD Biosciences, CA, USA), anti-synaptophysin (1:1000, Cell Signaling Technology, CA, USA), anti-α-synuclein (1:1000, Cell Signaling Technology, CA, USA), primary antibodies and then incubated with the corresponding secondary antibody (1:5,000; Epitomics, Hangzhou, Zhejiang Province, China) at room temperature for 1 h. Protein signals were detected using an enhanced chemiluminescence detection system (Pierce Biotechnology). A β-actin antibody (1:1,000; Santa Cruz Biotechnology, CA, USA) was used to normalize sample loading and transfer. Band intensities were densitometrically quantified using Gel-Pro Analyzer (Media Cybernetics, Bethesda, MD, USA). Data were expressed as mean ± SEM. The changes were presented as a percentage of band intensity of the control group sample. For purposes of comparing protein expression to that in Group Sev 1 h and Group Sev 6 h, protein expression in the control group was set at 100%.

2.4. Golgi staining

The hippocampus was collected bilaterally after execution at P21. Golgi-Cox staining to obtain hippocampal dendritic spine density was conducted with the FD Rapid GolgiStain™ Kit (FD Neuro Technologies Inc., Ellicott City, MD) following the manufacturer's instructions. Coronal tissue sections of 100-μm thicknesses were cut at room temperature using a vibratome (Leica VT1200S, Germany). After slides were dehydrated with a gradient of 50%, 75%, 95%, to 100% ethanol and cleared in xylene, we prepared the specimens with slide coverslips and sealed them with Permount. The slides were then viewed in detail with a light microscope (Leica DFC 420, Germany). We analyzed the stained spine using techniques similar to those described in other studies (Zhao et al., 2013; Han et al., 2013). Six pyramidal neurons that were well-impregnated and clearly distinguishable from others in each hippocampus were analyzed (20× objective lens). Five segments of 10 μm (or longer) of apical dendrites were randomly selected from each pyramidal neuron for inspection (via 100× oil immersion lens) to quantify the density of spines. Spine density of secondary apical dendrites was analyzed at proximal segments emerging at more than 50 μm away from the soma of the hippocampal CA1 neurons. All of these spines were required to have a clearly distinguishable base or origin and were isolated from neighboring dendrites. Spine density was calculated per 10 μm of dendritic length. The open-source ImageJ 1.48 r Java image-viewing and image-processing program (Wayne