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Effects of postnatal sevoflurane exposure on learning and memory in pre-adolescent rats

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Abstract

Introduction and Objective. Sevoflurane, a commonly used inhalation anaesthetic in paediatric procedures, has been associated with cognitive impairments, particularly affecting learning and memory. Despite its widespread use, the mechanisms underlying its neurocognitive effects remain unclear. The aim of the study is to investigate the impact of sevoflurane exposure at various developmental stages and dosages on learning and memory in pre-adolescent rats.

Materials and Method. Ninety male Wistar rat pups were randomly assigned to six groups: Control, Sham, S1 (single dose on postnatal day 7 [P7]), S2 (repeated doses on P7, P8, P9), S3 (single dose on P21), and S4 (repeated doses on P21, P22, P23). Morris Water Maze (MWM) Test was performed to LTP was recorded to assess synaptic plasticity., while Open Field Test (OFT) was utilized to assess locomotor activity and anxiety-like behaviours. Long-term potentiation (LTP) recordings were performed to assess synaptic plasticity.

Results. Sevoflurane exposure impaired learning and memory, with deficits most pronounced in the early exposure (S2) and repeated-dose (S2 and S4) groups. In the MWM, S2 showed significantly prolonged escape latency and reduced target-quadrant time, indicating impaired spatial memory.OFT revealed decreased locomotor activity and increased anxiety-like behaviours in S2, suggesting greater neurocognitive impairment following early developmental exposure and repeated dosing. LTP recordings demonstrated reduced fEPSP slopes and population spike (PS) amplitudes, particularly in S2 and repeated-dose groups, further supporting impaired synaptic plasticity, learning, and memory.

Conclusions. Early-stage and repeated-dose sevoflurane exposure disrupts learning, memory, and locomotor activity, highlighting the importance of timing and dosage in paediatric anaesthesia.

Key words

sevoflurane, paediatric anaesthesia, learning and memory, developmental neurotoxicity, pre-adolescent rats, long-term potentiation, Dentate gyrus

INTRODUCTION

Infants are routinely exposed to anaesthesia during various surgical and diagnostic procedures [1]. The administration of anaesthetic agents during the neonatal period has received considerable attention because the brain undergoes rapid maturation and extensive development of neuronal connections during this stage. Inhalational anaesthesia is frequently preferred in neonates and young children, mainly because establishing intravenous access before induction can be challenging [2]. Among the inhalational anaesthetics used in paediatric practice, sevoflurane is one of the most commonly preferred agents. Its low blood gas partition coefficient provides a relatively rapid induction and recovery, while its low airway irritation and relatively pleasant smell make it suitable for use in this age group [3].

The effects of sevoflurane on the developing nervous system have been investigated in several experimental studies. Although the results are not completely consistent, some studies have suggested that exposure to sevoflurane may influence cognitive functions, particularly learning and memory [4]. Repeated exposure during early life, for instance, has been reported to impair spatial memory in neonatal rats [5, 6]. Such findings have led to increasing interest in understanding how anaesthetic exposure may influence the neural processes involved in cognitive development.

Learning and memory depend on a number of interconnected cellular and molecular mechanisms, including changes in synaptic strength, gene expression, protein synthesis, and NMDA receptor trafficking [7, 8]. Previous experimental studies have indicated that sevoflurane can interfere with NMDA receptor function and reduce glutamatergic neurotransmission [9, 10]. In addition, inflammatory and neurotoxic responses associated with sevoflurane exposure may affect synaptic plasticity and other processes involved in long-term memory formation [3]. Such effects may be particularly relevant during early brain development, when synaptic connections are continuously formed, reorganized, and strengthened.

Long-term potentiation (LTP) is widely regarded as one of the important physiological mechanisms involved in learning and memory. It refers to a persistent increase in

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synaptic strength following high-frequency stimulation of afferent pathways [11]. In experimental studies, behavioural assessments and electrophysiological recordings are often used together to investigate the effects of early-life anaesthetic exposure on cognitive and neural function. The Morris water maze (MWM) is commonly used to examine spatial learning and memory, whereas the open field test (OFT) provides information about locomotor activity and anxiety-like behavior [12, 13].

The presented study examines whether different exposure frequencies and timing of postnatal sevoflurane exposure affect learning and memory in preadolescent rats. Behavioural changes were evaluated using established behavioural tests, while electrophysiological recordings were performed to investigate potential alterations in neural function.

MATERIALS AND METHOD

The study protocol received approval from the Akdeniz University Local Ethics Committee for Experimental Animal Research (Approval No. 142, dated 10 August 2020). All procedures were carried out at the Akdeniz University Laboratory Animals Application and Research Centre in compliance with relevant institutional and regulatory standards and the principles set forth in the Guide for the Care and Use of Laboratory Animals. The study was planned and reported in accordance with the ARRIVE guidelines [14–15].

Animals and procedure. The experiments were conducted between August 2021 – October 2021. A total of 90 male Wistar rat pups were obtained from the Laboratory Animals Application and Research Centre of Akdeniz University. Throughout the study, the animals were maintained in stainless-steel cages under standardized laboratory conditions. The environmental temperature was kept at 23 ± 1 °C, and a 12-h light/12-h dark photoperiod was applied continuously during the experimental period. The rats had unrestricted access to standard laboratory chow and tap water.

The 90 animals were randomly allocated into 6 experimental groups, each comprising 15 rat pups. The control group (C) received no postnatal intervention. The sham group (Sham) was exposed to 50% O_2 without sevoflurane for 2 h on postnatal day 7 (P7), in the same chamber and under the same gas-flow conditions (10 L/min) as the sevoflurane-exposed groups. Animals in the S1 group received 2.6% sevoflurane (AbbVie Inc., USA) in 50% O_2 on P7. The S2 group underwent the same exposure on 3 consecutive days (P7, P8, and P9). In the S3 group, sevoflurane exposure was performed on P21, whereas animals in the S4 group were exposed on P21, P22, and P23. An overview of the experimental design is presented in Figure 1. Sevoflurane or sham exposure was performed at P7–P9 or P21–P23. The MWM was conducted between P45 (acquisition, P45–P49; probe trial, P50), the OFT was performed on [P], and LTP recording was performed on P51.

The sample size for each group was calculated a priori using G*Power software (version 3.1.9.7; University of Düsseldorf, Germany). The calculation assumed an effect size (f) of 0.4, an α level of 0.05, and a statistical power of 0.80 (1–β).

For anaesthetic exposure, the pups were placed individually in a 500-mL chamber fitted with separate gas inlet and outlet ports. Sevoflurane was delivered at a concentration of 2.6% together with 50% oxygen using a vaporiser. The total gas flow was maintained at 10 L/min, and exposure was continued for 2 h. The concentrations of the inhaled gases were continuously monitored with an anaesthetic gas analyzer. Throughout the exposure period, the animals were observed for changes in respiratory rate and skin colour to detect possible apnea or hypoxia. At the end of the exposure period, sevoflurane administration was discontinued, and recovery was supported with 100% oxygen delivered at a flow rate of 10 L/min. Once recovery was complete, the pups were returned to their mothers. Recovery was defined as the return of the righting reflex (spontaneous return to the prone position from the supine position), after which the pups were returned to their mothers.

Anaesthetic depth was verified by the absence of pedal withdrawal and corneal reflexes. Once deep anaesthesia had been confirmed, the animals were decapitated, and hippocampal tissue was harvested for subsequent analyses.

Behavioural tests. Behavioural assessments were carried out in a temperature-controlled and sound-attenuated room to minimize environmental disturbances. All behavioural procedures were performed between 09:00–17:00. Animals from different groups were tested in a balanced/randomised order across this time window to avoid time-of-day bias.

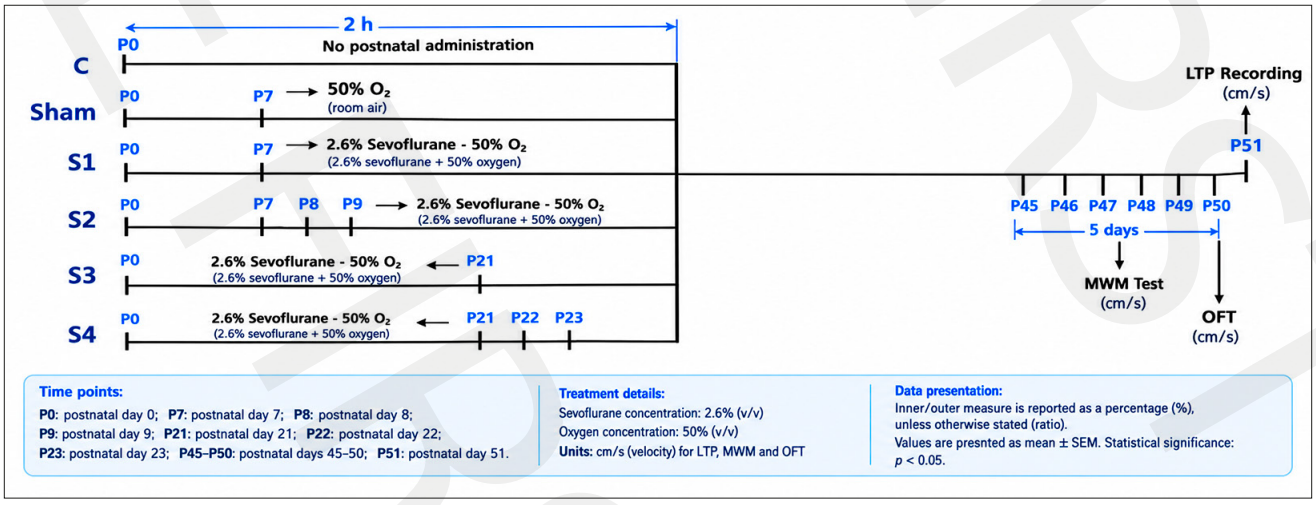


Figure 1. Experimental design and timeline of the study protocol

The test sessions were continuously monitored and video-recorded, and behavioural parameters were subsequently evaluated using the Noldus EthoVision XT tracking system (Noldus, Wageningen, The Netherlands).

Morris water maze. Spatial learning and memory performance was evaluated using the Morris water maze. The apparatus was composed of a black circular tank measuring 150 cm in diameter and 60 cm in height. The tank was filled with water to a depth of 31 cm, and water temperature maintained at 22 ± 1 °C. For data analysis, the pool was conceptually separated into 4 equal quadrants. A submerged circular platform with a diameter of 9 cm was positioned in the middle of the target quadrant, approximately 2 cm beneath the water surface.

The training protocol was conducted over 5 consecutive days, during which each animal completed 4 trials per day. A 5-min rest period was provided between successive trials, and the duration of each trial was limited to 120 s. Animals that successfully located the hidden platform were allowed to remain on it for 10 s. When an animal was unable to find the platform within the 120-s trial period, it was gently directed towards the platform and kept there for an additional 10 s [16].

On the sixth day, a probe trial was conducted to assess spatial memory. The platform was removed from the pool, and each animal was tested for 30 s. The amount of time spent in the previously designated target quadrant was subsequently recorded. Escape latency during the acquisition trials was considered an indicator of spatial learning, whereas the proportion of time spent in the target quadrant during the probe trial was used to assess spatial memory [16].

Open field test. Locomotor behaviour was evaluated by means of the open field test (OFT). The testing apparatus was a black Plexiglas enclosure measuring 80 × 80 cm, with walls 40 cm high. The arena floor was subdivided into 16 identical squares, each measuring 20 × 20 cm. For each assessment, the rat was positioned in the central area of the apparatus and permitted to explore the arena without restriction for 5 min. Animal activity was continuously monitored and recorded throughout the testing period.The number of squares entered, total distance traveled, and mean velocity were evaluated as measures of locomotor activity. In addition, the proportion of time spent in the inner and outer areas of the arena was calculated to assess anxiety-like behaviour [17].

Long-term potentiation recording. After completion of the OFT, the rat pups were anaesthetized with urethane (1.5 g/kg, i.p.; Sigma-Aldrich, USA) and secured in a stereotaxic apparatus (Kopf Instruments, USA) maintained on a heating pad at 37 ± 1 °C. A bipolar stimulating electrode (Plastics One, USA) was positioned in the left medial perforant pathway using the following coordinates relative to bregma: AP –8.0 mm, ML 4.4 mm, and DV 2–2.5 mm from the dura. A second bipolar electrode (Plastics One, USA) was placed in the dentate gyrus at AP –3.8 mm and ML 2.3 mm, and advanced ventrally to a depth of 2.7–3.2 mm until the maximal field excitatory postsynaptic potential (EPSP) was obtained. Electrophysiological signals were acquired using the PowerLab system (Version 8/35, ADInstruments, Sydney, Australia) and LabChart software (ADInstruments) [18].

The electrodes were advanced in 0.1-mm steps while test stimuli were delivered and the evoked responses were simultaneously recorded. Signals were acquired at a sampling rate of 40 kHz with a 1 Hz–3 kHz band-pass filter. The medial perforant pathway was then stimulated at progressively increasing intensities to determine the maximal fEPSP response, after which an input–output curve was constructed [19].

Long-term potentiation (LTP) was subsequently induced using a high-frequency stimulation (HFS) protocol comprising 10 bursts of 20 pulses delivered at 400 Hz. Each pulse had a duration of 0.2 ms and was generated as a biphasic square-wave stimulus, with a 10-s interval between consecutive bursts [20]. Evoked responses were continuously monitored for 60 min using test stimuli delivered at 0.1 Hz with a pulse duration of 0.1 ms [19]. For analysis, the mean of 6 consecutive responses obtained over each 1-min period was calculated for both baseline and LTP recordings [21]. Changes in the fEPSP slope and population spike (PS) amplitude during the 60-min recording period were expressed as percentages of the corresponding values obtained during the 10-min baseline period. At the conclusion of the experimental period, deep anaesthesia was induced with urethane (1.5 g/kg, intraperitoneally).

Statistical analysis. Data are expressed as the mean ± standard error of the mean (SEM). Statistical analyses were performed using SPSS Statistics, version 23.0 (IBM), following established statistical procedures described in the literature [22–24]. Prior to statistical testing, the distribution of the variables was examined to determine whether the normality assumption was satisfied. For normally distributed variables, differences between the groups were analyzed using one-way analysis of variance (ANOVA), with Tukey's test subsequently applied for post-hoc pairwise comparisons. When the data deviated from a normal distribution, group differences were assessed using the Kruskal–Wallis test, followed by the Mann–Whitney U test for comparisons between individual groups.

RESULTS

Behavioural experiments

Morris water maze test results. A visual representation of the rats' navigational path recorded during the MWM test is presented in Figure 2.

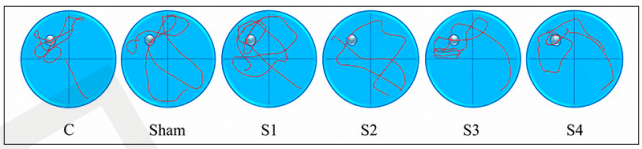


Figure 2. The representative path taken by rats during the Morris Water Maze (MWM) test. The platform (gray circle) indicates the target location. The path length and time spent in the targent quadrant were used as criteria to evaluate spatial learning and memory performance

Figure 3 illustrates the escape latency scores during the training days in the MWM test. Statistical analysis revealed significant differences between groups throughout the last 3 days of the training phase (Fig. 3). The Sham group demonstrated a significantly longer latency to find the platform compared to the C group on the third day ($p < 0.05$) (Fig. 3). The S3 and S4 groups exhibited significantly reduced

Table 1. Open field test (OFT) parameters in the experimental groups

Group	Total distance (cm)	Velocity (cm/s)	Number of squares entered	Time spent in the inner/outer squares (%)
C	2511.22 ± 84.54	8.76 ± 0.34	118.90 ± 7.85	2.91 ± 0.93
Sham	2114.91 ± 8.12	8.16 ± 0.36	72.93 ± 4.77***	4.19 ± 0.73
S1	2264.42 ± 102.65	8.08 ± 0.37	109.80 ± 8.12$^{###}$	6.81 ± 1.10
S2	1625.42 ± 169.53$^{***,\#,\alpha\alpha}$	5.87 ± 0.52$^{***,\#,\alpha\alpha}$	69.53 ± 6.61$^{***,\alpha\alpha\alpha}$	1.97 ± 0.33$^{\#,\alpha\alpha}$
S3	2364.73 ± 173.37$^{\beta\beta}$	8.27 ± 0.60$^{\beta\beta}$	98.13 ± 9.33$^{\#\#,\beta\beta}$	4.77 ± 1.00$^{\beta}$
S4	2363.79 ± 56.10$^{\beta\beta\beta}$	7.89 ± 0.19$^{\beta\beta\beta}$	111.70 ± 5.64$^{\#\#\#,\beta\beta\beta}$	5.20 ± 0.51$^{\beta\beta}$

*Data are presented as mean ± SEM (n = __ per group). Statistical significance defined as ***p < 0.001 vs. the C group; #p < 0.05, ##p < 0.01, and ###p < 0.001 vs. the Sham group; ααp < 0.01 and αααp < 0.001 vs. the S1 group; and βp < 0.05, ββp < 0.01, and βββp < 0.001 vs. the S2 group

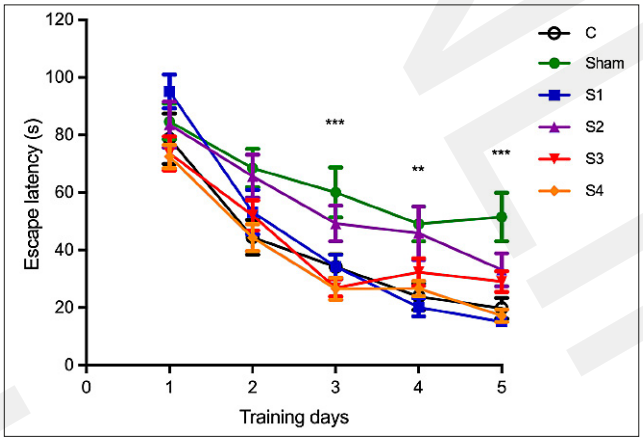


Figure 3. The escape latency scores during the training days. ** p<0.05, *** p<0.001 indicates a significant difference across all groups

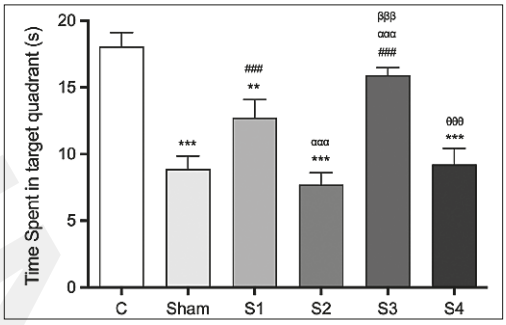


Figure 4. The time spent in the target quadrant on the probe trial (6th day). ** p<0.01, *** p<0.001 indicate a significant difference compared to the C group; ### p<0.001 indicates a significant difference compared to the Sham group; ααα p<001 indicates a significant difference compared to the S1 group; βββ p<0.001 indicates a significant difference compared to the S2 group; and τττ p<0.05 indicates a significat difference compared to the S3 group

escape latency compared to those in the S2 group (p < 0.01) (Fig. 3). The escape latency scores of the C (p < 0.01), S1 (p < 0.001), S3 (p < 0.05), and S4 (p < 0.01) groups were found to be shorter than those in the Sham group on the fourth day (Fig. 3). On the fifth day, the escape latency scores of the Sham (p < 0.01) and S2 (p < 0.05) groups were determined to be higher compared to the C group (Fig. 3). The S2 (p < 0.05) and S3 (p < 0.01) groups demonstrated significantly longer escape latencies compared to the S1 group (Fig. 3).

The duration scores of all experimental groups in the target quadrant on the probe trial are represented in Figure 4. The Sham (8.91 ± 0.94; p < 0.001), S1 (12.74 ± 1.34; p<0.01), S2 (7.73 ± 0.88; p < 0.001), and S4 (9.23 ± 1.19; p < 0.001) groups spent significantly less time in the target quadrant compared to the C (18.08 ± 1.05) group (Fig 4). The S2 group, subjected to repeated anaesthesia, exhibited a significantly reduced duration in the target quadrant compared to the S1 and S3 (15.92 ± 0.58) groups (p < 0.001) (Fig 4). The duration spent in the target quadrant by the S4 group was significantly lower compared to the S3 group (p < 0.001) (Fig 4).

Open field test results. A representative trajectory exhibited during the OFT is shown in Figure 5.

Table 1 summarizes the OFT findings, including total distance travelled, mean velocity, number of squares entered, and the proportion of time spent in the central and peripheral areas of the arena.

The S2 group showed significantly lower total distance than the C (p < 0.001), Sham (p < 0.05), and S1 (p < 0.01) groups, whereas the S3 (p < 0.01) and S4 (p < 0.001) groups showed higher total distance than the S2 group (Tab. 1). Velocity was significantly lower in the S2 group than in the C (p < 0.001), Sham (p < 0.01), and S1 (p < 0.001) groups, and higher in the S3 and S4 groups than in the S2 group (both p < 0.01) (Tab. 1).

The number of squares entered was significantly lower in the Sham (p < 0.001) and S2 (p < 0.001) groups than in the C group. Compared with the Sham group, the S1 (p < 0.001), S3 (p < 0.01), and S4 (p < 0.001) groups entered more squares. The S2 group entered fewer squares than the S1 group (p < 0.001), and the S3 (p < 0.01) and S4 (p < 0.001) groups entered more squares than the S2 group (Tab. 1). The S2 group spent a significantly lower proportion of time in the inner/outer zones than the Sham (p < 0.05), S1 (p < 0.01), S3 (p < 0.05), and S4 (p < 0.01) groups (Tab. 1).

The S2 group showed a significantly reduced number of square entries compared with the S1 group (p < 0.001) (Tab. 1). In contrast, square-entry frequency was significantly higher in the S3 (p < 0.01) and S4 (p < 0.001) groups than in the S2 group (Tab. 1). Furthermore, the S2 group spent a significantly lower proportion of time in the inner/outer zones than the Sham (p < 0.05), S1 (p < 0.01), S3 (p < 0.05), and S4 (p < 0.01) groups (Tab. 1).

Long term potentiation results. Time-dependent alterations of percentages in fEPSP slope (A) and mean fEPSP slopes (B) for all groups in the dentate gyrus are presented in Figure 6.

The percentage change in EPSP slope decreased significantly in groups S2 (104.23 ± 1.17) (p < 0.001), S3 (114.49 ± 3.81) (p < 0.01), and S4 (109.87 ± 4.11) (p < 0.01) compared to the C (139.85 ± 4.56) and Sham group (137.39 ± 7.80) (Fig. 6B). Additionally, a significant reduction in percentage change in the EPSP slope was observed in the S2 group compared to the S1 group (p < 0.01) (Fig. 6B).

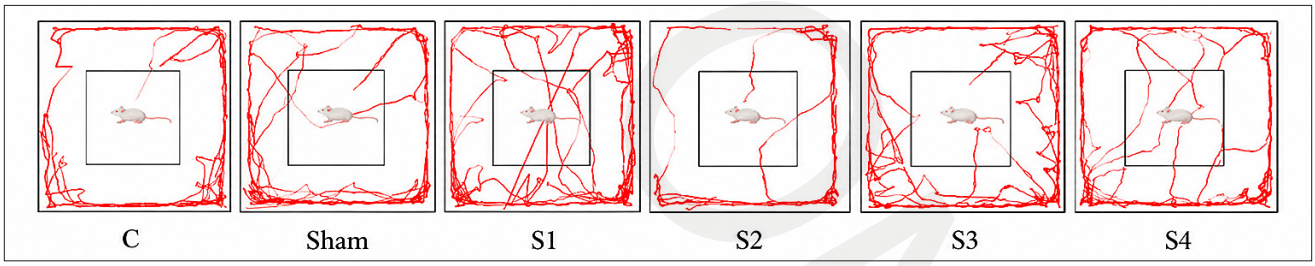


Figure 5. An illustration of the path recorded in the OFT. The platform (center zone, gray square) indicates the target area. The path length and time spent in the center zone were used as criteria to evaluate anxiety-like behaviour and exploratory activity

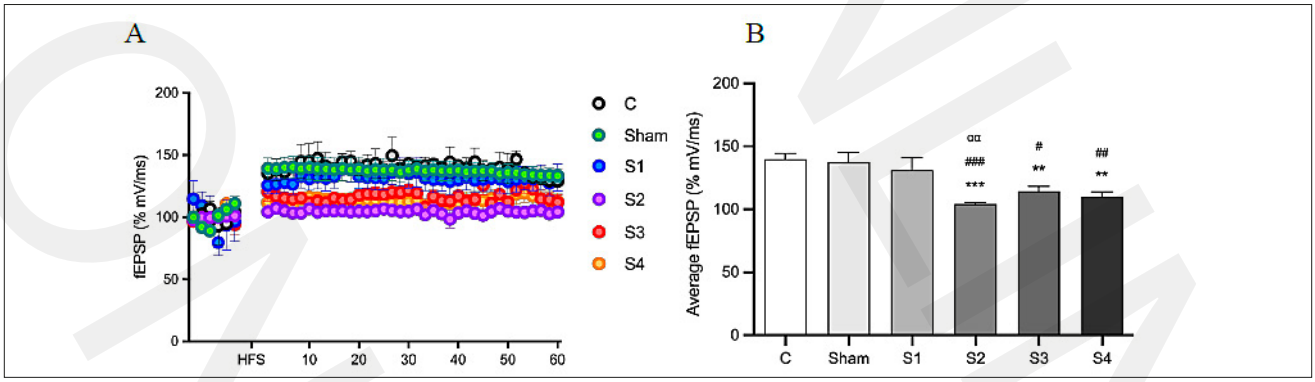


Figure 6. Time-dependent alterations of percentages in fEPSP slope (A) and mean fEPSP slopes for all groups in the dentate gyrus (B). Percentage change in EPSP slope is calculated in comparison to the mean value of the baseline recording. ** p<0.01, *** p<0.001 indicates a significant difference compared to the C group; # p<0.05, ## p<0.05, ### p<0.001 indicate a significant difference compared to the Sham group; αα p<0.01 indicates a significant difference compared to the S1 group

Figure 7 represents the alterations of percentage in PS amplitude over time (A) and the mean PS amplitude (B) for all groups obtained in the dentate gyrus. PS amplitude is expressed as a percentage of the baseline value (% of baseline).

The S1 (135.79 ± 3.76; p<0.01), S2 (108.89 ± 3.86; p < 0.001), S3 (139.07 ± 3.40; p < 0.01), and S4 (114.64 ± 2.78; p < 0.01) groups revealed significantly lower PS amplitudes compared to the C group (155.38 ± 0.35) (Fig. 7B). In addition, the percentage alteration in PS amplitude were significantly decreased in the S2 and S4 groups compared to the Sham group (152.74 ± 9.98; p < 0.01) (Fig. 7B). The amplitude of the percentage change in the S2 and S4 groups was observed to be lower compared to that in the S1 and S3 groups (Fig. 7B).

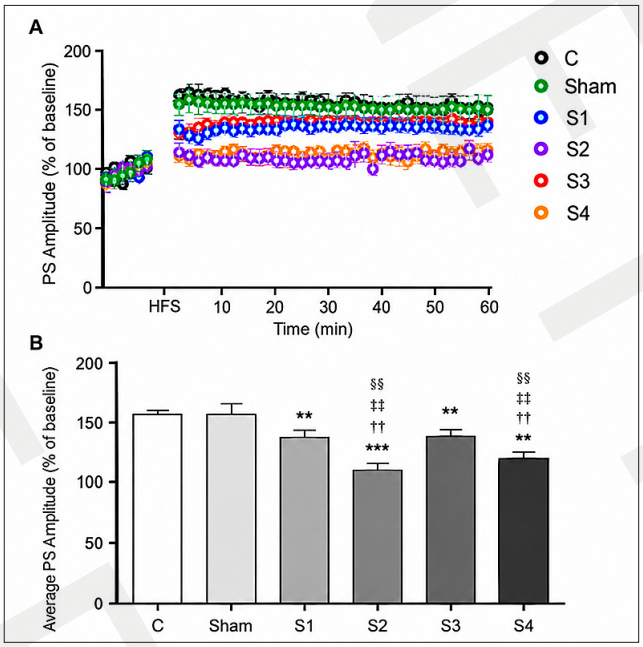


Figure 7. Effect of sevoflurane exposure on population spike (PS) amplitude in the dentate gyrus. (A) Time course of PS amplitude (% of baseline) before and during the 60 min after the high-frequency stimulation (HFS); (B) mean PS amplitude (% of baseline) in each group. Data are presented as mean ± SEM. C, control; Sham, sham exposure; S1, single sevoflurance exposure at P7; S2, repeated exposure at P7–P9; S3, single exposure at P21; S4, repeated exposure at P21–P23. ** p<0.01 and *** p<0.001 vs. C; †† vs. Sham; ‡‡ vs. S1; §§ vs. S3

DISCUSSION

Anaesthetic effects on cognitive development, particularly learning and memory in neonates and children, remain insufficiently understood, although growing evidence indicates that anaesthetic agents can significantly affect these functions during critical stages of brain development [4–6, 25–28]. Sevoflurane is considered one of the most frequently administered anaesthetic agents to paediatric patients [26]. In this regard, the effects of sevoflurane on learning and memory were investigated by subjecting pre-adolescent rats to sevoflurane at different postnatal ages through single and repeated exposures.

Early exposure was set at P7, a peak synaptogenic window, and late exposure at P21 [29]. Morris water maze testing was conducted between P45 and P50, and LTP recording was performed on P51, a period marked by heightened locomotor and exploratory activity [27]. The training-phase data of the MWM test revealed significant differences emerging distinctly on the third, fourth, and fifth days. Unexpectedly, the Sham group showed longer escape latencies than the C group on days 3 and 5, and longer latencies than the C, S1, S3, and S4 groups on day 4. In the probe trial, the Sham group also spent less time in the target quadrant than the C group.

Because Sham pups were separated from their dams for 2 h at P7 and were exposed to the chamber and gas-flow conditions without sevoflurane, these differences may reflect early-life stress related to maternal separation and handling rather than an effect of the anaesthetic [30]. Importantly, probe-trial performance in the Sham group (8.91 ± 0.94) was in a similar range to that of the S2 (7.73 ± 0.88) and S4 (9.23 ± 1.19) groups; therefore, the contribution of sevoflurane to the probe-trial deficit cannot be fully separated from the contribution of the experimental procedure itself. Nevertheless, the S2 and S4 groups spent significantly less time in the target quadrant than the corresponding single-exposure groups (S1 and S3, respectively), which is consistent with previous evidence that repeated anaesthetic exposure increases the risk of neurotoxicity [5].

Furthermore, compared with the Sham group, sevoflurane exposure did not significantly alter total distance or velocity, with the exception of the S2 group, which exhibited a decrease. Evidence indicates that anaesthetic agents exert detrimental effects on multiple brain regions that are documented to be involved in the regulation of locomotor activity [31, 32]. In addition, repeated sevoflurane applications were reported to induce hypomyelination [3]. These findings account for the specific occurrence of locomotor activity impairment in the S2 group among the sevoflurane-treated groups. In the OFT, the S2 group showed reduced total distance, velocity, and number of squares entered, indicating decreased locomotor and exploratory activity. The lower proportion of time spent in the inner relative to the outer zones in this group may be consistent with anxiety-related behavioural alterations. However, the Sham group also showed a reduced number of square entries compared with the C group, so a contribution of the experimental procedure cannot be excluded.

LTP is defined as a sustained increase in synaptic strength induced by high-frequency stimulation, commonly assessed by measuring the fEPSP slope and PS amplitude [11]. The fEPSP measures the effectiveness of synaptic transmission, whereas the population spike reflects the synchronized firing of neurons [33]. The fEPSP slope was reduced in the S2, S3, and S4 groups compared with the C and Sham groups, with the most pronounced decline in the S2 group. These results suggest that groups with repeated exposure experienced more pronounced impairment in LTP than groups with single exposure, and that early exposure may be associated with greater dysfunction than late exposure, consistent with the MWM findings. Analysis of the PS data confirmed the results of the fEPSP data.

Predominantly, these findings are in agreement with the existing literature. Despite contradictory evidence, the majority of the literature supports the notion that sevoflurane impairs learning and memory [4, 9–10, 26]. Liang et al. [26] administered sevoflurane in repeated doses on P7, P14, and P21 days and concluded that LTP was markedly suppressed in rats exposed to sevoflurane in the neonatal period compared to those in group C at both juvenile and adult stages. Shen et al. [4] demonstrated that repeated administration of sevoflurane impaired LTP and reduced the expression of synaptogenesis-associated proteins: GluR1 and PSD95.

Sevoflurane significantly enhances inhibitory GABAergic transmission while suppressing excitatory NMDA receptor-mediated glutamatergic activity, leading to altered neurotransmission [9]. Mapelli et al. [9] demonstrated that sevoflurane can inhibit NMDA receptor activity at clinically relevant concentrations. Since increases in NMDA receptor number and functional conductance play an important role in the induction and maintenance of LTP, alterations in NMDA receptor function may influence synaptic plasticity [8, 10].

Previous studies have generally focused on anaesthetic exposure during either the neonatal or juvenile period. In the present study, both single and repeated sevoflurane exposures were examined at 2 different developmental stages under comparable experimental conditions. This design allowed evaluation of the effects of exposure timing and frequency within the same experimental framework. In addition to behavioural assessments, in vivo hippocampal LTP was recorded following exposure, allowing changes in synaptic plasticity to be considered alongside learning and memory performance. Notably, repeated exposure at P21, a developmental stage that may be relatively less sensitive to anesthetic effects, was also associated with alterations in learning and memory and reduced LTP. These findings suggest that both developmental stage and repeated exposure may contribute to the neurobehavioral effects associated with sevoflurane.

Most previous studies examining the neurocognitive effects of neonatal sevoflurane exposure have focused on LTP in the CA1 region, particularly the Schaffer collateral–CA1 pathway, which has an established role in spatial learning and memory [4, 26, 34–36]. In comparison, relatively few studies have investigated LTP in the dentate gyrus following early-life sevoflurane exposure, and the effects of repeated exposure in this region have not been well characterized [37]. The present study examined synaptic plasticity at perforant pathway–dentate gyrus synapses. This pathway is involved in dentate gyrus functions such as pattern separation and is closely associated with hippocampal neurogenesis, which may also be affected by sevoflurane exposure [38]. The findings obtained therefore provide additional evidence that changes in dentate gyrus synaptic plasticity may be associated with the cognitive effects of early-life sevoflurane exposure, and may complement findings obtained from studies of the CA1 region.

Limitations of the study. This study has several limitations. First, only male Wistar rat pups were used; therefore, potential sex-dependent differences in the behavioural and electrophysiological effects of sevoflurane could not be evaluated, and the findings should not be generalized to female animals. This is an important limitation because sex is a recognized biological variable in anaesthetic-induced neurotoxicity. Sex differences in GABAergic and glutamatergic signalling, NMDA receptor function, gonadal hormone exposure, and the timing of neurodevelopmental milestones have been reported, and these differences could modify the vulnerability to sevoflurane at both P7 and P21. In particular, the effects of exposure at P21, which is closer to the onset of puberty, may interact with sex-specific hormonal changes. The use of males only may therefore have over- or underestimated the effects that would be seen in a mixed-sex population, and the magnitude of the LTP and behavioural changes reported here may not apply to females. Future studies should include both sexes and analyze sex as an independent factor. Second, a Sham group was included only for the P7 exposure, and no corresponding Sham group was included for the P21 exposure (S3 and S4). Consequently,

procedural effects such as maternal separation and chamber exposure at P21 could not be assessed independently. The Sham-related differences observed at P7 (for example, longer escape latencies and shorter target-quadrant time than in the C group) indicate that handling, maternal separation, and chamber exposure can by themselves influence behaviour. Similar procedural effects are therefore likely to be present at P21, and may differ in magnitude from those at P7 because maternal dependence and stress reactivity change with age. Accordingly, the S3 and S4 findings should be interpreted cautiously, because they could not be compared with an age-matched Sham group and part of the observed effect may reflect procedural stress rather than sevoflurane itself. The reduced LTP responses and the greater impairment in the repeated-exposure groups (S2 and S4) than in the single-exposure groups (S1 and S3), which were exposed under identical chamber and gas-flow conditions, are in line with a sevoflurane effect; however, this cannot be confirmed without a separate Sham group for the P21 period.

Future studies should include an age-matched Sham group for each exposure window. Third, maternal separation and other procedural factors may independently influence learning, memory, and anxiety-related behaviour, as suggested by the differences observed between the Sham and C groups.

CONCLUSION

In conclusion, repeated sevoflurane exposure during early development may adversely affect cognitive and behavioural functions in pre-adolescent rats. The observed alterations in learning, memory, locomotor activity, anxiety-like behaviour, and electrophysiological responses were more pronounced following earlier and repeated exposure. These findings suggest that both the timing and frequency of sevoflurane exposure may influence its effects on the developing brain. Therefore, repeated anaesthesia exposure during critical periods of neurodevelopment should be carefully considered.

Recommendations

- Unnecessary and repeated sevoflurane exposure during early developmental periods should be minimized when clinically appropriate.
- Future studies should investigate the molecular mechanisms underlying the neurobehavioural effects of sevoflurane.
- The effects of sevoflurane on synaptogenesis-related proteins and ion-channel regulation should be further evaluated.
- Histopathological analyses should be performed to identify possible structural changes in the developing brain.
- Different developmental stages and exposure frequencies should be investigated to identify potentially sensitive periods.
- Long-term follow-up studies should be conducted to determine whether the observed effects persist into adulthood.
- Future research should explore anaesthesia strategies that maintain adequate anaesthetic efficacy while minimizing potential effects on neurodevelopment.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Data Availability

The raw data supporting the findings of this study are available from the corresponding author (Hanife Kabukçu, hanifekabukcu@akdeniz.edu.tr) upon reasonable request.

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Ethics Approval and Compliance

The research protocols in this study were reviewed and approved by the Akdeniz University Local Ethics Committee for Experimental Animal Research (Approval No: 142, Date: August 10, 2020). All experimental procedures were conducted at the Akdeniz University Laboratory Animals Application and Research Center, in strict accordance with the international principles outlined in the Guide for the Care and Use of Laboratory Animals. Furthermore, to ensure transparency, reproducibility, and rigorous scientific reporting, the design and execution of the experiments, as well as the preparation of this manuscript, were performed in full compliance with the ARRIVE guidelines [14, 15]. The welfare of the animal subjects was monitored throughout the study to minimize pain, distress, and discomfort, adhering to institutional and regulatory ethical standards.

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