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Effects of repeated sevoflurane and rivastigmine on spatial learning and memory in weanling rats

Tekrarlı sevofluran ve rivastigminin yavru sıçanların uzaysal öğrenme ve hafıza üzerindeki etkileri

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Abstract

Aim: It has been reported that repeated sevoflurane exposure induces cognitive impairment. On the other hand, there is evidence that rivastigmine can attenuate or antagonize the cognitive dysfunctions caused by anesthetic agents. The aims of this study were to determine the effect of repeated sevoflurane exposure on spatial learning and memory (SLM) in weanling rats and to assess whether rivastigmine provides protection against the neurotoxic effects of sevoflurane at this early developmental stage.
Methods: Thirty-two weanling rats were randomly divided into four equal groups: sevoflurane (S: 2% sevoflurane for 2 hours), sevoflurane + rivastigmine (SR: 2% sevoflurane and 2 mg/kg rivastigmine), rivastigmine (R: 2 mg/kg), and control (C: 100% oxygen for 2 hours). Rats were treated four times over 10 days. Four days after the last treatment, the rats were subjected to a Morris water maze test protocol to examine SLM.
Results: The escape latencies of all groups gradually decreased day by day during the training trials performed to evaluate spatial learning ($\rho<0.05$). Group R showed more improvement than other groups as the rats in this group learned significantly more slowly on the first and second days of the training trials but reached the same levels as Group S and Group SR on the third and last days ($\rho<0.05$). In the probe trial to evaluate spatial memory, no significant difference was found among the groups for time spent in the 'platform' quadrant ($\rho>0.05$).
Conclusion: Sevoflurane negatively affects learning in weanling rat pups but has no detrimental effect on spatial memory. On the other hand, it can be claimed that sevoflurane offsets the memory-sparing effects of rivastigmine.
Keywords: Sevoflurane, rivastigmine, spatial learning and memory, rat.

Öz

Amaç: Literatürde tekrarlı sevofluran uygulamalarının bilişsel işlev bozukluklara neden olduğu bildirilmektedir. Diğer yandan, rivastigminin, anestezik ajanların neden olduğu bilişsel işlev bozukluklarını hafifletebileceğine dair bulgular da literatürde yer almaktadır. Çalışmanın amacı tekrarlı sevofluran uygulamasının yavru sıçanların uzaysal öğrenmesine ve hafızasına etkisini ve rivastigmin'in sevofluranın söz konusu nörotoksik etkilerine karşı koruma sağlayıp sağlamadığını araştırmaktır.
Yöntemler: Otuz iki yavru sıçan rastgele olarak dört eşit gruba ayrılmıştır: sevofluran grubu (S: 2 saat boyunca % 2 sevofluran), sevofluran ve rivastigmin grubu (SR: % 2 sevofluran ve 2 mg / kg rivastigmin), rivastigmin grubu (R: 2 mg / kg) ve kontrol grubu (C: 2 saat boyunca % 100 oksijen). Sıçanlara, söz konusu ajanlar 10 gün boyunca dört kez verilmiştir. Son tedaviden dört gün sonra, sıçanlar uzaysal öğrenmeyi ve hafızayı incelenmek üzere Morris Su Labirent Testi protokolüne tabi tutulmuştur.
Bulgular: Uzaysal öğrenmeyi değerlendirmek için yapılan eğitim denemelerinde tüm grupların labirentten kurtulma süreleri günden güne yavaş yavaş azalmıştır ($\rho<0.05$). Uzaysal hafızayı değerlendirmek için yapılan eğitim testlerinde ise platform kadranında geçirilen süre için gruplar arasında anlamlı bir fark bulunamamıştır ($\rho>0.05$).
Sonuç: Çalışmada sevofluran, emzirme dönemindeki yavru sıçanların uzaysal öğrenmelerini olumsuz yönde etkilemesine karşın uzaysal hafızaları üzerinde zararlı bir etkisi çıkmamıştır. Diğer yandan sevofluranın, rivastigminin sağladığı hafıza koruyucu etkileri dengelediği gözlemlenmiştir.
Anahtar kelimeler: Sevofluran, rivastigmin, uzaysal öğrenme ve hafıza, sıçan.

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Introduction

There is growing concern about the side effects of general anesthetics, especially in neonates [1]. Recent studies show that commonly used anesthetic agents such as sevoflurane and desflurane can cause neuronal cell death during neonatal brain development and can bring about developmental disability or cognitive dysfunction in both infants and adults. As a consequence, neurocognitive impairments may arise in children exposed to anesthetic agents [2, 3], and repeated dosing may elevate the risk. Infants can be exposed to repeated inhalational anesthetics for a variety of reasons, including complex surgeries, burn treatments, and interventional or diagnostic radiological procedures [4].

In an animal model, repeated exposure of neonatal rats (between 1 and 2 weeks old) to isoflurane was associated with greater memory impairment compared to single exposure [5]. Similarly, multiple exposures of neonatal rats (beginning at 6 days old) to sevoflurane induced cognitive impairment, whereas no cognitive decline was evident with a single exposure. Moreover, in the same study, adult rats (60 days old) subjected to the same experimental protocol exhibited no cognitive impairment [6]. Another study showed that repeated exposure of pregnant rats to sevoflurane caused a significant increase in apoptosis of neurons in the hippocampus of the offspring [7]. Thus, repeated dosing of these inhalational anesthetics has a demonstrable neurotoxic effect on the developing central nervous system.

In addition, several studies have reported that anesthetic agents have detrimental effects on long-term spatial cognitive functions, but not on short-term spatial cognitive functions [8, 9]. However, another study concluded that sevoflurane or propofol has no effect on both short- and long-term memory in children 7-13 years of age [10]. Controversial results have also emerged in studies of the effects of anesthetic agents on spatial cognitive functions [11, 12].

The detrimental effects of general anesthetics are partially explained by suppression of acetylcholine (ACh) release in the brain [13]. These detrimental effects are more pronounced with the use of volatile inhalation agents such as desflurane and sevoflurane [14]. On the other hand, certain neurodegenerative diseases including Alzheimer's, Parkinson's, and delirium are associated with reduced ACh levels in the brain [15, 16, 17]. Cholinesterase inhibitors are the mainstay in the treatment of these diseases, and their action elevates the cerebral levels of Ach [18]. Efforts to treat the symptoms of such diseases have included the use of acetylcholinesterase inhibitors such as rivastigmine, a semi-synthetic derivative of physostigmine and carbonate [19, 20]. Thus, it can be argued that the reduced ACh level induced by repeated sevoflurane can be offset by using a cholinesterase inhibitor such as rivastigmine. Moreover, rivastigmine can also be used to attenuate or antagonize the cognitive dysfunctions caused by anesthetic agents [13, 21]. Furthermore, some experimental studies have indicated that cognitive impairments induced by ketamine, ethanol, or scopolamine can be similarly reversed by rivastigmine [22, 23, 24].

Although rivastigmine is effective at improving cognitive functions, the dose used is also important. Overdoses of rivastigmine have adverse effects on cognitive functions in rats, whereas doses between 0.1 and 2.5 mg/kg eliminate or reduce the neurotoxic side effects of some anesthetic agents and help ameliorate symptoms in Alzheimer's disease [19, 23-25].

Thus, we sought to investigate the toxicity of sevoflurane to the neonatal brain and its potential amelioration by rivastigmine. The aims of this study were to determine the effect of repeated sevoflurane exposure on spatial learning and memory in weanling rats and to assess whether rivastigmine provides protection against the cognitive impairment induced by sevoflurane.

Material and methods

This study was conducted at the Istanbul Bagcilar Education and Research Hospital Experimental Research and Ability Development Center (BADABEM®) with the approval of the Istanbul Bagcilar Education and Research Hospital Experimental Animals Ethical Commission (Protocol No: 2014/4, approved February 19, 2014). As the nursing period of neonatal rats can last up to 21 days [26], thirty-two healthy weanling Wistar Hannover male rats (40-50 g) at postnatal day 21 (P21) were used in the experiment. Male rats were chosen because they are less affected by physiological factors [27], and physiological factors such as blood pressure, heart rate, and blood gases were not measured. The rats were housed under standard laboratory conditions from birth (12-hour day/night cycle, 20-22 °C, 50-60% relative humidity). Health reports from the vendor (BADABEM®) indicated that the rats were free of known viral, bacterial, and parasitic pathogens.

Memory and learning, frequently used in assessments of cognitive functions in animal experiments, were also used in this study due to their ease of measurability. In addition to its effects on Ach level, sevoflurane was preferred as a volatile anesthetic agent in this study due to its low side effects and odorless, non-irritating, and soft anesthesia induction. Sevoflurane is also widely used in children because it does not induce airway irritation and does not stimulate the cough reflex, and in this context we decided to use sevoflurane in weanling rats.

Rat pups were randomly divided into four groups, each consisting of eight rats.

Group S: Rat pups were exposed to 2% sevoflurane with oxygen for 2 hours, after which 1 mL of saline was injected subcutaneously.

Group SR: Rat pups were exposed to 2% sevoflurane with oxygen for 2 hours, after which 2 mg/kg rivastigmine was injected subcutaneously [i.e. 1 mL of an Exelon® 1.5 mg tablet (Novartis) dissolved in 15 mL of saline]. The subcutaneous route was used to prolong the duration of rivastigmine's effect. Rivastigmine doses used in studies in the literature are between 0.1 and 2.5 mg/kg [19, 23-25].

Group R: Rat pups were exposed to 100% oxygen for 2 hours, after which 2 mg/kg rivastigmine solution was injected subcutaneously.

Group C: Rat pups were exposed to 100% oxygen for 2 hours, after which 1 mL of saline was injected subcutaneously.

For sevoflurane application, each group was put into an induction box of 3000 mL in volume equipped with a gas input and output system. Sevoflurane (Sevorane®, Abbott Lab., Istanbul, Turkey) was fed into the induction box at 2% concentration in oxygen at a rate of 6 L/min with a vaporizer. In accordance with the duration and doses applied in many studies, the rat pups were exposed to 2% sevoflurane for 2 hours [28, 29]. Anesthesia level was monitored by checking respiratory pattern,

speed, and reflexes. Volatile anesthesia application was halted after 2 hours and oxygen was provided with a flow rate of 6 L/min for recovery of the rats. After recovery, the rat pups were returned to their cages.

Experimental timeline

Starting on postnatal day 22, Group S and Group SR animals were exposed to 2% sevoflurane on days 22, 25, 28, and 31 for 2 hours each day [5]. Groups R and C were exposed to 100% oxygen for 2 hours on the same days as above. After the exposure, 2 mg/kg rivastigmine was prepared, and 1 mL of this solution was injected subcutaneously into rats of Groups R and SR, whereas 1 mL of saline was injected into Groups C and S.

Beginning on postnatal day 35 and on each of the following three days (days 36, 37, and 38), the rat pups were subjected to training trials using the Morris water maze test (MWMT) [30]. The platform-finding periods of the rats were recorded. One day after the completion of training trials (day 39), probe trials for spatial memory function were carried out. In the training trials, the platform was fixed in the north quadrant, but the rat pups were released into the tank from different quadrants. The experimental timeline is illustrated in Figure 1.

In cognitive studies of experimental animals, long-term spatial cognitive functional tests such as the MWMT are performed after more than one week following administration of the experimental treatment. In short-term studies, tests are performed within 24 hours. In the present study, we selected 4 days (from day 31 to day 35) between the medications and trials to conduct the spatial learning and memory tests.

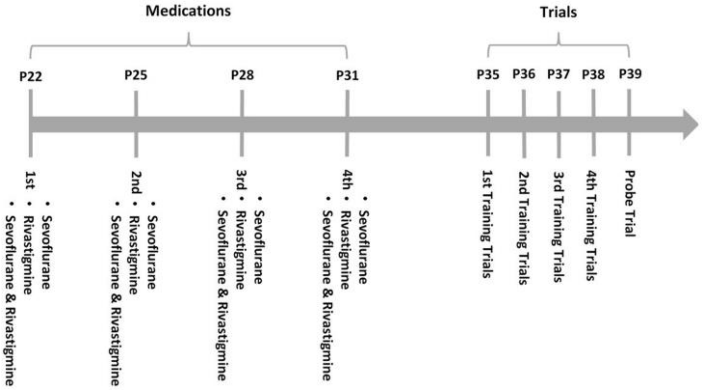

Figure 1. Experimental Timeline.

Assessment of Spatial Learning and Memory

Before the MWMT, the weanling rat pups were transferred to the room containing the test apparatus. During the training (learning) trials, the location and shape of the objects and platform were not changed. The experiments were also conducted by the same person. During the experiments, the clothes, hairstyle, jewelry, scent, etc. of the researcher were not changed. Red, black, and white papers were applied to three different sides of the tank so that the rat pups were able to use these visual cues as a means of navigating the maze. All trials were done in a black Plexiglas pool 120 cm in diameter and 80 cm deep. Milk powder (1 kg) was put into the tank to make the water opaque. The tank was divided into four imaginary quadrants: west, east, south, and north. A hidden platform, the same color as the tank and 10 cm in diameter, was put into the north quadrant and submerged 3-4 mm below the water level. The temperature of the water was adjusted to 25±1 °C.

Training Trials (Learning Function): In the training trials, the rat pups were released into the water from different quadrants (west, east, south) at 10-min intervals on each of the 4 days (P35, P36, P37, P38). The escape latency, which is the time it takes a rat to find the platform, was noted for each training trial. The average of these escape latencies was calculated for each day and each experimental group. One minute was given to each rat pup to find the hidden platform. If a rat pup could not find the hidden platform within one minute, the researcher helped the rat pup find the platform and it was kept for 30 seconds on this platform. Afterwards, each rat was taken from the platform and dried with paper towels.

Probe Trial (Memory Function): A day after the MWMT (day 39), the submerged platform was removed from the apparatus for the memory test. The rat pups were allowed to swim in the tank for 60 seconds. In this experiment, the amount of time the rat spent at the targeted quadrant (the quadrant in which the hidden platform was located in the training sessions) was noted as a percentage of one minute.

Statistical analysis

The results were analyzed using IBM SPSS Statistics 20 software. Since each group was subjected to repeated trials in four different directions for each day, the number of observations for each group and each day was over 30. For this reason, it was assumed that the data were normally distributed [31]. One-way and two-way ANOVA were used to analyze differences among the groups. If a group was found to be different from the others, it was then compared bilaterally with post hoc tests. Before the ANOVA, Levene's test (at 1% significance level) was carried out to test the homogeneity of variances. After the ANOVA, pairwise comparison by a least significant difference (LSD) test was carried out for multiple comparisons of the independent variables in order to find significant differences between two compared group averages. A value of $\rho<0.05$ was considered significant.

Results

One rat pup from Group C died (the cause of death could not be identified). It was excluded from the study.

No difference was observed among the groups in terms of average recovery time from anesthesia.

All groups' escape latencies (measured as the average for each day) gradually decreased day by day during the trials ($\rho<0.05$). However, Groups S, SR, and R all had significantly longer escape latencies compared to Group C (24.29±17.31, $\rho<0.01$). The escape latencies of Groups S, SR, and R were not significantly different from one another ($p>0.05$) (Table 1).

A significant difference was found in the average escape latencies of each group between the first (44.84±19.87) and second trial days (38.93±20.89). In addition, highly significant differences were found among the other experimental days ($\rho<0.01$) (Table 1).

On the first two experimental days, the escape latencies of Group R (55.29±8.69, 49.38±19.06) were longer ($\rho<0.05$) than those of Group S (42.57±20.06, 36.71±18.09), Group SR (43.5±23.22, 37.71±19.39), and Group C (38±21.05, 31.92±23.76). Group R showed more improvement than other groups as the rats in this group learned significantly more slowly on the first and second days of the training trials, but reached the same level as Group S and Group SR on the third and last days ($\rho<0.05$). On the third experimental day, the escape latency of Group C (16.63±15.04) was shorter ($\rho<0.05$) than that of the other groups (Group S: 30.38±17.72, Group SR: 29.92±21.78, and Group R: 28.58±23.37). On the fourth experimental day, the

escape latency of Group C (10.59±9.39) was highly significantly different ($p<0.01$) from those of the other groups (Group S: 20.21±17.1, Group SR: 18.88±15.88, and Group R: 19.17±17.21) (Table 1, Figure 2).

Table 1. Escape Latencies (Seconds) in the Training Trials.

Groups		1.Day	2.Day	3.Day	4.Day	Average
S[¥]		42.57±20.06	36.71±18.09	30.38±17.72	20.21±17.1	32.47±18.24
ρ values	Group SR	0.867	0.865	0.935	0.543	0.767
	Group R	0.023	0.032	0.753	0.634	0.142
	Group C	0.409	0.413	0.018	0.001	0.001
SR[¥]		43.5±23.22	37.71±19.39	29.92±21.78	18.88±15.88	32.50±20.07
ρ values	Group S	0.867	0.865	0.935	0.543	0.767
	Group R	0.035	0.048	0.816	0.895	0.078
	Group C	0.321	0.323	0.022	0.001	0.001
R[¥]		55.29±8.69	49.38±19.06	28.58±23.37	19.17±17.21	38.11±17.08
ρ values	Group S	0.023	0.032	0.753	0.634	0.142
	Group SR	0.035	0.048	0.816	0.895	0.078
	Group C	0.002	0.004	0.039	0.001	0.001
C[¥]		38±21.05	31.92±23.76	16.63±15.04	10.59±9.39	24.29±17.31
ρ values	Group S	0.409	0.413	0.018	0.001	0.001
	Group SR	0.321	0.323	0.022	0.001	0.001
	Group R	0.002	0.004	0.039	0.001	0.001
Average[¥]		44.84±18.26	38.93±20.08	26.38±19.48	17.21±14.90	
ρ values	1.Day		0.018	0.001	0.001	
	2.Day	0.018		0.001	0.001	
	3.Day	0.001	0.001		0.001	
	4.Day	0.001	0.001	0.001		

[¥]:mean±standard deviation.

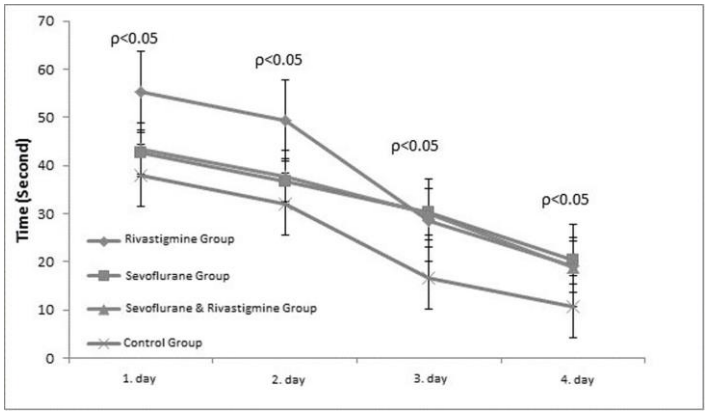

Figure 2. Average learning durations per experimental group.

In the probe trial carried out to evaluate spatial memory, no significant difference was found among the groups in terms of time passed in the north quadrant ($\rho>0.05$) (Table 2).

Table 2. Time spent in the north quadrant in probe trial.

Groups	Time Spent in the North Quadrant (Seconds)[¥]
Rivastigmine	43.75±11.76
Sevoflurane+ Rivastigmine	40.13±14.89
Sevoflurane	38.43±12.4
Control	45.17±11.76

[¥]:mean±standard deviation.

Discussion

Although negative effects have been reported in the vast majority of studies on this subject [1], some studies indicate that sevoflurane has no effect or even a positive effect on spatial cognitive functions [8, 9, 11]. Also, as noted, rivastigmine has been shown to be effective in reversing cognitive impairments, depending on the dose used [19, 23-25]. Within this framework, the effect of repeated sevoflurane exposure on the spatial learning and memory of weanling rats was investigated in this study, as well as whether cognitive impairments induced by sevoflurane can be suppressed by rivastigmine treatment.

We found that repeated sevoflurane exposure impaired spatial learning in weanling rat pups; however, it produced no adverse effect on spatial memory. Considering that sevoflurane has long-term detrimental effects on both learning and memory [5, 8-10], it can be argued, according to the results of the present study, that sevoflurane initially affects learning, and then memory. In other words, the effect of sevoflurane on spatial memory is rather long-term [28], so this effect was not observed in our study. In addition to this, we found that rats of the rivastigmine group showed more improvement than any other group as the rats in this group learned significantly more slowly at the beginning of the training, but reached the same level as the rats in the sevoflurane and sevoflurane + rivastigmine groups at the end.

On the other hand, we found that rats treated with sevoflurane alone (Groups S) had poorer spatial memory numerical values compared to control rats (Group C). In addition, although not statistically significant, the spatial memory values of the sevoflurane and rivastigmine group (Group SR) were better than those of the sevoflurane group (Group S).

The use of weanling rat pups distinguishes this work from other repeated-dose sevoflurane studies, which generally used adult rats or pre-weaned rat pups (most studies on infant rats used postnatal day 7 pups) [6, 7, 26, 28]. Also, the durations between medications and trials are important factors in this study, as well as the doses of sevoflurane or rivastigmine. New studies on the subject could be carried out by increasing the sample sizes, using different durations between medications and trials, using experimental animals of different ages, or changing the doses of sevoflurane and/or rivastigmine.

In conclusion, we found that sevoflurane negatively affects learning in weanling rat pups but has no detrimental effect on spatial memory. On the other hand, it can be claimed that sevoflurane offsets the memory-sparing effects of rivastigmine.

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