Behavioural effects of chronic exposure to subanesthetic concentrations of halothane, sevoflurane and desflurane in rats

[Effets comportementaux d'une exposition chronique à des concentrations sous-anesthésiques d'halothane, de sévoflurane et de desflurane chez les rats]

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Background: A double-blind, randomized trial was conducted to determine the behavioural effects of chronic exposure to subanesthetic concentrations of halothane, sevoflurane and desflurane in rats.

Methods: Halothane, sevoflurane and desflurane group rats received 0.1%, 0.3%, and 0.6% concentrations in a flow rate of 3 $L{\cdot}min^{-1}$ O_2, respectively. Control animals also received 3 $L{\cdot}min^{-1}$ O_2 in another investigation room, which had the same properties as the study group rooms. Rats breathed inhaled agents or oxygen between 09:00–13:00 hr every day for 30 days. After 30 days of inhalation of subanesthetic doses of inhaled agents or oxygen, behavioural tests were applied.

Results: Tests of exploratory activity and curiosity (hole-board test), anxiety (elevated plus maze test) and learning and memory functions (multiple T maze test), demonstrated that chronic exposure to subanesthetic concentrations of all three anesthetics alters behavioural functions in rats. However, impairment of learning ($P<0.05$) and memory function ($P<0.05$) were greater in association with desflurane, in comparison to halothane and sevoflurane-treated rats.

Conclusion: Chronic exposure to subanesthetic concentrations of halothane, sevoflurane and desflurane is associated with behavioural change in rats. Of the three drugs, desflurane was associated with the lowest learning and memory function test scores.

***Objectif** : Déterminer, par une étude randomisée à double insu, les effets comportementaux de l'exposition chronique à des concentrations sous-anesthésiques d'halothane, de sévoflurane et de desflurane chez les rats.*

***Méthode** : L'halothane, le sévoflurane et le desflurane ont été administrés à des groupes de rats selon des concentrations respectives de 0,1 %, 0,3 % et 0,6% à un débit 3 $L{\cdot}min^{-1}$ d'O_2. Les animaux témoins ont aussi reçu 3 $L{\cdot}min^{-1}$ d'O_2 dans un local d'expérimentation qui avait les même propriétés que le local du groupe à l'étude. Les rats ont respiré les anesthésiques d'inhalation ou de l'oxygène entre 9 h et 13 h tous les jours, pendant 30 jours. Après quoi, des tests de comportement ont été appliqués.*

Résultats : Les tests d'activité exploratrice et de curiosité (planche trouée), d'anxiété (test de labyrinthe «elevated plus maze test») et des fonctions cognitive et mnésique («multiple T maze test») ont démontré qu'une exposition chronique à des concentrations sous-anesthésiques des trois agents altèrent les comportements des rats. L'atteinte cognitive ($P < 0,05$) et mnésique ($P < 0,05$) a été plus importante avec le desflurane, comparé à l'halothane et au sévoflurane.

***Conclusion** : L'exposition chronique à des concentrations sous-anesthésiques d'halothane, de sévoflurane et de desflurane est associée à un changement de comportement chez les rats. Le desflurane a produit les scores les plus bas aux épreuves cognitives et mnésiques.*

NUMEROUS studies have suggested that chronic exposure to trace levels of anesthetic gases may be genotoxic, teratogenic, and may cause immunologic and systemic toxic problems.[1–7] In pediatric anesthesia, mask induction with volatile agents and the use of pediatric semiclosed anesthesia circuits may result in even higher levels of anesthetic gases in the operating

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room. Furthermore, uncuffed endotracheal tubes are used widely in routine pediatric anesthesia practice and can cause considerable volatile anesthetic contamination in the operating room. This problem is especially important for operating room personnel who work in places with inadequate waste gas scavenging and air-conditioning systems.

Long-term exposure to inhalation agents may cause headache, depression, anxiety, loss of appetite, loss of memory, and also changes in the intellectual function.[8–11] Although the harmful effects of anesthetic gases on human health has been documented in several studies, behavioural effects of these agents have not been thoroughly examined. The aim of this study was to compare, using a rat model, the effects of chronic exposure to subanesthetic concentrations of halothane, sevoflurane and desflurane.

Methods

The experimental protocol was approved by the Institutional Animal Investigation Committee of Ondokuz Mayis University School of Medicine. Forty Wistar albino rats, eight months old (Ondokuz Mayis University Laboratory Animals, Samsun, Turkey), weighing 250–280 g were used. The animals were housed in groups of two or three in aluminium cages, and were maintained on a standard pellet food, tap water and normal day/night cycles, at an ambient temperature of 22 ± 2°C. Rats were divided into four groups of ten animals and allocated to halothane, sevoflurane, desflurane or control groups. All anesthetic exposures were conducted in a chamber of 50 cm width, 50 cm height and 100 cm length. The inspiratory gas inlet was on the upper border of the one side of anesthesia chamber. The expiratory gas outlet was located at the bottom of the anesthesia chamber on the controlateral side of the inspiratory gas inlet. A second exposure chamber with identical properties was used for the control animals.

Rats breathed inhaled agents or oxygen between 09:00–13:00 hr every day for 30 days. The equivalent of one minimal alveolar concentration of halothane, sevoflurane and desflurane in rats were considered to be 1.02, 2.9 and 6%, respectively, based upon previous studies.[12–14] The target exposed minimal alveolar concentrations of inhaled anesthetics were 1/10. Halothane, sevoflurane and desflurane groups received 0.1%, 0.3%, and 0.6% concentrations, respectively, at a flow rate of 3 $L \cdot min^{-1}$ O_2 delivered through an anesthesia machine (Dameca, Denmark). Control subjects inhaled 3 $L \cdot min^{-1}$ O_2 only. The day after the 30-day inhaled anesthetic exposure behavioural tests were conducted in all groups at the same time of the day, as described below.

Hole-board test

The Hole-board test, which was described by File and Wardill in 1977,[15] is used for exploration, curiosity and locomotor activity in rodents, and provides an overview of an animal's behaviour in a single short test. The test arena consisted of a box (width 60 cm, length 60 cm and height 40 cm) with a board containing 16 round holes (diameter 4 cm). Each rat was observed in the hole-board test arena for five minutes. The number of times the rat demonstrated the activity of head–dipping into the holes, and the duration of immobility, was assessed by a video camera and expressed in minutes.

Elevated plus maze test

Elevated plus maze test is a standard tool of research for anxiety in rats. The apparatus was identical to that described by Pellow *et al.*[16] The test area is made of wood and consisted of two open arms (length 50 cm, width 10 cm) and two enclosed arms (length 50 cm, width 10 cm, height 40 cm), which extended from a central 10 cm × 10 cm square platform, configuring the maze into a 'plus' shape. The plus maze was elevated 50 cm above the floor. Each rat was placed in the centre of the maze facing an open arm, and was observed for five minutes. The times spent on the open and closed arms were recorded; the total time spent on open arms is considered to be related to anxiety.

Multiple T maze test

The multiple T maze test evaluates learning and memory functions.[17] The test arena consisted of one starting box (20 cm × 20 cm × 20 cm), nine straight passages (20 cm × 10 cm × 10 cm), three blind boxes (20 cm × 10 cm × 10 cm) and two goal boxes (20 cm × 20 cm × 20 cm). All rats received water once a day at 14:00 hr for three consecutive days before this test. In order to acclimatize the rats to the test area, each rat was allowed to explore the maze for five minutes with no reward, on the day prior to the experiment. During the test, a cup of water was placed in one goal box in the same position for both trials. Rats were then placed on the starting box. Time to locate the water cup, and the number of incorrect turns (leading to blind boxes) in five minutes were calculated from the video camera record. This test was repeated twice on consecutive days.

Following each experiment the test arenas were cleaned carefully with a disinfectant (alcohol) to remove any source of smell. All tests were continuously recorded with a video camera and evaluated by an observer blinded to group identity.

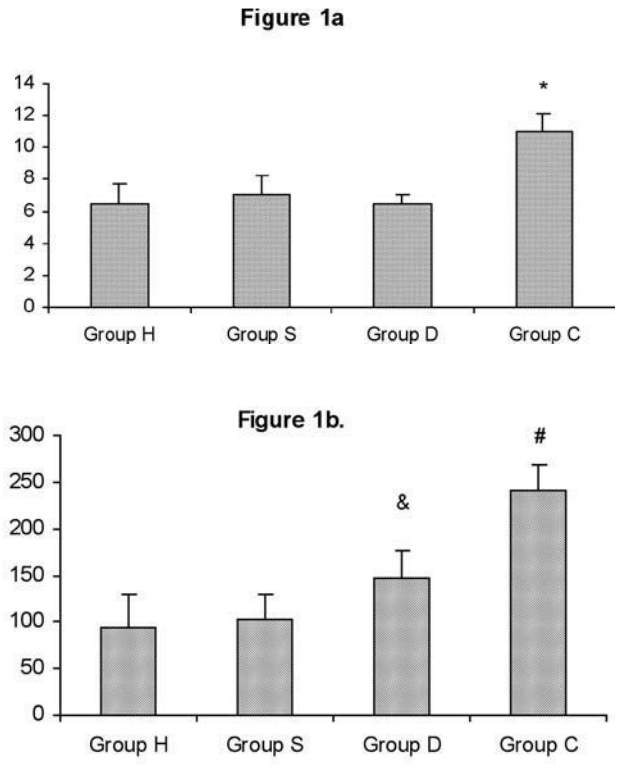

FIGURE 1A and 1B Head-dipping scores (1a) and exploratory time (1b) of groups in the hole board test. Group C: control group, Groups H, S and D: halothane, sevoflurane and desflurane groups, respectively. *$P < 0.05$ *vs* halothane and desflurane groups in Figure 1a. #$P < 0.0001$ *vs* other groups, &$P < 0.005$ *vs* halothane and sevoflurane groups in Figure 1b.

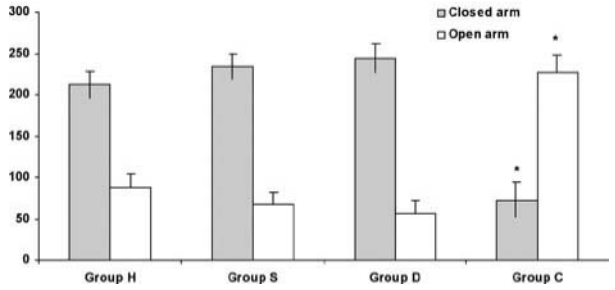

FIGURE 2 Time spent on the open and closed arms of the elevated plus maze test. Group C: control group, Groups H, S and D: halothane, sevoflurane and desflurane groups, respectively. *$P < 0.001$ *vs* other groups.

Statistical considerations

Data were evaluated by Kruskal Wallis analysis of variance (ANOVA). When a factor was found significant ($P < 0.05$), subgroups were compared with the Mann-Whitney U test. Data are presented as mean ± standard deviation. A $P < 0.05$ was considered statistically significant. The SPSS for Windows, version 10.0, software package was used (SPSS Inc, Chicago, IL, USA).

Results

On the hole-board test, control animals demonstrated a greater number of head-dippings than all three anesthetic groups; the differences were significant in the halothane and desflurane groups ($P < 0.05$), but not in the sevoflurane group (Figure 1a). Control rats had longer exploratory times than the study groups ($P < 0.0001$, Figure 1b). Amongst the anesthetic groups, exploratory time was longest in the desflurane group ($P < 0.05$ *vs* sevoflurane and halothane).

In the elevated plus maze test, the percentage of time spent within the open arms in study groups was shorter than the control group (23.7%, 20.9%, 16.6% and 72.2% in halothane, sevoflurane, desflurane and control groups, respectively, $P < 0.0001$, Figure 2). In the multiple T maze test, control group rats found the water cup more quickly than did sevoflurane and desflurane-treated rats ($P < 0.001$) in the first trial (Figure 3a). In the second trial, time to finding the water cup was shorter in the control group in comparison to the other three groups in the multiple T maze test ($P < 0.005$). In the multiple T maze test, the number of wrong turns were significantly fewer in the control subjects than with the animals from other groups in the first trial (Figure 3b). The numbers of wrong turns was greatest in the desflurane group ($P < 0.005$ compared to groups sevoflurane and halothane) in the second trial.

Discussion

The results of this study indicate that chronic exposure to subanesthetic concentrations of halothane, sevoflurane and desflurane results in modified exploratory behaviour and increased anxiety in rats. In addition, subanesthetic levels of halothane, sevoflurane and desflurane are associated with poorer scores in learning and memory tests.

The systemic effects of chronic exposure to inhalation anesthetics have been well documented, especially with respect to hepatic and renal function.[7,18–20] However, behavioural changes associated with long-term exposure to these agents have not been investi-

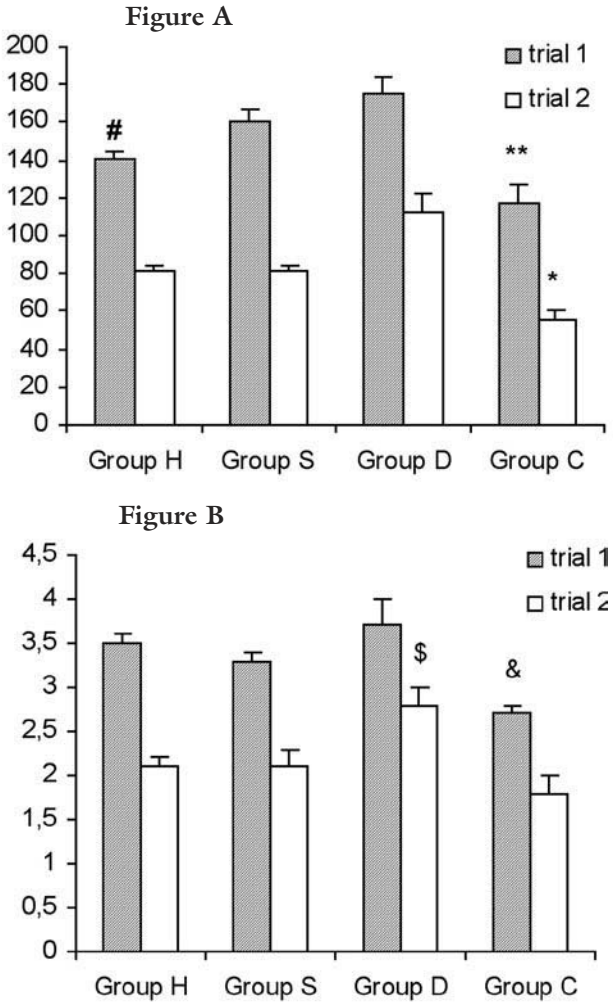

FIGURE 3A and 3B Time spent to find the water cup (3a) and the number of wrong turns (3b) of the groups in the multiple T-maze test. Group C: control group, Groups H, S and D: halothane, sevoflurane and desflurane groups respectively. $^{**}P < 0.001$ *vs* sevoflurane and desflurane groups in trial 1, $\#P < 0.05$ *vs* desflurane group in trial 1, $^{*}P < 0.005$ *vs* all other groups in trial 2, $\&p < 0.05$ *vs* all other groups in trial 1, $\$P < 0.005$ *vs* all other groups in trial 2.

gated in detail. The known adverse behavioural effects of inhalation anesthetics include impaired mental function, increased anxiety, impairment in psychomotor performance and learning.[8–22] In some European countries, concentrations of inhalation anesthetics in operating rooms with active scavenging and air-conditioning systems have been reported to vary between 53-2140 ppm for N_2O, 0.7–11.3 ppm for halothane, 0.3–1.4 ppm for isoflurane, 0.02–0.2 ppm for desflurane, and 0.03–0.16 ppm for sevoflurane.[3,23] Concentrations of anesthetics to which rats were exposed in this study were substantially higher than trace concentrations encountered in most operating rooms with scavenging systems. On the other hand, if an operating room is not equipped with active scavenging devices or air conditioning systems, concentrations of inhalation anesthetics in the operating room air could approach subanesthetic concentrations in the immediate work area of the anesthesiologist. This is especially true for pediatric anesthesia, where inhalation induction via mask or uncuffed endotracheal tubes, or unsealed airway devices are used frequently. Thus, operating room personnel (anesthesiologists, surgical technicians and nurses) could potentially be at some risk of behavioural impairment and systemic toxicity due to chronic exposure to inhalation anesthetic agents.

Levin *et al.*[9] showed that chronic exposure (30 or 60 days) to low levels of halothane (12.5 ppm) during intra-uterine development is associated with both neural and behavioural alterations in rats. In another clinical study, it was suggested that even low levels of exposure to anesthetic gases cause impairment of neurobehavioural performance in operating room personnel.[24] In accordance with these findings, our results demonstrate that chronic exposure to subanesthetic levels of halothane, sevoflurane and desflurane may induce behavioural impairment in rats. Gentili *et al.*[25] have suggested that anesthesia with sevoflurane can pose a hazard of chronic exposure especially for anesthesiologists. Occupational exposure limits for sevoflurane and desflurane are not established, and behavioural changes related to chronic exposure and risks posed by their systemic toxicity on the health and professional performance of health-care personnel have not been thoroughly investigated.

Limitations in translating knowledge from animal experiments to humans must be considered in context of the benefits of these types of studies. Animals experiments can be performed in a more standardized fashion, verified easily, facilitate accurate data recording, and, finally allow use of objective parameters of behavioural function. There are numerous tests for studying curiosity, exploration, anxiety, learning and memory functions in rats. The classical hole-board test is used to investigate exploration, curiosity and locomotor activity in rodents.[15] This test is based on the assumption that head-dipping of the animals is directly proportional to their curiosity and exploratory activity.[26] In our study, 30-day exposure to subanes-

thetic levels of inhaled agents decreased head-dipping in the hole-board test suggesting impairment in their curiosity. Exploratory time was also reduced in all study groups with respect to control groups, especially in halothane and sevoflurane exposed animals. These results suggest that halothane, sevoflurane and desflurane all suppress exploratory activity.

Elevated plus maze test is a widely used and well-validated test of anxiety.[16] Exposure to the elevated plus maze test induces behavioural and physiological effects in rodents consistent with fear and anxiety. The animal is placed in the centre of an elevated four-arms maze where only two of the arms are enclosed and the reduction of time spent in the open arms reflects increased anxiety. Our results indicate that halothane, sevoflurane and desflurane caused anxiety. There is no previous publication evaluating the effects of inhalation agents on anxiety.

The multiple T maze test is used to assess the learning and memory functions in rodents[17] but it has not been previously used to evaluate the effects of chronic exposure to inhalation agents on memory. Nevertheless, there are several studies performed for benzodiazepines and 5-HT_3 receptor antagonists, and it is a valid and reliable tool.[27–29] Our multiple T maze test results indicate that learning and memory functions were depressed by inhalation agents, more obviously by desflurane.

New anesthetic chamber methods developed for anesthetizing non-intubated animals include monitoring CO_2 levels.[30,31] The lack of CO_2 monitoring in the anesthetic chamber is a limitation in our study. On the other hand, although high CO_2 levels cause adverse effects on the psychomotor responses of rats, our control group rats were also exposed to the same conditions. We therefore believe that results from the anesthetic groups and the control group can be attributed to anesthetic exposure. Furthermore, a fresh gas flow rate of 3 $L \cdot min^{-1}$ to the chamber provides twice the minute ventilation of rats (minute ventilation is 160 mL for one rat and 1600 mL for a group of ten rats) and it is therefore unlikely that CO_2 retention was a confounding variable in our study. Finally, we performed the behavioural tests only after 30 days of exposure to subanesthetic levels of inhalation anesthetics. Therefore, we cannot speculate as to whether these changes are transient or persistent in nature. However, operating room personnel may be exposed to trace levels of these agents throughout their occupational life, and subanesthetic concentrations could occur.

In conclusion, our study shows that 30-day exposure to subanesthetic concentrations of halothane, sevoflurane and desflurane is associated with impaired curiosity, exploratory behaviour, increased anxiety, and impaired learning and memory functions in rats. These findings emphasize the importance of procedures and periodic monitoring to ensure that exposure to these agents in the operating room is minimized. Effects of more prolonged exposure of both trace and subanesthetic levels of inhalation agents warrant further investigation.

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