The effect of nicotine on the recovery of rats receiving anesthesia

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ABSTRACT

Objective: To determine the effect of intraperitoneal (ip) nicotine on the recovery of rats receiving general anesthesia compared with placebo.

Methods: The placebo controlled experimental study was conducted in the Faculty of Medicine, Gazi University, Turkey, between April and May 2005. Twenty-one male and 21 female rats were randomly divided into 3 groups. Group C (n=14), comprising a female group CF (n=7), and male group CM (n=7) received ip 0.9% sodium chloride (NaCl), group P (n=14), comprising a female group PF (n=7), and male group PM (n=7) received ip propofol 150 mg/kg, and group NP (n=14), comprising a female group NPF (n=7), and male group NPM (n=7) received 0.4 mg/kg ip nicotine followed by 150 mg/kg propofol after 15 minutes. For the evaluation of recovery period, tail pinch test was used, and for cognitive performance, the radial arm maze test was used.

Results: The number of entrances and exits decreased in group P significantly compared to group C (p<0.05), and the decrease in group PF was higher than it was in group PM. Entrance and exit in group NP increased significantly compared to group P (p<0.05). The increase in entrance and exit in group NPF was much higher compared to group NPM. The recovery period in group NP was significantly shorter than in group P (p<0.05).

Conclusion: The ip administration of nicotine in rats shortens the recovery from propofol anesthesia and improves cognitive performance.

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It is known that after the administration of anesthesia, cognitive functions are affected negatively with varying degrees of severity and time.[1-3] The negative effects, such as prolonged recovery time in the clinic and post-operative cognitive dysfunction (POCD) are all undesirable.[4-6] Neuronal nicotinic acetylcholine receptors (nAchRs) are commonly present on central and autonomic nervous systems. The nAchRs have a positive effect on upper cortical activities, and it has been reported that nAchRs in the reticular activating system are associated with alertness, conscious attention, and learning functions.[7,8] Central and peripheric nAchRs have been very sensitive to volatile anesthetics and intravenous anesthetic agents such as barbiturate, etomidate, and propofol have been shown to exert inhibitory effects on nAchRs.[9] However, it is not clear whether nAchRs inhibition results from the anesthetic effects of volatile anesthetics, or not.[10,11] It is considered that nAchRs inhibition on the medial habenula neurons of halogenous compounds in rats is not important in the anesthetic effects of volatile anesthetics, but it causes amnesia effects.[12] Furthermore, it has been shown that the cognitive performance of the rats is affected negatively after the general anesthesia is administered with isoflurane and nitrous oxide (N_2O).[13] Nicotine is a typical nicotinic receptor agonist that binds with nAchRs. The effects in humans are an increase in loco-motor activity and the stimulation in brain reward areas, an improvement in learning and memory functions, stimulation of epinephrine release in periphery, which is the result of the stimulation of sympathetic nervous system, and an increase in blood pressure, respiration, and heart rate.[14] It has been reported that the administration of chronic and acute nicotine to rats develops the cognitive performance evaluated by radial arm maze test (RAMT),[15] whereas the nAchRs antagonist, mecamylamine weakens memory, and affects cognitive functions negatively.[16-18] Although nicotine and its analogues have been shown to have beneficial effects experimentally in such diseases as Alzheimer's, in which cognitive functions are affected,[19,20] we have not found any studies showing its effect on cognitive performance after anesthesia in comparison with placebo. We hypothesized that using one of the nicotine agonist agents may prevent

the inhibitory effects of anesthetics on neurotransmitter release in the central level. For this reason, we aimed to determine the efficacy of nicotine on recovery and cognitive performance during the recovery from propofol anesthesia in comparison with placebo.

Methods. This study was carried out in the Animal Laboratory of Ankara Education and Research Hospital with the permission of Gazi University Medical Faculty Ethics Committee, Ankara, Turkey from April to May 2005. The study included 44 Wistar albino rats (22 male and 22 female rats) of 5-6 months of age and 270-350 gms in weight. To measure cognitive functions we used the RAMT,[21,22] which can evaluate spatial memory, permanent memory, and the integration between frontal cortex and hippocampus, which is suitable for repeated practice, and which can determine slight differences in memory and learning defects resulting from aging and medication; while conducting the test, a radial arm maze (RAM) with 8 channels opening to a platform in the middle, and with a part where the food was hidden was used. For the evaluation of cognitive performance, the rats had limited access to the food to suspend their motivation. However, they had free access to water in the spaces they were kept before they were subjected to the test. Rats were trained for the RAMT for 3 days, for 10 minutes per day. At the beginning after the food was hidden, the rats were put in the middle of the platform and they were made to go into the 8 channels in 10 minutes. The aim was to make them find the correct place, and at which trial they achieved their goal was taken as the criterion for their success. After the training, 2 rats were excluded from our study because they failed in their attempts to find the food. The remaining 21 male and 21 female rats were included in our study. Rats went through the RAMT after they were kept hungry for 2 hours. After the test in the first group, the female rats (group CF, n=7) and male rats (group CM, n=7) received intraperitoneal (ip) 0.9% sodium chloride (NaCl) (group C, n=14). In the second group the female (group PF, n=7) and male (group PM, n=7) rats received ip 150 mg/kg propofol (propofol 1% Fresenius Kabi ilaç San, İstanbul, Turkey) (group P, n=14). In the third group the female (group NPF, n=7) and male (group NPM, n=7) rats received ip 0.4 mg/kg nicotine in 30 seconds, and after 15 minutes they received ip 150 mg/kg propofol (group NP, n=14). After the recovery was determined with tail pinch test, which measures the response to the painful stimulus, cognitive functions were evaluated by RAMT. Cognitive functions were measured just after recovery (T_1) and then the first (T_2), second (T_3), third (T_4), fourth (T_5), fifth (T_6), and sixth (T_7) hours following recovery.

Data were presented as mean values ± SD. Data were analyzed using SPSS version 11.0.5 for Windows, Chicago, IL, USA. Analysis of variance (ANOVA) was used to compare parametric data, followed by post hoc with the Bonferroni adjustment. $P<0.05$ was determined to be statistically significant.

Results. The groups had equal numbers of female and male rats, and did not differ from each other regarding demographic data such as age and weight. In group P, the entrances and exits significantly decreased when compared to those in group C ($p<0.05$). The recovery time (minutes) in group NP (group NPM - 35.8±6.6, group NPF - 34.2±7.4) was significantly shorter than in group P (group PM - 53.3±12.5, group PF - 55±14.1) ($p=0.026$, p=0.015). In group NP, the entrances and exits significantly increased in comparison with those in group P ($p<0.05$) (**Tables 1 & 2**). At all measuring times in group NPM, the entrances and exits significantly

Table 1 - Radial arm maze channel entrances and exits data in propofol groups of the male (PM) and female rats (PF).

Groups	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Group PF	0.8±0.7	1.2±07	1.0±0.6	1.8±0.8	2.3±0.8	1.8±0.5	1.8±0.8
Group PM	2.5±2.4	2.5±2.3	2.2±1.4	2.3±0.8	2.8±0.9	3.7±0.5*	4.0±0.6*

T1 - just after recovery, and then the first (T_2), second (T_3), third (T_4), fourth (T_5), fifth (T_6), and sixth (T_7) hours following recovery,
*$p<0.05$ - group PF in comparison with group PM.

Table 2 - Radial arm maze channel entrances and exits data in nicotine-propofol groups of the female (NPF) and male rats (NPM).

Groups	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Group NPF	6.5±3.6	6.7±2.3	6.3±1.9	7.5±1.1	6.7±1.9	6.5±1.2	6.5±1.5
Group NPM	9.5±3.9	9.7±3.0*	9.0±2.3*	9.0±1.5	8.2±1.9	8.5±1.6	7.8±1.2

T1 - just after recovery, and then the first (T_2), second (T_3), third (T_4), fourth (T_5), fifth (T_6), and sixth (T_7) hours following recovery,
*$p<0.05$ - group NPF in comparison with group NPM.

Table 3 - Radial arm maze channel entrances and exits data in control groups of the female (CF) and male rats (CM).

Groups	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Group CF	4.0±1.3	4.0±1.4	3.3±1.2	3.5±1.4	4.3±1.1	3.0±1.0	3.5±0.6
Group CM	5.3±2.6	5.7±3.0	3.2±1.2	4.3±2.1	4.7±2.1	4.2±1.2	4.8±1.9

T1 - just after recovery, and then the first (T_2), second (T_3), third (T_4), fourth (T_5), fifth (T_6), and sixth (T_7) hours following recovery,
*p<0.05 - group CF in comparison with group CM.

increased compared to group PM (p=0.010, p=0.011, p=0.004, p=0.003, p=0.003, p=0.003, p=0.003). Except for T_1 and T_2, at all measuring times the entrances and exits in group NPM were found significantly more than group CM (p=0.004, p=0.003, p=0.015, p=0.003, p=0.007), (**Tables 2 & 3**). Although they decreased in group PM compared to group CM, it was not a significant decrease (p>0.05). According to the evaluations within the groups, everything was similar at T_1 and other measuring times. In group PF, the RAM channel entrances and exits decreased significantly in comparison with those in group CF at the measuring times of T_1, T_2, T_3, T_6, and T_7 (p=0.005, p=0.006, p=0.004, p=0.008, and p=0.006) (**Tables 2 & 3**). They increased significantly in group NPF in comparison with group PF (p=0.004, p=0.002, p=0.003, p=0.004, p=0.003, p=0.004, p=0.003) and group CF at all measuring times, except the first measuring time for group CF (p=0.026, p=0.007, p=0.004, p=0.022, p=0.004, p=0.003) (**Tables 2 & 3**). According to the evaluations within the groups, everything was similar at T_1 and other measuring times. In both groups CF and CM, RAM entrances and exits were similar at all measuring times (p>0.05) (**Table 3**). It was found to have decreased significantly in group PF compared with group PM at the measuring times of T_6 (p=0.002) and T_7 (p=0.004). At other measuring times it was found to have decreased, but it did not have a statistical significance (**Table 1**). In group NPF, RAM entrances and exits decreased significantly compared to group NPM at the measuring times of T_2 (p=0.026) and T_3 (p=0.041). At other measuring times it was found to have decreased, but it did not have a statistical significance (**Table 2**).

Discussion. In this study, we concluded that ip administration of propofol affected the cognitive performance of rats negatively, especially of the female rats more negatively, and the use of nicotine reversed this effect in both of the genders, and the ip administration of nicotine shortened the time of recovery from anesthesia.

Although the effects of general anesthesia are considered to be temporary, recent studies have indicated that anesthesia and surgery cause early post-operative cognitive dysfunction, especially in old patients, and this does not result from intra-operative or perioperative hypotension and hypoxemia.[1-3,6,23,24] However, there are some findings showing the permanent effects of general anesthesia. It has been reported that Halothane and N_2O anesthesia cause behavioral retardation, and learning defect during the perinatal period.[25] In their experimental study including 344 rats aged between 6-18 months, Culley et al[13] found that when the old rats receiving 1.2% isoflurane in 70/30% N_2O/O_2 for 2 hours were compared with another group of old rats receiving 90% O_2, in the group receiving anesthesia blood pressure and arterial blood gases were at normal physiological levels, whereas they suffered from a reversible but long lasting insufficiency in spatial memory tests. In our study, we also found that rats had poor cognitive performance in the first 6 hours after general anesthesia administered with propofol compared with the control group.

Gender has been reported to be a factor that affects the time of recovery from anesthesia. In their study carried out with 160 patients aged between 20-60 years, Tercan et al[26] reported that gender was an effective factor in the early recovery from desflurane or sevoflurane anesthesia, and this recovery time was shorter in the male patients, and it did not make any difference in Aldrete scores, delayed memory recall tests. Wilhelm et al[27] administered propofol-remifentanil anesthesia to 120 patients and found that the recovery time in the male patients was longer than it was in female patients who received the same amount of propofol. We have not found any studies evaluating the effect of gender on cognitive functions during the post-anesthetic period in rats. In our study, we compared the cognitive performance after the recovery from anesthesia according to gender, and determined that at T_6 and T_7 measuring times, cognitive performance of female rats was poorer than that of male rats.

Age is one of the most important factors that affects cognitive performance after anesthesia. Experimental and clinical studies suggest that aging affects cognitive performance negatively.[28] Blokland et al[29] showed that repeated pentobarbital anesthesia in rats caused an increase in the impulsivity evaluated by choice reaction time, and reported that repeated anesthesia

was a vulnerability factor for cognitive aging. However, Culley et al[30] carried out a striking study that indicated that RAM and spatial memory performance after the administration of 1.2% isoflurane in 70/30% N_2O/O_2 anesthesia to adult and old rats decreased the RAM and spatial performance without making any difference according to their groups. In this study, cognitive performance of old rats not receiving anesthesia was also low, but the decrease after anesthesia was not much more than it was in adult rats. In our study, to exclude the aging factor that affects cognitive performance after anesthesia, we preferred to use young rats of 5-6 months old. We found that in the groups that received propofol anesthesia, the recovery time was longer and the cognitive performance was poorer than the control group. For the diseases such as Alzheimer's and Parkinson's, which affect cognitive performance negatively, the agents affecting nicotinic receptors are used experimentally and some positive results have been identified.[19] Katner et al[18] indicated that monkeys receiving 3.2 and 5.6 µg/kg intramuscular nicotine had better cognitive performance. In our study, we observed that the recovery time in male and female rats receiving nicotine and propofol was significantly shorter than it was in the propofol group and cognitive performance evaluated by RAMT was again better in the nicotine-propofol group. The speed and dose of the administration of nicotinic agents are also factors determining the efficacy. For that reason, in our study we administered 0.4 mg/kg dose of nicotine in 30 seconds and there was no difference between both of the groups.

Gender is a factor determining the efficacy of nicotinic agonists. The neuron-chemical and behavioral effects of nicotine are seen on nAchRs, which use acetylcholine, dopamine, norepinephrine, serotonin, glutamate, and GABA as neurotransmitters. The administration of nicotine for a short time increases acetylcholine release and extracellular dopamine. Gender has been reported to cause differences for the agents having an effect through the dopaminergic system.[17,19] Thus, in our study, rats were randomly allotted into 3 groups with equal numbers of male and female rats. Since we observed differences in the cognitive performances of male and female rats, we compared and contrasted the data of male and female rats in both of the groups. When the cognitive performances of the control groups were compared with their gender, no difference was found between male and female groups. There was a decrease in the cognitive performance of the male and female rats that received propofol when compared to male and female rats in the control group. The cognitive performance of female groups receiving propofol and nicotine was better than it was in the male and female groups receiving propofol. However, T_6 and T_7 measurements revealed that the decrease in the cognitive performance of female rats that received propofol was significantly more than that of male rats. Also, T_2 and T_3 measurements showed that entrances and exits in the group of female rats that received nicotine and propofol decreased significantly in comparison with that of male rats that received propofol.

Our study has several limitations: The sample size was a major limitation of study. And this research is observational. We believe more research about nicotinic receptors is needed to better define the role of nicotine on recovery from anesthesia.

Consequently, we consider the administration of 0.4 mg/kg nicotine shortens the time of recovery from propofol anesthesia and improves cognitive performance in both female and male rat groups.

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