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Memory-related effects of cholinergic receptor ligands in mice as measured by the elevated plus maze test

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Abstract:
The purpose of our experiments was to examine the influence of cholinergic receptor ligands on memory-related behavior in mice using the elevated plus maze (EPM) test. The EPM test allows the exploration of different memory processes (acquisition and consolidation), depending on the time of drug treatment. The time necessary for mice to move from the opened arm to the enclosed arm (i.e., transfer latency, TL) was used as an index of memory. Our findings reveal that for both the processes of acquisition and consolidation, treatment with nicotine (0.035 or 0.175 mg/kg, free base, *sc*) shortened TL on the second day of the experiments (TL2), thus improving memory processes. Treatment with scopolamine (0.3 or 1.0 mg/kg, *ip*) significantly increased TL2 values, thus impairing cognitive processes. Moreover, we found that treatment with nicotine, at the non-effective doses used during testing, prevented scopolamine-induced memory impairment by inducing a decrease in TL2 values. Next, we evaluated the influence of bupropion (10 or 20 mg/kg, *ip*), a drug currently used for smoking cessation in humans, on memory-related behavior induced by treatment with nicotine and scopolamine. An acute injection of bupropion (10 or 20 mg/kg) prior to injection with either nicotine (0.035 mg/kg) or scopolamine (1.0 mg/kg) significantly prevented nicotine-induced memory improvement or scopolamine-induced memory impairment. Bupropion treatment can diminish the rewarding (dependence-producing) effects of nicotine and also the cognitive effects that are related to addiction. Our studies further indicate the great involvement of the cholinergic system in memory processes and the potential for the development of more effective pharmacotherapies for memory impairment-like human disorders in which the cholinergic pathways have been implicated.

Key words:
nicotine, scopolamine, bupropion, memory and learning, elevated plus maze, mice

Abbreviations: 5HT – serotonin, A – adrenaline, ACh – acetylcholine, AD – Alzheimer's disease, DA – dopamine, EPM – elevated plus maze, GABA – γ-aminobutyric acid, mAChRs – muscarinic cholinergic receptors, nAChRs – nicotinic cholinergic receptors, VTA – ventral tegmental area

Introduction

Diverse findings in the literature have indicated that dementia is one of many age-related mental problems and is a characteristic symptom of various neurodegenerative diseases, including Alzheimer's disease (AD). One of the pathways of treatment of neurodegenerative disorders involves the cholinergic hypothesis, which predicts that the decline of mental function in dementia is predominantly related to a decrease in cholinergic neurotransmission [17].

Many previous studies in the literature have indicated that there is a strong relationship between the central cholinergic pathways and learning and memory. It has been demonstrated that the neurotransmit-

ter acetylcholine (ACh) is essential for cognitive function. Previous studies have concluded that there is a strong correlation between the levels of synaptic ACh and improvements in cognitive function [17, 21]. The data reveal that the inhibition of the activity of cholinesterase, an enzyme that breaks down ACh, leads to increased levels of ACh in the brain, especially in the two major areas that are involved in cognitive processes (i.e., the central cortex and hippocampus). Moreover, dysfunction of the cholinergic system, a decline in the number of cholinergic neurons in the basal forebrain and a decrease in the activity of choline acetyltransferase have been observed in patients with AD. Thus, many of the acetylcholinesterase inhibitors have been shown to improve performance in several cognitive models in humans and rodents, whereas anticholinergic drugs have been demonstrated to impair learning and memory in a variety of experimental paradigms [8, 30].

It has been commonly accepted that there are two types of cholinergic receptors, muscarinic (mAChRs) and nicotinic (nAChRs), which mediate the action of ACh and play important roles in memory processing [29, 44, 49]. The first clinical trials in patients suffering from AD revealed that there are no changes in the number, structure or function of mAChRs, whereas a significant decrease in nAChRs density was observed, especially in the areas of the central cortex and hippocampus [34]. Thus, the influence of nAChRs and mAChRs on memory-related behavior has been evaluated in many behavioral studies [31, 33].

Based on the results mentioned above, we investigated the influence of cholinergic receptor ligands on memory-related responses using the recently developed elevated plus maze (EPM) animal memory model. The aim of our experiments was to ascertain whether the acquisition or consolidation processes of memory were affected by nicotine and scopolamine using the EPM test. Additionally, based on previous findings that indicate that bupropion is utilized as a first-line pharmacotherapy for smoking cessation in humans and that there is commonality in the molecular mechanisms and the brain regions involved in drug addiction and memory-related processes [4], we investigated the effect of pre-treatment with bupropion on memory-related responses induced by nicotine and scopolamine using the EPM test. Our results were interpreted with regard to the role of the cholinergic system in learning and memory. Our experiments may contribute to a better understanding of neuronal mechanisms that are important for the modulation of memory processes induced by nicotine and scopolamine.

Materials and Methods

Animals

Experiments were carried out on naive male Swiss mice (Farm of Laboratory Animals, Warszawa, Poland), weighing 20–30 g. The animals were maintained under standard laboratory conditions (12-h light/dark cycle, room temperature 21 ± 1°C) with free access to tap water and laboratory chow (Bacutil, Motycz, Poland) in their home cages and were adapted to the laboratory conditions for at least one week. Each experimental group consisted of 7–10 animals. All behavioral experiments were performed between 8:00 and 15:00 h and were conducted according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and the European Community Council Directive for the Care and Use of Laboratory Animals of 24 November 1986 (86/609/EEC). All experiments were approved by the local ethics committee.

Drugs

The following compounds were tested: (–)-nicotine hydrogen tartrate (0.035, 0.175 or 0.35 mg/kg, reported in freebase nicotine weight; Sigma-Aldrich, St. Louis, MO, USA), scopolamine (0.1, 0.3 or 1.0 mg/kg; Sigma-Aldrich) and bupropion hydrochloride (10, 20 or 40 mg/kg; Sigma-Aldrich). All compounds were dissolved in saline solution (0.9% NaCl). Except for nicotine, the drug doses refer to the salt form. The pH of the nicotine solution was adjusted to 7.0. Fresh drug solutions were prepared on each day of experimentation. All agents were administered subcutaneously (*sc*) or intraperitoneally (*ip*) at a volume of 10 ml/kg. Control groups received saline injections of the same volume and *via* the same route of administration.

Experimental procedures

Memory-related responses were measured using the elevated plus maze (EPM) test. The experimental apparatus was shaped like a plus sign and consisted of a central platform (5 × 5 cm), two open arms (5 × 30 cm) and two enclosed arms (5 × 30 × 15 cm) opposite to each other. The whole apparatus was constructed of dark Plexiglas and elevated to a height of 50 cm above the floor. Additionally, the EPM test was conducted under dim red lighting.

For the EPM test, the time that the mice took to move from the open arm to the enclosed arm was used as an index of learning and memory and defined as transfer latency (TL). The mice were placed individually at the end of the open arm facing away from the central platform. Each group was submitted to the same procedure twice (the interval between the trials was 24 h). During the first trial (pretest), the time each mouse took to move from the open arm to either of the enclosed arms was recorded as TL1. If the mice failed to enter the enclosed arm within 90 s, they were placed at an enclosed arm and permitted to explore the plus maze for additional 60 s; in these cases, the TL1 value was recorded as 90 s. For the next trial (retention trial) 24 h later, the test was performed in the same manner as the first trial, and the TL was recorded as TL2. If the mouse did not enter the enclosed arm within 90 s on the second day, the test was stopped and the TL2 was recorded as 90 s.

We used the TL2 values as indices of memory and learning effects. Improvement in memory was characterized by a reduction in the time necessary for the mouse to move from the open arm to either of the enclosed arms on the second day relative to the control group. Impairments in memory and learning were characterized by increases in these measurements.

The EPM task allowed us to investigate different stages of memory depending on the time of drug treatment. Thus, administration of a drug before the first trial (before pretest) should interfere with the acquisition of information, while administration immediately after the first trial (after pretest) should affect the processes of consolidation. In our experiments, the drugs were administered 30 min before the pretest or immediately after the pretest, and the effects of each compound on both acquisition and consolidation of memory were investigated.

Treatment

The first experiment was designed to examine the influence of nicotine, scopolamine or bupropion on memory-related responses using the EPM test in mice. Nicotine (0.035, 0.175 or 0.35 mg/kg, *sc*), scopolamine (0.1, 0.3 or 1.0 mg/kg, *ip*), bupropion (10, 20 or 40 mg/ kg, *ip*) or saline was administered 30 min before the first trial or immediately after the first trial. The second set of experiments was designed to investigate the influence of nicotine on memory-related responses induced by scopolamine administration. For these experiments, nicotine (0.35 mg/kg, *sc*) or saline was administered 15 min prior to scopolamine (1.0 mg/kg, *ip*), and then the mice were tested 30 min later and re-tested after 24 h. The final experiment was designed to examine the influence of bupropion on memory-related responses induced by acute nicotine or scopolamine administration. Bupropion (10 or 20 mg/kg, *ip*) or saline was administered 15 min prior to nicotine (0.035 mg/kg, *sc*) or scopolamine (1.0 mg/ kg, *ip*), and the mice were then tested after 30 min and re-tested after 24 h.

Experimental doses and procedures used were chosen according to those commonly used in the literature, including our previous study, in which we examined the cognitive effects of nicotine in mice and the interaction between nicotine and bupropion [5, 6, 41, 50].

Statistics

The data were expressed as the means ± SEM. For the EPM test, we measured TL, i.e., the time necessary for the mice to move from the open arm to either of the enclosed arms. Statistical analyses were performed using one- or two-way analysis of variance (ANOVA) for the factors of pretreatment, treatment and treatment interaction. *Post-hoc* comparison of means was carried out using Tukey's test for multiple comparisons, when appropriate. The data were considered statistically significant at a confidence limit of $p < 0.05$.

Results

Across all experiments, the time (in s) that each mouse took to move from the open arm to either of the enclosed arms on the first trial (pre-test), i.e., TL1, did not significantly differ among groups (data not presented).

Influence of nicotine, scopolamine or bupropion on memory-related processes in the EPM model in mice

One-way ANOVA revealed that the acute *sc* doses of nicotine (0.035; 0.175 or 0.35 mg/kg) had a statistically significant effect on TL2 values [F(3,30) = 6.114; p = 0.0023], with respect to memory acquisition during the retention trial. Indeed, *post-hoc* Tukey's test revealed that mice treated with nicotine, at doses of 0.035 or 0.175 mg/kg, had significantly decreased TL2 values compared with saline-treated mice, indicating that nicotine improves memory and learning processes ($p < 0.01$) (Fig. 1A). Similarly, for memory consolidation during the retention trial, the mice receiving acute *sc* doses of nicotine (0.035, 0.175 or 0.35 mg/kg) had significantly decreased TL2 values compared to the saline-treated mice ([F(3,27) = 4.245; p = 0.0140], one-way ANOVA). Indeed, *post-hoc* Tukey's test revealed a statistically significant effect ($p < 0.05$ for 0.035 mg/kg nicotine; $p < 0.01$ for 0.175 mg/kg nicotine) (Fig. 1B), indicating that nicotine, at the doses used, also improved this stage of memory and learning processes. For both the acquisition and consolidation trials, the highest dose of nicotine (0.35 mg/kg) did not induce any effect in this paradigm.

The active doses of 0.035 or 0.175 m/kg of nicotine were chosen for the subsequent experiments involving the use of bupropion. In addition, the inactive dose of nicotine 0.35 mg/kg was chosen for the subsequent experiments examining the effects of the administration of scopolamine to show the antagonistic effects of nicotine on the amnestic effects of scopolamine.

For memory acquisition during the retention trial, one-way ANOVA revealed that administration of the acute *ip* doses of scopolamine (0.1, 0.3 or 1.0 mg/kg) had a statistically significant effect on TL2 values [F(3,35 = 8.305; p = 0.0003]. Indeed, treatment with scopolamine (0.3 or 1.0 mg/kg) significantly increased TL2 values in mice compared to those in the saline-treated control group ($p < 0.05$ for scopolamine 0.3 mg/kg; $p < 0.001$ for 1.0 mg/kg scopolamine, Tukey's test) (Fig. 2A), indicating that scopolamine, at the doses used, impaired the acquisition of memory and learning. Similarly, Fig. 2B shows that for memory consolidation during the retention trial, administration of the acute *ip* doses of scopolamine (0.1, 0.3 or 1.0 mg/kg) significantly increased the TL2 values [F(3,36) = 4.498; p = 0.0088, one-way ANOVA] compared to the saline-treated mice. Furthermore, a *post-hoc* Tukey's test revealed a statistically significant effect caused by treatment with 1.0 mg/kg scopolamine ($p < 0.01$) (Fig. 2B), which indicates that scopolamine, at the dose used, also impaired this stage of the memory and learning processes.

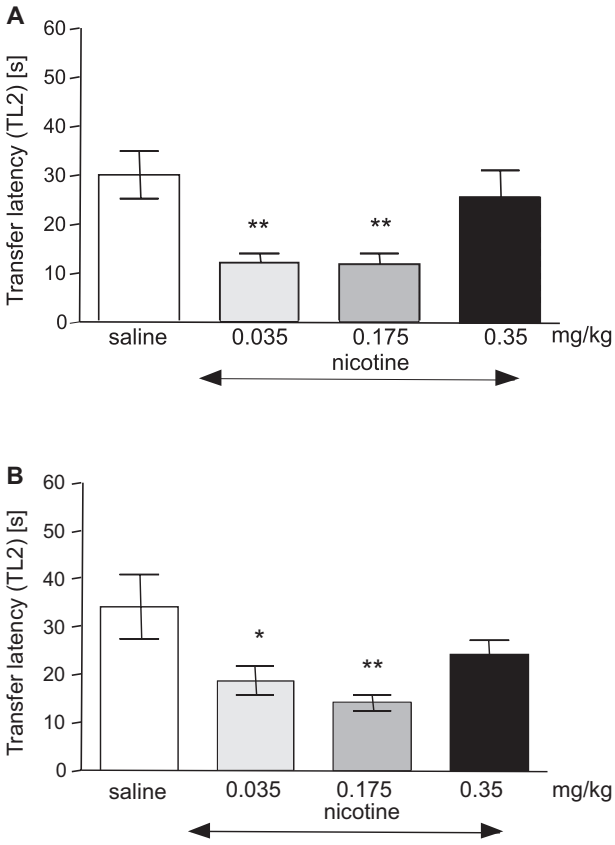

Fig. 1. Effects of acute nicotine or saline injection on the transfer latency to the enclosed arm in the acquisition trial (**A**) or consolidation trial (**B**) using the EPM test in mice. Nicotine (0.035, 0.175 and 0.35 mg/kg; *sc*) or saline were administered 30 min before the first trial (**A**) or immediately after the first trial (**B**); n = 7–9; the data are shown as the means ± SEM; * $p < 0.05$; ** $p < 0.01$ *vs.* the saline control group; Tukey's test

The active dose of 1.0 mg/kg of scopolamine was then chosen for the subsequent experiments examining the effects of bupropion.

Our data indicate that for both acquisition [F(3,34) = 1.604; p = 0.2066, one-way ANOVA] and consolidation trials [F(3,33) = 1.404; p = 0.2591, one-way ANOVA], at any dose tested (10, 20 or 40 mg/kg), bupropion did not significantly alter the TL2 values in the EPM test (Figs. 3A and 3B).

The inactive doses of 10 and 20 mg/kg of bupropion were then chosen for the subsequent experiments with nicotine and scopolamine.

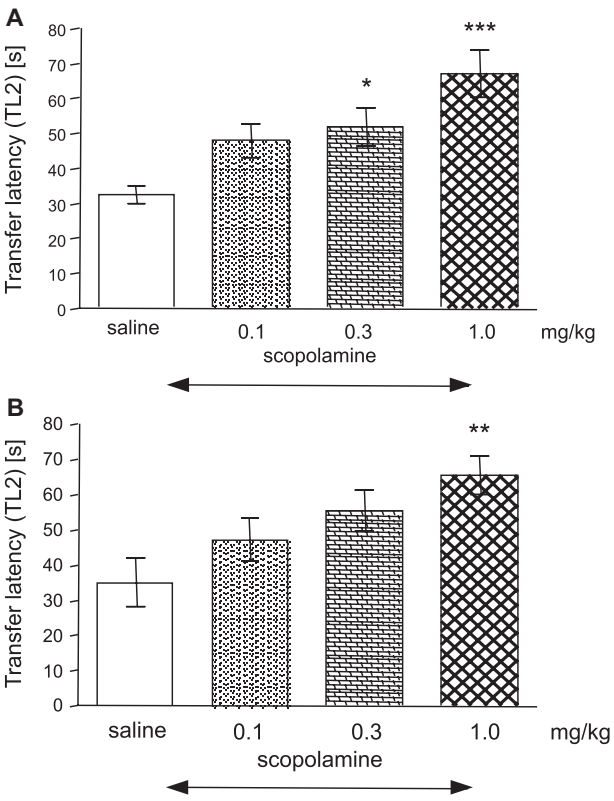

Fig. 2. Effects of acute scopolamine or saline injection on the transfer latency to the enclosed arm in the acquisition trial (**A**) or consolidation trial (**B**) using the EPM test in mice. Scopolamine (0.1, 0.3 and 1.0 mg/kg; *ip*) or saline were administered 30 min before the first trial (**A**) or immediately after the first trial (**B**); n = 9–10; the data are shown as the means ± SEM; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ *vs.* the saline control group; Tukey's test

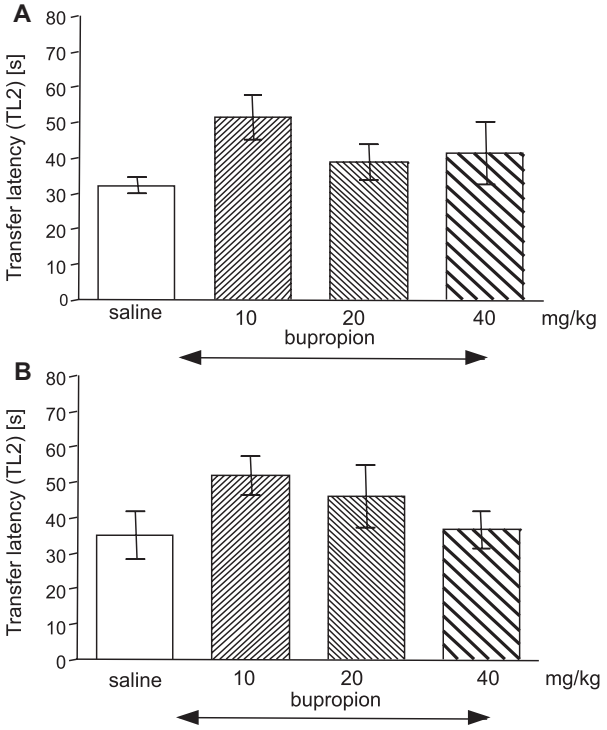

Fig. 3. Effects of acute bupropion or saline injection on the transfer latency to the enclosed arm in the acquisition trial (**A**) or consolidation trial (**B**) using the EPM test in mice. Bupropion (10, 20 and 40 mg/kg; *ip*) or saline was administered 30 min before the first trial (**A**) or immediately after the first trial (**B**); n = 8–10; the data are shown as the means ± SEM

Influence of nicotine on memory-related responses induced by scopolamine using the EPM test in mice

An interesting effect was observed when nicotine (0.35 mg/kg, *sc*) was injected 15 min before scopolamine administration (1.0 mg/kg, *ip*). For memory acquisition during the retention trial, two-way ANOVA revealed that there was a statistically significant effect caused by nicotine pretreatment [$F(1,32) = 9.17$, $p = 0.0048$] and scopolamine treatment [$F(1,32) = 5.0$, $p = 0.0325$], but there was not an interaction between nicotine pretreatment and scopolamine treatment [$F(1,32) = 2.45$, $p = 0.1271$]. However, in this experiment, nicotine, at the dose used, significantly reversed the impairment of memory provoked by acute injection of scopolamine, thus resulting in a decreased TL2 time ($p < 0.01$) (Fig. 4A).

Furthermore, for memory consolidation during the retention trial, two-way ANOVA revealed a statistically significant effect caused by nicotine pretreatment [$F(1,30) = 6.24$, $p = 0.0182$] and scopolamine treatment [$F(1,30) = 30.93$, $p < 0.0001$], and there was an interaction between nicotine pretreatment and scopolamine treatment [$F(1,30) = 13.0$, $p = 0.0011$]. Nicotine significantly reversed the impairment of memory provoked by acute injection of scopolamine and caused a decrease in the TL2 time ($p < 0.001$) (Fig. 4B).

Influence of bupropion on memory-related responses induced by nicotine using the EPM test in mice

Finally, we examined the effects of combined administration of bupropion and nicotine. For memory acquisition during the retention trial, two-way ANOVA revealed a statistically significant effect caused by bupropion pretreatment [$F(2,48) = 8.29$, $p = 0.0008$] and nicotine treatment [$F(1,48) = 28.22$, $p < 0.0001$];

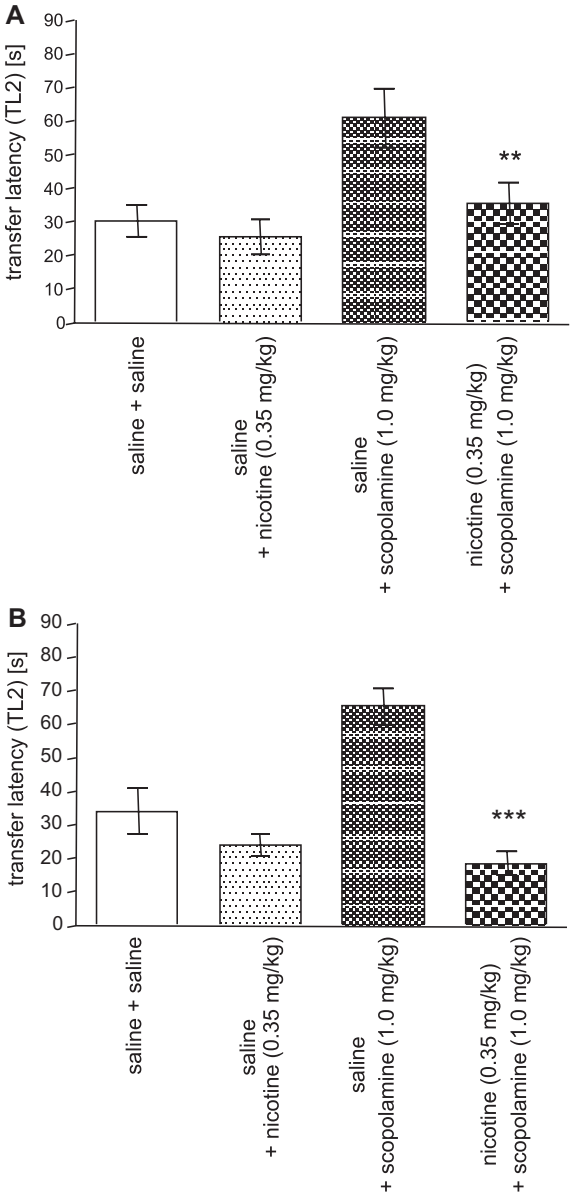

Fig. 4. Influence of nicotine on the memory-related response induced by acute scopolamine administration in the acquisition trial (**A**) or consolidation trial (**B**) using the EPM test in mice. Nicotine (0.35 mg/kg, *sc*) or saline was administered 15 min prior to scopolamine (1.0 mg/kg, *ip*) injection, 30 min before the first trial (**A**) or immediately after the first trial (**B**); n = 7–10; the data are shown as the means ± SEM; ** p < 0.01; *** p < 0.001 *vs.* the scopolamine-treated group; Tukey's test

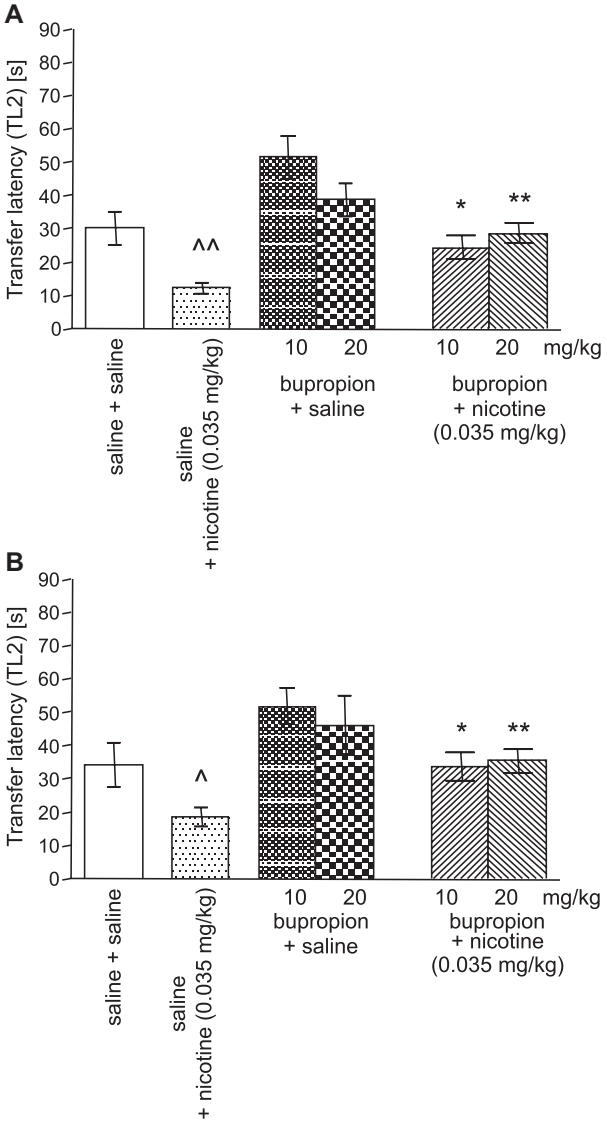

Fig. 5. Influence of bupropion on the memory-related responses induced by acute nicotine administration in the acquisition trial (**A**) or consolidation trial (**B**) using the EPM test in mice. Bupropion (10 and 20 mg/kg, *ip*) or saline was administered 15 min prior to saline or nicotine (0.035 mg/kg, *sc*) injection, 30 min before the first trial (**A**) or immediately after the first trial (**B**); n = 9; the data are shown as the means ± SEM; ^ p < 0.05; ^^ p < 0.01 *vs.* the saline-treated group; and * p < 0.05; ** p < 0.01 *vs.* the nicotine-treated group; Tukey's test

however, there was no interaction between bupropion pretreatment and nicotine treatment [F(2,48) = 1.97, p = 0.1501]. Nevertheless, bupropion (10 or 20 mg/kg, *ip*) prevented memory improvement after administration of 0.035 mg/kg nicotine, resulting in an increased TL2 value (p < 0.05 for 10 mg/kg bupropion, p < 0.01 for 20 mg/kg bupropion) (Fig. 5A).

Similarly, for memory consolidation during the retention trial, two-way ANOVA revealed that there was a statistically significant effect caused by bupropion pretreatment [F(2,46) = 4.87, p = 0.0121] and nicotine treatment [F(1,46) = 10.15, p = 0.0026]; however, there was no interaction between bupropion pretreatment and nicotine treatment [F(2,46) = 0.23, p = 0.7937]. Treatment with bupropion (10 or 20 mg/kg, *ip*) prevented memory improvement after treatment

with 0.035 mg/kg nicotine, resulting in an increased TL2 value ($p < 0.05$ for 10 mg/kg bupropion, $p < 0.01$ for 20 mg/kg bupropion) (Fig. 5B).

Influence of bupropion on memory-related responses induced by scopolamine using the EPM test in mice

For the next experiments, we examined the effects of combined administration of bupropion and scopolamine. For memory acquisition during the retention trial, two-way ANOVA revealed that there was a statistically significant effect caused by scopolamine treatment [$F(1,50) = 4.16$, $p = 0.0466$] and an interaction between bupropion pretreatment and scopolamine treatment [$F(2,50) = 4.67$, $p < 0.0138$], while there was no effect caused by bupropion pretreatment [$F(2,50) = 1.28$, $p = 0.2876$]. However, bupropion (20 mg/kg) prevented memory impairment after treatment with 1.0 mg/kg scopolamine, resulting in a decreased TL2 value ($p < 0.01$) (Fig. 6A).

Additionally, for memory consolidation during the retention trial, two-way ANOVA revealed that there was only a statistically significant effect caused by an interaction between bupropion pretreatment and scopolamine treatment [$F(2,47) = 9.50$, $p = 0.0003$], while there was no effect caused by scopolamine treatment [$F(1,47) = 0.003$, $p = 0.9502$] or bupropion pretreatment [$F(2,47) = 1.30$, $p = 0.2809$]. However, bupropion (10 or 20 mg/kg) prevented memory impairment after treatment with 1.0 mg/kg of scopolamine, resulting in a decreased TL2 value ($p < 0.001$ for 10 mg/kg or 20 mg/kg bupropion) (Fig. 6B).

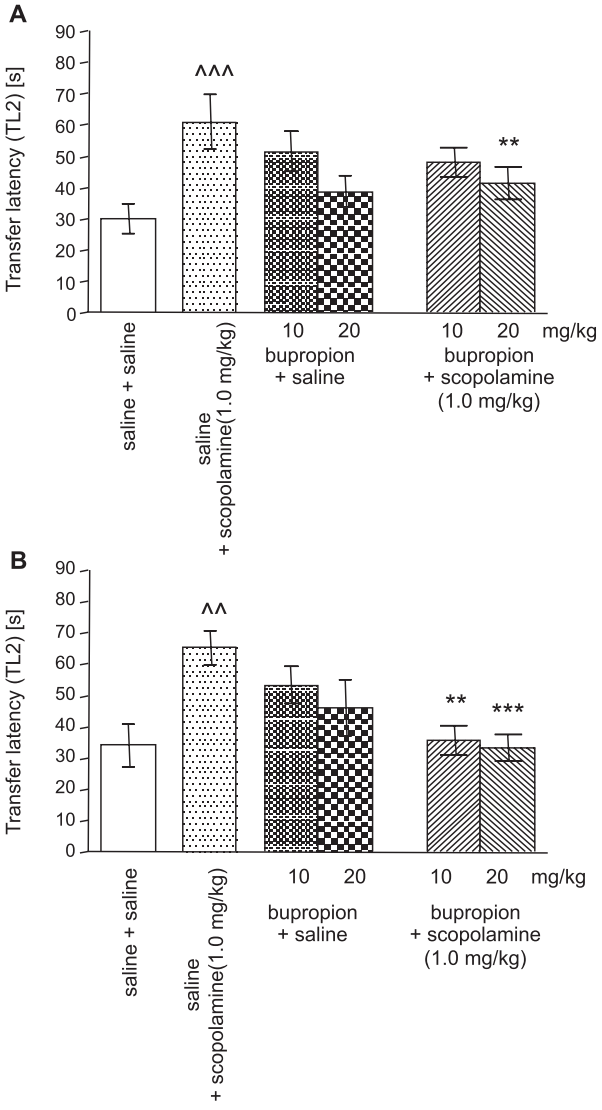

Fig. 6. Influence of bupropion on the memory-related responses induced by acute scopolamine administration in the acquisition trial (**A**) or consolidation trial (**B**) using the EPM test in mice. Bupropion (10 and 20 mg/kg, *ip*) or saline was administered 15 min prior to saline or scopolamine (1.0 mg/kg, *ip*) injection, 30 min before the first trial (**A**) or immediately after the first trial (**B**); n = 9–10; the data are shown as the means ± SEM; ^^ $p < 0.01$; ^^^ $p < 0.001$ *vs.* the saline-treated group and ** $p < 0.01$; *** $p < 0.001$ *vs.* the scopolamine-treated group; Tukey's test

Discussion

The aim of our present research was to estimate the influence of substances that affect the cholinergic system (nicotine and scopolamine) on cognitive effects (stages of acquisition and consolidation of memory) using the EPM test in mice. We examined the mechanisms involved in the formation of memory pathways *via* evaluation of the impact of bupropion on the procognitive effects of nicotine and the amnestic effects of scopolamine. In the present study, we revealed for the first time that nicotine improved memory and learning processes during the different stages of memory (acquisition and consolidation) in mice. In contrast, scopolamine impaired those cognitive processes. Moreover, we found that bupropion attenuated nicotine-induced improvements and scopolamine-induced impairments in memory.

The EPM test was originally developed to estimate anxiety in rodents [37]. However, it was recently modified to evaluate spatial learning and memory in rodents. The parameters measured are not the same:

the number of entries into the open and closed arms, the time spent in the open arms for anxiety, and transfer latency (TL), which reflects the time the mice took to move from the open arm to either of the enclosed arms for the memory processes.

In the context of our present data, it should be noted that nicotine can affect both types of parameters, but with our modification of the test, we can focus on the learning and memory capacity for the spatial configuration of the arms. Our previous experiment [5, 6], as well as other results already published, demonstrated the effectiveness of the EPM test to evaluate memory-related behavior in mice and in the context of pharmacological manipulations of the cholinergic system [26, 27, 41, 47].

The great influence of the cholinergic systems on memory-related processes had been revealed previously by various experiments and clinical studies. Some studies have reported that acute nicotine treatment improves memory in rodents [5, 6, 34–36], while others have reported no effects or even negative influences of nicotine on cognitive functions [16]. In contrast, there are numerous pharmacological reports that have suggested that scopolamine interferes with memory and cognitive functions in humans [18, 46, 51], and experimental animals [10, 14, 39]. Other studies have suggested that scopolamine induces dose-dependent significant decreases in both long-term memory (explored through the passive avoidance test) and short-term memory (evidenced in a Y-maze task) [57] and causes similar degrees of impairment in both reference and working memory using the models of the Morris water maze [3] or the novel object recognition task [14].

Our results in the present study are in accordance with the data in the literature and our previous research [5, 6], in which the positive influence of nicotine on cognitive effects was observed.

The mechanisms responsible for the cognitive improvement induced by nicotine or for the cognitive impairments induced by scopolamine are complex. Nicotine exerts its behavioral effects through the nAChRs, which have been implicated in many processes, such as learning and memory processes, reward, antinociception and anxiety [32, 37, 54]. Among all central nAChR subtypes, both the $\alpha 4\beta 2$ combination and the $\alpha 7$ subunits appear to play important roles in memory-related responses [19, 32]. It is possible that nicotine treatment results in increased receptor activity (i.e., upregulation of $\alpha 4\beta 2$ and $\alpha 7$ nAChR expression) in the central nervous system, especially in the hippocampus, which appears to be an important target site for the nicotinic effects on memory function [43, 52].

Although the cholinergic system and the direct interaction between nicotine and the nAChRs play important roles in nicotine-induced memory-related behavior, these effects can also result from the release of several neurotransmitters. As indicated, through activation of the presynaptic nAChRs, nicotine induces the release of ACh, which is essential for cognitive processes, and also dopamine (DA), γ-aminobutyric acid (GABA), noradrenaline (NA), adrenaline (A), serotonin (5HT) and glutamate [54]. The data in the literature have shown that dopaminergic mechanisms affect learning, and brain DA plays a crucial role in both rewarding and memory-related processes [23, 24]. Dopaminergic neurons from the ventral tegmental area (VTA) are equipped with both nAChRs and mAChRs, and systemic or *in vitro* administration of nicotine excites dopaminergic neurons in the VTA [13, 55]. There is copious evidence indicating that the D1 dopaminergic receptor antagonist SCH 23390, but not the D2 receptor antagonist sulpiride, increases the effects of nicotine on passive avoidance learning. In this context, it is possible that dopaminergic mechanisms, through the D1 receptors, exert negative influences on the improvement of retrieval induced by nicotine [13, 25]. Previous studies have also confirmed that nicotine has an antiamnestic effect on long-term memory in rodents with muscarinic, nicotinic or dopaminergic D2 receptor blockade [1, 23, 24].

We are unable to explain the mechanism underlying cognitive impairments induced by scopolamine without further analysis of this phenomenon [28]. Scopolamine is an anticholinergic drug that antagonizes the mAChRs (subtypes: M1 and M2). In particular, this drug is quite selective for M1 receptors, potentially indicating that impairments of cognitive processes are associated with the blockade of mAChRs in the basal forebrain regions. Therefore, it is possible that cholinergic transmission through the mAChRs is important for synaptic plasticity and memory processes. It has been well documented that the effects of scopolamine may affect both mAChRs and nAChRs [45].

It is particularly important to note in our present study that cholinergic transmission through both mAChRs and nAChRs is important for synaptic plasticity and memory processes. We also investigated

the effects of nicotine on cognitive functions in mice treated with scopolamine to block mAChRs. Our data reveal that nicotine, at the dose that is ineffective in the test used, prevented scopolamine induced memory impairment, thus confirming that both mAChRs and nAChRs play a role in memory processing [20, 33]. In addition, extensive work performed in rats suggests that acetylcholinesterase inhibitors, such as metrifonate, physostygmine, tacrine, rivastigmine and donepezil, are able to reverse the scopolamine-induced deficit in spatial memory in the radial arm maze, the Morris water maze and the passive avoidance tests, thereby indicating that this cognitive deficit has a cholinergic nature [7, 12, 56].

Finally, consistent with previous results that demonstrated the influence of bupropion on the nicotine response [6], we evaluated the effects of bupropion on memory-related responses induced by nicotine and scopolamine. The results from our experiments indicated that bupropion was able to attenuate both the antiamnestic effect induced by nicotine and the amnestic effect induced by scopolamine in the acquisition and consolidation trials.

Bupropion is an atypical antidepressant drug that alleviates the symptoms of nicotine withdrawal in humans, such as irritability, depression and difficulty in concentration [48]. Additionally, in animal models of nicotine addiction, an acute administration of bupropion decreases nicotine self-administration [53] and reduces somatic signs of nicotine withdrawal in rats, including teeth chattering, gasping, writhing, tremors, chewing and ptosis [9], and memory-related responses induced by nicotine [50].

The mechanisms of action through which bupropion produces its therapeutic effects and the effects of the combination of bupropion with other agents and are not completely understood [11, 40]. Many studies have suggested that the effectiveness of bupropion in the treatment of tobacco dependence is independent of its antidepressant properties [22]. The data in the literature have shown that the action of bupropion is mediated by two different mechanisms. The central mechanism is based on the inhibition of the re-uptake of monoamines, especially DA and NA. Moreover, this drug enhances dopaminergic activity in the mesolimbic system [2]. Because a D1 dopaminergic receptor antagonist, SCH 23390, and a D2 dopaminergic receptor antagonist, sulpiride, decreased bupropion-induced sniffing, it can be speculated that both D1 and/or D2 dopamine receptor mechanisms are involved in the response of bupropion [25]. Other data have indicated that bupropion induces a dose-dependent attenuation of the spontaneous firing rate of NA and an increase in serotoninergic firing neurons, without altering the firing rate of dopaminergic neurons in the mesolimbic/cortical regions [15]. Additionally, recently published data have provided evidence that reserpine, a drug that depletes catecholamines, decreases climbing induced by bupropion, indicating that bupropion has indirect catecholaminergic effects [42]. Alternatively, the mechanism of action of bupropion is mediated by nAChRs. Bupropion can act as a non-competitive nicotinic-receptor antagonist of rat $\alpha3\beta2$, $\alpha4\beta2$ and $\alpha3\beta4$ ganglionic-type of nAChRs expressed in a human neuroblastoma cell line. Therefore, the presynaptic action of bupropion on the release of monoamines, as mentioned above, can be nAChR-mediated [50]. Because atropine significantly increases bupropion-induced sniffing, it is likely that the mAChRs exert influence, as well [58, 59]. Therefore, many studies have suggested that the antagonistic effects of bupropion on the amnestic effects of scopolamine may be a result of action through both nAChRs and mAChRs. It should be noted that the influence of bupropion on the effects induced by scopolamine is caused by anticholinergic action and also by the interaction with the serotoninergic or noradrenergic systems. Moreover, the influence of bupropion on the effects of nicotine and scopolamine can be attributed to its major metabolite hydroxybupropion, which is behaviorally active [38]. However, the interactions of bupropion with scopolamine-induced behavior have not been thoroughly investigated. Thus, more research is still necessary to better understand the mechanisms influencing the efficacy of bupropion.

Considering that cognitive processes are associated with similar plasticity as the brain regions involved in learning and memory processes and that the processes underlying drug addiction may overlap [4], our results suggest that bupropion, a drug currently used for smoking cessation in humans, can alleviate the pro-cognitive effects closely associated with dependence as well as the symptoms of nicotine withdrawal. Additionally, it is possible to speculate on the interaction between nAChRs and mAChRs and their influence on memory processes. Our results are useful because they increase our knowledge regarding the processes underlying human cholinergic transmission disorders, including cognitive dysfunction.

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