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Participation of muscarinic receptors in memory consolidation in passive avoidance learning

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It is well-known that the cholinergic system and the muscarinic cholinergic receptors are associated with cognitive functions. Here we examined whether a non-selective muscarinic receptor antagonist scopolamine affects learning performance and/or synaptic plasticity during the memory consolidation period. Adult male Wistar rats (250–300 g) were injected with scopolamine (2 mg/kg) or saline immediately after training in a "passive avoidance" task. Memory retention test was conducted 24h after training. The changes in the latency of the first entry into a dark compartment of a test chamber was choosen as a criterion of learning. The efficacy of synaptic transmission was estimated by the changes in the basal level of focal potentials (fEPSP amplitude and slope ratio) before training (baseline), 90 min after the training (consolidation period), and 24 hour after the training (retention period). We found that foot-shock presentation by itself had no effect on fEPSP within the first 90 min after training, but in 24 hour fEPSPs were decreased. In untrained rats administration of scopolamine had no effect on the fEPSP amplitude within the first 90 min after the injection, but in 24 h we observed an increase in the fEPSP amplitude. In trained animals, scopolamine decreased the fEPSP amplitude in the hippocampal CA1 area during first 1.5 h after the injection. However, the drug had no effect on the memory retention in the passive avoidance task. Taken together our data suggest that scopolamine modifies the synaptic placticity of the hippocampal network but does not induce significant changes in the retention of the passive avoidance skill.

Key words: scopolamine, hippocampus, mAChR, consolidation, memory

INTRODUCTION

The hippocampus plays an important role in learning and memory processes, including learning in inhibitory avoidance and maze tasks (Izquierdo et al. 2002). Performance of cognitive functions involve muscarinic acetylcholine receptors (mAChR) of the hippocampus (Jerusalinsky et al. 1997). Several studies have reported that activation of mAChRs by selective M1 agonists improved the cognitive performance of mice in a novel object recognition assay (Bradley et al. 2010). The non-selective anticholinergic drugs, scopolamine or atropine, and selective muscarinic antagonist pirenzepine produced amnesia when given before or after training (Moss et al. 1980, Jerusalinsky et al. 1997, Herrera-Morales et al. 2007). Finally, mAChRs are involved in synaptic plastisity such as long-term potentiation (LTP), which is considered as a typical model of synaptic modifications in the hippocampus that occur during learning and memory (Bliss and Collingridge 1993). It was shown that muscarinic agonists enhance and antagonists disrupt LTP (Leung et al. 2003, Ye et al. 2001, Sanchez et al. 2009).

There are many studies that have used different acetylcholine agonists/antagonists to assess the role of acetylcholine in the diverse learning performances (Power et al. 2003, Klinkenberg and Blockland 2010). To illustrate, intraseptal administration of acetylcholine agonist carbachol prior to the training in the radial maze paradigm had no effect on memory formation. Nevertheless, its infusion immediately after the sample session produced acute amnesia (Bunce at al. 2004). Similarly, the effects of non-selective antagonist scopolamine also depend on various factors: the type of learning task, dose of the drug, time of the injection and age of animals (for review, see Klinkenberg and

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Blockland 2010). For instance, in an active avoidance test low doses of scopolamine improved memory retention whereas higher doses disrupted performance (Flood and Cherkin 1986). In passive avoidance studies, administration of scopolamine before training appeared to be more effective in impairment of retention than posttraining treatment (Nomura et al. 1994).

Previous findings of experiments, in which acetylcholine antagonists affected learning performance, are quite contradictory, which may be related to differences in test conditions such as time of drug administration (Moss et al. 1981, Diaz del Guante et al. 1991, Quirarte et al. 1994). Nevertheless, the use of posttraining drug administration is a good approach for evaluation of drug effects on memory consolidation. Here we examined the relationship between the synaptic plasticity in the hippocampus and reconsolidation of memory traces during the memory consolidation after passive avoidance training using high dose of scopolamine (2 mg/kg), a non-selective antagonist of mAChRs.

METHODS

The experiment was conducted on adult male Wistar rats (250–350 g) received from Reasearch Center of Biomedical Technology RAMS, nursery "Stolbovaya". A total of 40 rats were involved in the study. Animals were housed under standard vivarium conditions at 21±1°C with a 12 h light/dark cycle, food and water were provided *ad libitum*. All experiments were performed in accordance with the ethical principles stated in the European directive (86/609/EC) and were approved by the Ethical Committee of the Institute of Higher Nervous Activity and Neurophysiology of the Russian Academy of Sciences.

Stereotaxic surgery

Neurophysiological and behavioral studies were preceded by surgical operation. Rats were anaesthetized with chloralhydrate (400 mg/kg, i.p.) and mounted in a Kopf stereotaxic frame. Bipolar nickel-chrome electrode (diameter 80 micrometers) was implanted into the brain for Schaffer-collateral pathway (3.0 mm posterior, 3.0 mm lateral to bregma, approximately 2.8 mm ventral to dura) stimulation (Paxinos and Watson 1998). To record field excitatory postsynaptic potential (fEPSP) in response to paired pulse stimulation a recording nickel-chrome electrode was lowered into the CA1 area (2.7 mm posterior, 1.5 lateral to bregma, approximately 2.2 mm ventral to dura) (Paxinos and Watson 1998). The bregma and lambda were at the same horizontal level. No differential lead was used, therefore, one electrode in the frontal bone served as a ground and as a reference electrode. The electrodes were fixed on the skull of a rat using quick-setting dental plastic (protakril M) and stainless steel screw. Rats were allowed to recover 7 days after the surgery.

Passive avoidance (PA) learning

To study the effect of scopolamine (2 mg/kg, Sigma, United States), on the formation of passive avoidance (PA) we used the dark-light chamber paradigm. The test apparatus (OpenScience, Russia) consisted of a plastic box equally divided into two compartments (30 cm×30 cm×30 cm): one was wite-colored and brightly illuminated and the other one was black-colored and dark. The two compartments were not separated by door.

Behavioral procedures

During the first testing, rat was placed into the light compartment and allowed to move freely between the two parts of the chamber for 10 min (habituation trial). After the habituation trial, the fEPSP initial slope was recorded for 30 min in an individual recording cage. Immediately after the fEPSP recording rat was placed into the same chamber (acquisition trial), behavioral conditions were similar to the habituation trial but entry into the dark compartment was paired with a 10-s electric shock (0.5 mA) provided through the metal grid covering the floor of the test camera.

After the shock rat was immediately removed from the apparatus, received an intraperitoneal injection (1 ml/kg) of saline or 2 mg/kg scopolamine (dissolved in sterile 0.9% saline) and were placed into the recording cage for the fEPSP registration (90 min). In 24 h, during the retention trial, no foot shock was given and the step-through latency was recorded as a measure of retention. Before and after that rats were placed into the recording cage for fEPSP registration (30 min).

Electrophysiology

For habituation to the experimental conditions each rat was housed in the individual recording cage for 30 min prior to the start of the field EPSPs recording ses-

sion. The amplitude of the fEPSPs was measured in freely moving rats during the first 90 min after the trial and saline/scopolamine injection (consolidation period) and 24 h after (memory retention test).

The fEPSP amplitude in the CA1 field evoked by stimulation of the Schaffer collaterals (interstimulus interval 30 ms; intertrain time 20 s at intensity of 100–400 μA) was obtained from 10 succesive stimuli and was recorded every 10 min. The test paired pulse intensity was set to evoke 40–50% of maximum fEPSP amplitude. The efficacy of synaptic transmission was evaluated based on changes in the amplitude characteristics of the evoked responses in the hippocampal CA1 field in response to test stimulation of the Schaffer collaterals.

Statistical analysis

All data are presented as mean ± SEM. Across groups of electrophysiological data, statistical significance between means was determined using mixed-design analysis of variance followed (where applicable) by Fisher's LSD *post hoc* test to reveal group differences on separate time intervals; for within the group comparisons, a paired Student's *t*-test was used. The differences in behavioral parameters were tested with Student's *t*-test. To estimate the probability of appearance of the secondary response and for comparison within a group χ^2 criterion was used. Differences were considered significant at $P<0.05$.

RESULTS

For the analysis of the effects of scopolamine at a dose of 2 mg/kg on the characteristics of the fEPSP of the hippocampal CA1 field, we tested four groups of animals. In two of them, treatment with the drug or saline was preceded by electrical shock. In two other groups, drug or saline were administrated without electrical shock. According to the results of the retention test in 24 hour, the step-through latency in saline- or scopolamine-treated trained rats was significantly increased as compared to untrained animals. As shown in Figure 1 trained rats that received scopolamine did not significantly differ from trained rats that received saline (310.8±83.5 s in scopolamine-injected rats vs. 425.1±92.3 s in saline-injected group, n=10/group).

We also assessed the effect of post-training scopolamine treatment on fEPSPs during the consolidation period (after acquisition trial) and 24 hour after (retention period). Under our experimental conditions, fEPSPs evoked by Schaffer collateral stimulation were stable for at least 1 hour (data not shown). Saline injection (1 ml/kg i.p.) to untrained rats had no effect on fEPSP amplitude and slope ratio for over a 90 min recording period. In 24 hour, in saline-treated untrained rats the fEPSP magnitude was not significantly different from the basal level. Thus, placement of animals in dark-light chamber without electrical shock caused neither immediate nor delayed changes in electrophysiological responses in the hippocampus.

In the group of trained rats, systemic injection of saline did not affect fEPSP amplitude and slope ratio in the first 90 min compared with the saline-injected untrained rats and baseline. However, in 24 hour, the fEPSP slope ratio in trained rats treated with saline significantly decreased (to 85.5±5.2%, $P<0.05$) as compared to the basal level (Fig. 3B). The decrease in the fEPSP amplitude tended to be significant (89.3±7.4%, $P<0.1$). Thus, training of animals did not have immediate effect on fEPSP characteristics but resulted in the delayed (in our case by 24 h) decrease in fEPSP slope ratio.

When scopolamine was injected to untrained rats, it did not significantly alter the amplitude and slope ratio of fEPSP for the following 90 min recording period.

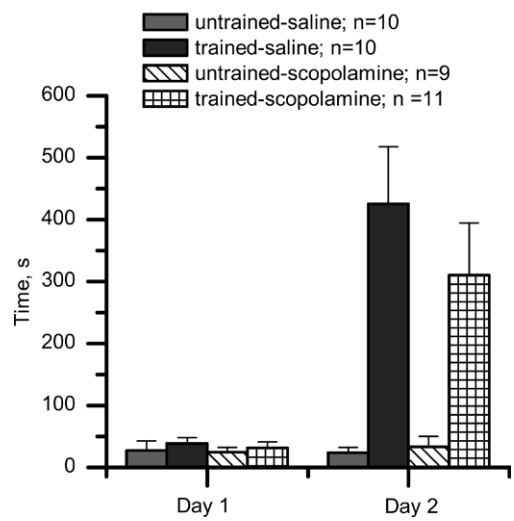

Fig. 1. The effect of scopolamine (2 mg/kg) on the retention of passive avoidance 24 h after training. Rats received saline or scopolamine immediately after training. Intraperitoneal injection of saline and scopolamine to untrained rats had no effect on behavioral latency. The latency in trained-scopolamine group did not differ significantly from the trained-saline group.

However, we found that, in 24 hour, in untrained rats scopolamine significantly increased the fEPSP amplitudes (133.2±13.2% and 132.7±14.8% before and after placement into the light-dark chamber, respectively, compared with basal level, Fig. 3A) compared with saline-injected untrained rats. Slope ratio was also significantly increased by scopolamine (141.7±18.8% and 140.3±25.1% before and after placement into the light-dark chamber, respectively, compared with basal level, Fig. 3B) compared to saline-injected untrained rats.

In the trained group, scopolamine decreased the amplitudes of the responses of the CA1 field in comparison with the saline-treated trained rats during the first 90 min after the drug injection ($F_{1,18}$=5.5, P<0.05) (Fig. 2A, B). The post hoc test revealed that the most distinct changes were observed from the 50th min of recording (an average decrease of 20%, P<0.05). The changes in the slope of the focal potentials were similar but insignificant ($F_{1,17}$=3.3, P<0.087). In 24 hour, in trained rats treated with scopolamine, the fEPSP amplitude (94.5±10.7% and 92.2±11.1% before and after camera session, respectively, compared with baseline)(Fig. 3A) and slope ratio (90.5±12.5% and 91±12.0% before and after camera session, respectively, compared with baseline) returned to the basal level (Fig 3B). Thus, time course of effect of scopolamine depended on the fact of training of animals: in untrained rats it had only delayed (in 24 h) effect whereas in trained rats we observed „fast" (within first 90 min) effect and no delayed effect.

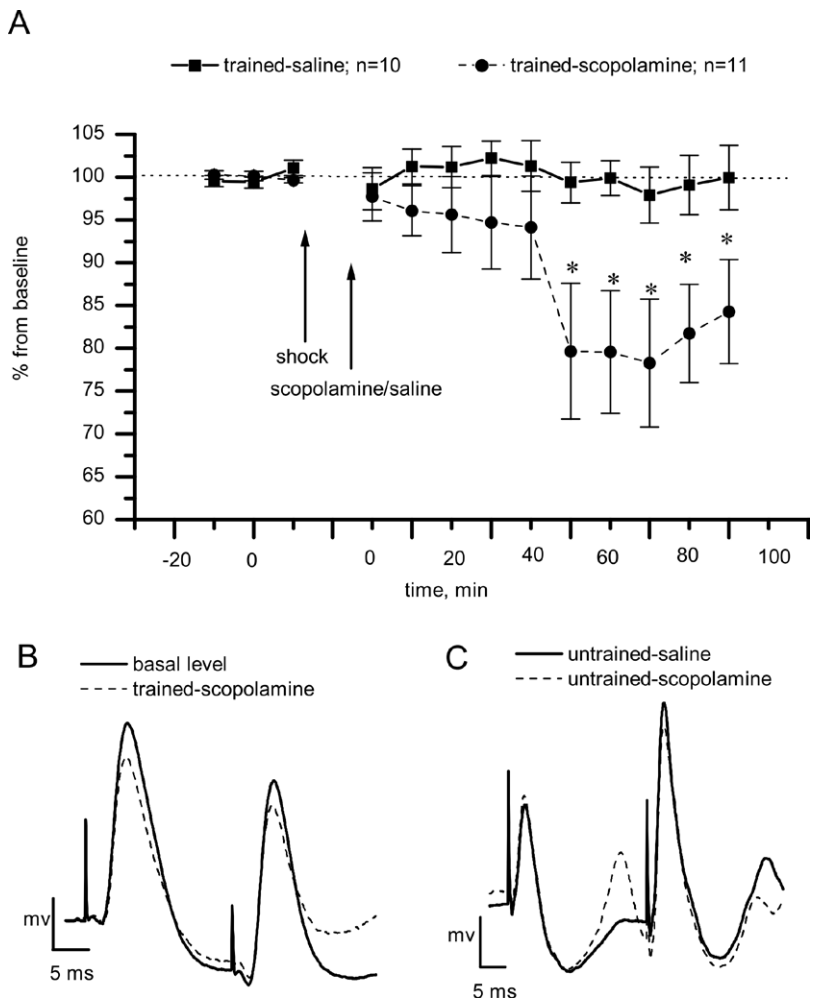

Fig. 2. The effect of scopolamine on fEPSP in the hippocampal CA1 region. (A, B) The time course of scopolamine and saline in trained rats, n=10–11/group, is shown for 90 min after scopolamine or saline application. Each point represents the mean ± SEM. percentage of basal fEPSP amplitude at 0 min. Black arrow marks intraperitonial injection of scopolamine. *Significant difference between groups (P<0.05). (C) Representative example of supplementary waves of excitation generated in the CA1 region.

Note that in both groups of scopolamine-treated rats field response to paired pulse stimulation sometimes had a specific feature: each fEPSP was followed by a secondary response (Fig. 2C), an additional excitation wave. Following scopolamine treatment, the frequency of appearance of the secondary phase after the first or second fEPSP increased as compared to control. The probability of occurrence of additional waves of excitation was higher in the scopolamine-treated group than in the control group (χ^2=42.8, df=1, P<0.01).

DISCUSSION

Here we have assessed the effects of scopolamine on synaptic plasticity and consolidation of memory traces in passive avoidance training. Our results demonstrate that scopolamine at a systemic dose of 2 mg/kg does not alter the amplitude of fEPSP in hippocampal CA1 area. These results on the acute scopolamine injection are consistent with previous investigations showing that scopolamine does not affect the basal level of fEPSP amplitude (Ye et al. 2001, Sanchez et al. 2009). However, in the presence of scopolamine we observed the appearance of additional excitation waves. Additional excitation waves may reflect the return of excitation and reactivation of hippocampal neurons that initiated the wave, i.e., reverberation (van Haeften et al. 2003). Perhaps, scopolamine induced disinhibition of hippocampal network facilitating the occurrence of the reverberation.

Scopolamine significantly increased the fEPSP amplitude in 24 h after the injection compared to the baseline. A possible mechanism of this increase is a compensatory increase in hippocampal ACh release *in vivo* (Stillman et al. 1996, Li et al. 2007) in response to scopolamine-induced blockage of M2/M4 presynaptic autoreceptors that regulate ACh release in the hippocampus (Zhang et al. 2002, Drever et al. 2011). In other words, we suspect that scopolamine induced an increase in the release of ACh and, as a result, activation of nicotinic receptors (nAChRs). Acute systemic application of nAChRs agonists *in vivo* induces LTP induction (Matsuyama and Matsumoto 2003, Drever et al. 2011). Thus, the activation of nAChRs after the increase in ACh level may finally result in LTP-like effect in our case. Previously, it was revealed that induction of nicotine-induced LTP depends on the activation of the two major nAChR subtypes which are composed of α7 and α4β2 subunits (Hunter et al. 1994, Alkondon et al. 2000). There is some indirect evidence that supports our idea of LTP-like nature of the fEPSP increase: treatment with scopolamine induces an increase in level of α7-containing nicotinic receptors (Nic 7) and NR1-containing NMDAR (Falsafi et al. 2012), which can lead to increase in fEPSP level. Taken together with previous data it can be speculated that scopolamine induced biochemical changes that finally resulted in LTP-like effect.

In contrast to the absence of the effect of scopolamine on fEPSP characteristics, acute scopolamine

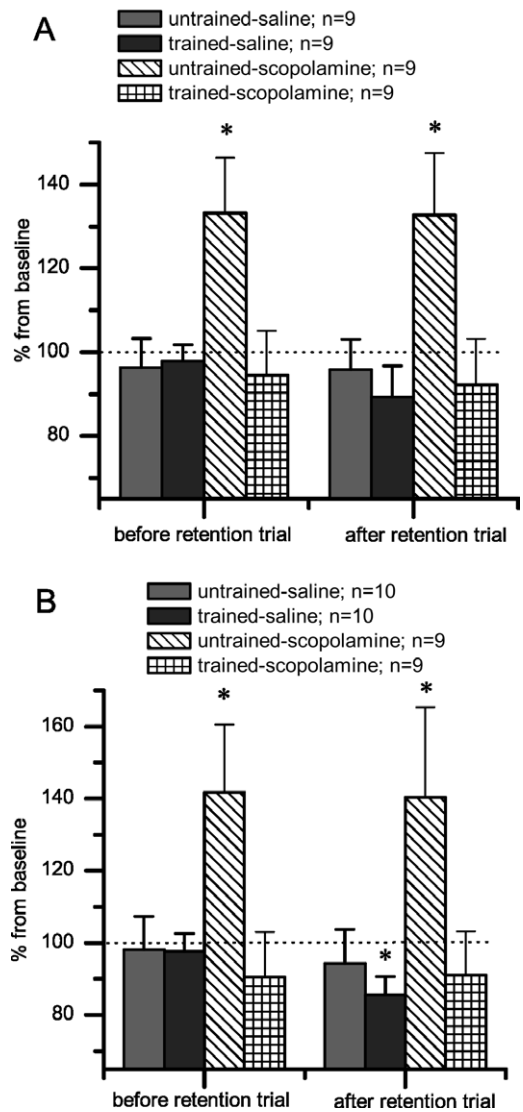

Fig. 3. The effect of scopolamine (2 mg/kg) on the SC/CA1 fEPSP amplitude (A) and fEPSP slope ratio (B), 24 hours after the injection (mean ± SEM, n=9/group) before and after the testing of memory retention (retention trial). Scopolamine greatly increases the fEPSPs amplitude and slope ratio 24 hour after the injection.* Significant difference as compared to basal fEPSP at 0 min (P<0.05).

treatment after exposure to electrical foot shock significantly reduced the amplitude of fEPSP compared to the trained animals treated with saline. Contextual fear conditioning by itself or paired with shock can induce an increase in extracellular GABA levels in the amygdala, mPFC, and hippocampus (Matsumoto et al. 2005, Venton et al. 2006, Hirata et al. 2009). In our study, electrical foot shock alone did not cause any significant decrease in fEPSP levels. Furthermore, we found that scopolamine alone also did not induce any changes in the fEPSP of the CA1 area. Scopolamine can inhibit M2/4 receptors, which are present on inhibitory interneurons (Ye et al. 2001, Drever et al. 2011) that synapse directly onto pyramidal cells. This effect of scopolamine may lead to the increase in GABA release, which, however, was too weak to influence fEPSP basal level. Thus, we hypothesized that, since GABA release is enhanced by stress-related processes and scopolamine, simultaneous activation of both mechanisms may be necessary for the long-term reduction of synaptic transmission in the hippocampus CA1 region. The lack of the delayed LTP-like effect in trained-scopolamine rats may be due to the supression of synaptic transmission in the CA1 region. It seems highly probable that enhanced GABA inhibition in this case would cancel effects induced by scopolamine, including LTP-like effect.

We also showed that post-training scopolamine administration tends to decrease memory consolidation in the passive avoidance skill (see Fig. 1). It has been discussed in several passive avoidance studies that the effective average dose of post-training scopolamine administration should be higher than the dose of scopolamine before training (Rush 1988, Quirarte et al. 1994). However, in our study scopolamine at the dose used induced locomotor hyperactivity (data not shown), which is strongly supported by previous findings (Chintoh et al. 2003, for review see Klinkenberg and Blokland 2010). It was previously shown that cholinergic signaling in the hippocampus, striatum or frontal cortex may positively correlate with the scopolamine-induced increase in locomotor activity (Day et al. 1991, Nomura et al. 1994). Thus, the effects of scopolamine on learning and memory vary between different studies, which could be related to term differences such as dose used and the time of the injection (Wiener and Messer 1973, Flood and Cherkin 1986, Quirarte et al. 1994).

CONCLUSION

The present study demonstrated that scopolamine at the dose used (2 mg/kg) induces changes in the fEPSP that can cause modulatory changes in synaptic plasticity of the hippocampal network. However, the drug had no significant effect on the elaboration of the passive avoidance skill. Perhaps, the described changes in the efficacy of synaptic transmission influence other forms of learning.

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