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Nicotine enhances the reconsolidation of novel object recognition memory in rats

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ABSTRACT

There is increasing evidence that nicotine is involved in learning and memory. However, there are only few studies that have evaluated the relationship between nicotine and memory reconsolidation. In this study, we investigated the effects of nicotine on the reconsolidation of novel object recognition memory in rats. Behavior procedure involved four training phases: habituation (Days 1 and 2), sample (Day 3), reactivation (Day 4) and test (Day 6). Rats were injected with saline or nicotine (0.1, 0.2 and 0.4 mg/kg) immediately or 6 h after reactivation. The discrimination index was used to assess memory performance and calculated as the difference in time exploring on the novel and familiar objects. Results showed that nicotine administration immediately but not 6 h after reactivation significantly enhanced memory performance of rats. Further results showed that the enhancing effect of nicotine on memory performance was dependent on memory reactivation, and was not attributed to the changes of the nonspecific responses (locomotor activity and anxiety level) 48 h after nicotine administration. The results suggest that post-reactivation nicotine administration enhances the reconsolidation of novel object recognition memory. Our present finding extends previous research on the nicotinic effects on learning and memory.

1. Introduction

According to the traditional memory consolidation hypothesis, newly acquired memory is initially present in a transient unstable state in which the memory trace can be disrupted by various treatments, but becomes resistant to disruption over time (Alberini, 2005). This process is called memory consolidation. However, a well-consolidated memory could be again rendered labile and susceptible to disruption upon its reactivation. Memory reconsolidation refers to the process by which memories that have been destabilized by reactivation are restabilized (Dudai, 2006). It is proposed that memory reconsolidation is a vital mechanism of memory modification by which the memory maintains relevant to present and future behaviors (Lee, 2009). Although the issue of memory reconsolidation remains controversial (Lattal and Abel, 2004; Miller and Matzel, 2000), the reconsolidation of memories has been observed in many species including invertebrates and vertebrates (Reichelt and Lee, 2013). Numerous studies have suggested that consolidation and reconsolidation share brain circuits and molecular processes, but the neuronal mechanisms involved do not completely overlap (Lee et al., 2004; Lee and Hynds, 2012; Taubenfeld et al., 2001).

Declarative memory refers to a conscious memory for events and facts and is often subdivided into semantic memory (memory for general information) and episodic memory (memory for personal events) (Squire and Zola, 1996). Declarative memory is usually thought to be acquired with relatively few exposures to the material to be learned. The novel object recognition (NOR) memory task is a simple behavioral assay of memory that relies primarily on the spontaneous tendency of rats to explore a novel object more than a familiar one in the absence of externally applied rules or reinforcement (Antunes and Biala, 2012). Currently the NOR task has become a widely used paradigm for the investigation of the neurobiology of mammalian declarative memory (Winters et al., 2008). Similar to other types of memories, NOR memory can be modified by various pharmacological treatments. For example, microinfusion of a protein synthesis inhibitor anisomycin into the dorsal hippocampus or the ventromedial prefrontal cortex impaired consolidation and reconsolidation of NOR memory (Akirav and Maroun, 2006; Rossato et al., 2007).

Recent studies have suggested an important role of nicotinic acetylcholine receptors (nAChRs) in a variety of learning and memory, such as fear conditioning (Tian et al., 2008), spatial learning (Sharifzadeh et al., 2005), trace eyeblink conditioning (Brown et al., 2010), and various forms of recognition memories (Boess et al., 2007; Froeliger et al.,

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2009; Kenney et al., 2011; Puma et al., 1999; Tinsley et al., 2011). Activation of nAChRs typically enhances the NOR memory by promoting stronger memory encoding, consolidation or/and retrieval (Boess et al., 2007; Melichercik et al., 2012; Obinu et al., 2002; Puma et al., 1999). To date, however, whether nicotine affects the reconsolidation of NOR memory remains to be elucidated. In the present study, we explored the effects of post-reactivation nicotine administration on the reconsolidation of NOR memory in rats.

2. Material and methods

2.1. Subject

The subjects were adult male Sprague-Dawley rats (230–250 g) obtained from the Laboratory Animal Center of University of South China, Hengyang, China. After arrival, the rats were housed individually in a temperature- and humidity-controlled room with ad libitum access to food and water. Animals were maintained on a 12 h light/dark schedule, with lights on at 7 A.M. After being housed, the rats were handled (3–5 min per rat per day) for 1 week to habituate them to the experimenter. Experiments were conducted according to the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*, and experimental protocols were approved by the animal care and use committee of University of South China.

2.2. Behavioral apparatus

As we have previously described (He et al., 2013), the training apparatus consisted of two similar black Plexiglas boxes (50 × 50 × 40 cm) which were used to test 2 animals at the same time respectively. Each box was placed in a sound-attenuating cabinet which was located in a brightly lit and isolated room. Illumination was provided by a 15 W white house light mounted on the ceiling of cabinet, and a 65 dB background noise was supplied by a ventilation fan in the cabinet. The floor of the box was covered with sawdust. The objects used in the test were made of water-repellant materials such as glass and plastic with differences in shape and color. The sizes of the objects were about 6 × 6 × 8 cm. Objects were fixed to the floor of the training apparatus, 10 cm from the walls. The location and objects were counterbalanced to control for any preferences that the rats might have had for one of the corners or of the objects. The sawdust was stirred and the box and the objects were cleaned with 40% ethanol solution between trials. Exploration of an object was defined as pointing the nose to the object at a distance of <1 cm and/or touching it with the nose.

2.3. Experiment design and procedure

Experiment 1 was designed to evaluate the effects of nicotine administration immediately after reactivation on NOR memory reconsolidation. The behavioral procedure involved four phases: habituation, sample, reactivation and the test phase. On Days 1 and 2 (habituation phase), rats were taken from their home cages and transported to the training box for 5 min with no objects presented to habituate them to the training box. On Day 3 (sample phase), rats were transported from their home cages to the training box, and were exposed to 2 objects (A and B) for 4 min as described above. The total time spent on exploring both objects was recorded. On Day 4 (reactivation phase), rats were exposed to the same 2 sample objects (A and B) for a 2-min period to reactivate the memory trace. The total time spent on exploring both objects was recorded. Immediately after reactivation, rats were injected intraperitoneally with saline or nicotine hydrogen tartrate salt (Sigma Co., St. Louis, USA) at doses of 0.1, 0.2 and 0.4 mg/kg respectively. All nicotine doses are expressed as those of the freebase. On Day 6 (test phase), rats were exposed to a duplicate of an object from the sample/reactivation trial and a novel object for 2 min. The time spent on exploring each object and the total time spent on exploring both objects were recorded. The discrimination index used to assess memory performance was expressed as the difference in time exploring on the novel and familiar objects divided by the total time spent on exploring both objects (Ennaceur and Delacour, 1988). As described below in experiment 1, we observed that rats treated with nicotine at doses of 0.1 and 0.2 mg/kg presented an enhancement of memory performance on Day 6, which implies that nicotine may enhance the reconsolidation of NOR memory. To further strengthen our conclusion, three additional experiments (2, 3 and 4) were added.

Experiment 2 was designed to evaluate the effects of nicotine administration 6 h after reactivation on NOR memory reconsolidation. Training procedures were as described in experiment 1, except that rats were injected intraperitoneally with saline or nicotine at a dose of 0.1 mg/kg, 6 h after reactivation on Day 4.

According to a previous note (Nader et al., 2000), a valid criterion to consider a potential effect on reconsolidation is that such manipulation must be effective only following memory reactivation rather than when memory is not reactivated. Thus, Experiment 3 was designed to assess whether nicotine enhances NOR memory performance without the reactivation of memory. Training procedures were as described in experiment 1, except that on Day 4 rats were transported from their home cages and only received intraperitoneally saline or nicotine at a dose of 0.1 mg/kg (no reactivation).

Experiment 4 was designed to study whether nicotine affects the nonspecific responses (locomotor activity and anxiety level) of rats 48 h after nicotine administration. The rats received intraperitoneally saline or nicotine at a dose of 0.1 mg/kg. Forty-eight hours after injection, rats were taken from their home cages and transported to the open field test chambers (60 × 60 × 50 cm) (Shanghai Jiliang Software Technology Co. Ltd., Shanghai, China) for 5 min and their behaviors were recorded as digital videos. The digital videos were then analyzed offline. The distance of rat traveling (defined as locomotor activity index) and the ratio of the time spent in the central zone to the time spent in the peripheral zone (defined as the anxiety level index) in the open field test chamber were analyzed by the commercial software provided by Shanghai Jiliang Software Technology Co. Ltd., Shanghai, China.

2.4. Statistical analyses

Statistical analyses were performed using one-way ANOVA (SigmaStat 3.1). Post-hoc comparisons were performed with the Tukey HSD method. All data were represented as mean ± SEM. Significant level was set at $p < 0.05$.

3. Results

3.1. Experiment 1: effects of nicotine administration immediately after reactivation on NOR memory performance

Fig. 1 shows the effects of nicotine administration immediately after reactivation on NOR memory performance in experiment 1. During the sample phase (Fig. 1A), a one-way ANOVA of the total time spent on exploring both objects found no significant differences between groups ($F_{(3, 28)} = 0.165$, $p > 0.05$), indicating that the four groups showed equivalent levels of exploring objects. During the reactivation phase (Fig. 1B), a one-way ANOVA of the total time spent on exploring both objects found no significant differences between groups ($F_{(3, 28)} = 0.222$, $p > 0.05$). During the test phase (Fig. 1C), a one-way ANOVA revealed a significant group effect ($F_{(3, 28)} = 6.199$, $p < 0.01$). Post hoc comparisons showed that compared with saline treated rats, rats injected with nicotine at 0.1 and 0.2 mg presented a significantly higher discrimination index ($p < 0.01$ and $p < 0.05$, respectively). There was no significant difference in the discrimination index between saline and nicotine injected at 0.4 mg ($p > 0.05$).