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Short Communication

A two-injection protocol for nicotine sensitization

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HIGHLIGHTS

- A two-injection protocol of nicotine exposure resulted in significant nicotine sensitization.
- Mecamylamine blocked the development of nicotine sensitization using a two-injection protocol.
- Two-injection nicotine sensitization was context-independent.

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ABSTRACT

Sensitization to the locomotor activating effect of drugs of abuse occurs following repeated exposure to a drug, and/or when limited exposure to a drug is paired with a specific environment. Conditioned, or context-dependent, sensitization has been well-characterized using limited exposure protocols in, for example, cocaine- and amphetamine-treated animals. However, little data exists regarding limited exposure protocols for other drugs of abuse, such as nicotine. The current experiment investigated whether a two-injection protocol of nicotine administration would result in locomotor sensitization. Mice administered two injections of nicotine (0.175 mg/kg, s.c.) 7 d apart demonstrated significant locomotor sensitization in response to the second exposure. Furthermore, the development of this sensitization was blocked by the administration of the nicotinic acetylcholine receptor antagonist mecamylamine (2 mg/kg) prior to the first nicotine exposure. In a follow-up study, we found that this two-injection nicotine sensitization was independent of context, as separate groups of mice given an initial nicotine exposure (0.175 mg/kg, s.c.) in either the specific environment in which locomotor activity was tested or in their home cages demonstrated equivalent levels of locomotor activity during subsequent testing 7 d later. These data suggest a novel approach to nicotine sensitization using limited nicotine exposure.

1. Introduction

In addiction research, locomotor sensitization refers to a progressive increase in the locomotor response to a drug of abuse with repeated exposure, and has long been suggested to model the neurobiological adaptations that occur following continued drug use in humans, especially long-lasting changes in mesolimbic, mesocortical, and corticolimbic circuits that underlie the reinforcing properties of drugs of abuse and drug-associated cues that precipitate craving and relapse in humans [reviewed in 1]. Thus, the examination of sensitization to the locomotor activating effects of drugs of abuse in rodents has been critical to understanding the neuroadaptive changes that occur following repeated drugs of abuse, as well as helping to identify treatment strategies that can impair or reverse these alterations. Because sensitization not only occurs to repeated exposure to drugs of abuse, but has also been demonstrated in specific situations to be contingent upon the environment in which a drug is administered, measures of sensitization have also been critical in identifying mechanisms of learning and memory that can contribute to the potential incentive learning associated with drugs of abuse [2,3].

Two-exposure paradigms have been well-characterized for drugs such as cocaine and amphetamine, and in each instance the sensitization that has occurred has been demonstrated to be context-dependent [4–7]. For example, Post et al. (1987) demonstrated in rats that a single high dose of cocaine (40 mg/kg) administered in a specific context produced a sensitized locomotor response to a subsequent challenge dose of cocaine 72 h later, but only when testing occurred in the same context. Furthermore, an elegant series of studies from Valjent et al. [4] demonstrated that a two-injection protocol also produced context-dependent sensitization in mice, and this sensitization occurred when the second

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cocaine exposure was as few as 2 d and as many as 84 d after the first exposure, with a peak in cocaine sensitization at 7 d.

Interestingly, few, if any, studies have examined limited exposure-induced sensitization for other drugs of abuse, such as nicotine. The purpose of the present study was to determine whether a low dose of nicotine (0.175 mg/kg) would induce a sensitized locomotor response following a single exposure. Because we have previously shown that animals sensitized to nicotine at this dose following repeated exposure demonstrated a further increase in nicotine-induced locomotor sensitization following a 7 d abstinence period [8], and studies with cocaine using a two-injection protocol in mice have demonstrated a peak in sensitization after 7 d [4], we used this duration of separation between the two nicotine exposures in the current experiment. We also evaluated the effect of the nonspecific nicotinic acetylcholine receptor (nAchR) antagonist mecamylamine on the development of sensitization via administration prior to the first nicotine exposure. Finally, we assessed whether nicotine sensitization induced using a two-injection protocol was context-dependent by administering separate groups of mice the first nicotine exposure in either the experimental context or in their home cages.

2. Methods

2.1. Subjects

Male C57Bl/6N mice (Charles River, Germany) aged 10–14 weeks old at the start of experiments served as subjects. Mice were single-housed in a temperature-controlled (21 °C) environment maintained on a 12-h light–dark cycle (lights on at 6 a.m.). Food and water were available *ad libitum*. All experiments were performed in accordance with EU guidelines on the care and use of laboratory animals and were approved by the local animal care committee (Regierungspräsidium, Karlsruhe, Germany). All behavioral testing was conducted during the light phase between 0800 h and 1700 h.

2.2. Drugs

Nicotine hydrogen tartrate salt (Sigma–Aldrich, Germany) was dissolved in physiological saline (0.9% NaCl) for subcutaneous (s.c.) injection of 0.175 mg/kg (10 ml/kg) based on free base weight (final solution adjusted to approximately pH 7 using NaOH). This dose of nicotine was based on previous results from our laboratory demonstrating a robust locomotor sensitization following repeated administration [8,9]. Mecamylamine hydrochloride (Sigma–Aldrich, Germany) was dissolved in physiological saline (0.9% NaCl) for intraperitoneal (i.p.) injection of 2 mg/kg (10 ml/kg) and based on previous studies [e.g., 10].

2.3. Apparatus

Locomotor activity was assessed in seven TruScan activity monitors (Coulbourn Instruments, USA). Each monitor consists of a clear acrylic plastic testing arena (27 × 27 × 39 cm) placed inside a monitoring unit that records via computer ambulatory beam interruptions from infrared photocell emitter/detector pairs evenly spaced along each axis 1.5 cm above the arena floor.

2.4. Behavioral procedures

2.4.1. Experiment 1

Mice were randomly assigned to one of four groups based on their treatment condition on Day 1: vehicle–nicotine ($n = 6$), mecamylamine–nicotine ($n = 6$), vehicle–saline ($n = 7$), or mecamylamine–saline ($n = 7$). All mice were then habituated to the locomotor activity monitors over 3 daily 60 min sessions. Twenty-four hours following the last habituation session, mice received their respective treatment injections. Vehicle (10 ml/kg, i.p.) or mecamylamine (2 mg/kg; 10 ml/kg, i.p.) was administered 30 min prior to nicotine (0.175 mg/kg, s.c.) or saline (10 ml/kg, s.c.) injections, which were immediately followed by a 60-min exposure to the locomotor chamber. Seven days later, mice were administered nicotine (0.175 mg/kg, s.c.) or saline (10 ml/kg, s.c.), followed by a 60-min locomotor activity session. Locomotor activity was measured as distance traveled (cm).

2.4.2. Experiment 2

Mice were randomly assigned to one of four groups based on their initial treatment condition (Day 1): (1) saline administered in the locomotor chambers (Saline-LC; $n = 7$), (2) nicotine administered in the locomotor chambers (Nicotine-LC; $n = 7$), (3) saline administered in the home cages (Saline-HC; $n = 7$), or (4) nicotine administered in the home cages (Nicotine-HC; $n = 7$). All mice were then habituated to the locomotor activity monitors over 3 daily 60 min sessions. Twenty-four hours following the last habituation session, mice received their respective treatment injections (Day 1): vehicle (saline, 10 ml/kg, s.c.) or nicotine (0.175 mg/kg, s.c.) immediately followed by a 60-min exposure to the locomotor chamber, or vehicle or nicotine in their home cages. Seven days later (Day 8), all mice were administered nicotine (0.175 mg/kg, s.c.), followed by a 60-min locomotor activity session. Locomotor activity was measured as distance traveled (cm).

2.5. Data analysis

Statistical analyses were conducted with SPSS software (Chicago, IL, USA). For Experiment 1, locomotor activity was analyzed using repeated measures two-way ANOVAs (treatment × day), with significant interactions followed by paired *t*-tests to confirm sensitization, where indicated (with a Bonferroni-corrected α for multiple comparisons), and a one-way ANOVA to measure between-groups differences in sensitization score. For Experiment 2, locomotor activity on Days 1 and 8 was analyzed in LC groups using a repeated measures two-way ANOVA (initial treatment × day) and a two-way ANOVA (Day 1 treatment × Day 1 treatment location) was used to analyze locomotor activity on Day 8 in all groups. Significant interactions were followed by paired *t*-tests to confirm sensitization, where indicated (with a Bonferroni-corrected α for multiple comparisons). Significance was set at $p < .05$.

3. Results

3.1. Experiment 1

A two-injection nicotine protocol resulted in nicotine sensitization that was impaired in mecamylamine-treated mice. Fig. 1A and B shows the mean (±SEM) distance traveled (cm) during the 60-min locomotor sessions on Days 1 and 8 in nicotine- and saline-treated groups, respectively A two-way repeated measures ANOVA of nicotine-treated animals (treatment × day) revealed a significant interaction [$F(1,10) = 13.4$, $p < .005$] and main effect of day [$F(1,10) = 11.0$, $p < .01$], but no main effect of treatment ($F < 1$). Paired *t*-tests confirmed that the mice administered vehicle prior to nicotine on Day 1 showed a significant sensitization on Day 8 [$t(5) = 5.1$, $p < .005$; Bonferroni-corrected $\alpha = .025$], while mice treated with mecamylamine on Day 1 failed to differ significantly in locomotor activity on Day 8 [$t(5) = 0.2$, $p = .82$; Bonferroni-corrected $\alpha = .025$]. Thus, a two-injection protocol resulted in nicotine sensitization that was blocked by mecamylamine (Fig. 1A). A two-way repeated measures ANOVA of saline-treated animals