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Restraint stress attenuates nicotine's locomotor stimulant but not discriminative stimulus effects in rats

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ABSTRACT

Stress enhances the locomotor stimulant and discriminative stimulus effects of several addictive drugs (e.g., morphine) in rodents, yet interactions between stress and nicotine's effects in these behavioral models have not been well established. To this end, the current studies examined the effects of restraint stress on nicotine-induced locomotor activity and nicotine discrimination in rats. We used a novel approach in which onset of stress and nicotine administration occurred concurrently (i.e., simultaneous exposure) to simulate effects of stress on ongoing tobacco use, as well as a more traditional approach in which a delay was imposed between stress and nicotine administration (i.e., sequential exposure). Simultaneous exposure to stress reduced the rate of locomotor sensitization induced by daily injections of nicotine (0.4 mg/kg, s.c.). A lower dose of nicotine (0.1 mg/kg, s.c.) produced modest effects on activity that were generally unaffected by simultaneous exposure to stress. Sequential exposure to stress and nicotine (0.4 mg/kg, s.c.) slightly suppressed nicotine-induced activity but did not influence rate of locomotor sensitization. Neither simultaneous nor sequential exposure to stress influenced the discriminative stimulus effects of nicotine (0.01–0.2 mg/kg, s.c.). These data show that restraint stress reduces nicotine's locomotor stimulant effects, particularly when onset of stress and nicotine exposure occurs simultaneously, but does not influence nicotine discrimination. These findings contrast with the ability of stress to enhance the effects of other drugs in these models. This study also suggests that studying the influence of simultaneous stress exposure on drug effects may be useful for understanding the role of stress in addiction.

1. Introduction

Stress contributes to addiction to nicotine and other drugs (Cleck and Blendy, 2008; Goeders, 2003; Kassel et al., 2003; Koob, 2013). For example, stressful life events increase drug consumption and are a common cause of relapse (e.g., Niaura et al., 2002; Sinha, 2009; Sinha et al., 2011). Elucidating the behavioral and neurobiological mechanisms mediating the relationship between stress and addictive drugs could lead to more effective prevention and treatment of drug addiction.

Preclinical models have been useful for understanding the role of stress in drug addiction. It is well established that stressors (e.g., restraint, food restriction) increase the locomotor stimulant effects of single or repeated injections of addictive drugs such as amphetamine, cocaine, and morphine (e.g., Ahmed et al., 1995; Antelman et al., 1980; Deroche et al., 1993; Shaham et al., 1995). Stress can also increase the discriminative stimulus (interoceptive) effects of certain drugs (e.g., cocaine) and/or produce drug-like discriminative stimulus effects itself (Fowler et al., 1993; Kohut et al., 2012; Mantsch and Goeders, 1998; Miczek et al., 1999). These effects may have relevance to the facilitation of drug addiction by stress (e.g., Lu et al., 2003; Marinelli and Piazza, 2002).

Effects of stress on nicotine's locomotor stimulant and discriminative stimulus effects have not been well established. Across different studies, restraint or other stressors enhanced, inhibited, or had no effect on nicotine-induced locomotor activity (e.g., Cadoni et al., 2003; Cruz et al., 2008; Kita et al., 1999; Leao et al., 2012; McCormick and Ibrahim, 2007). It is unclear which of the many methodological factors that differed across studies (e.g., nature of stressor, age and sex of the animals, nicotine dosing regimen) account for these mixed findings. Regardless, the inability of stress to consistently enhance nicotine's locomotor stimulant effects suggests that the relationship between stress and nicotine may be unique. No studies have examined effects of stress in a model of nicotine discrimination.

In most studies examining effects of stress on the locomotor stimulant or discriminative stimulus effects of drugs, a delay ranging from minutes to days is imposed between offset of stress and administration of drug/behavioral testing. While this approach has been very valuable, humans may also be exposed to stress and drugs simultaneously rather than sequentially (e.g., smoking in the presence of social stress). Models involving the use of simultaneous exposure to stress and drugs may

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therefore provide additional insights into the role of stress in addiction (see Zago et al., 2012). In addition, the duration of the interval between stress and drug administration can influence magnitude of stress effects on drug-induced locomotor stimulation (e.g., Stohr et al., 1999; Vanderschuren et al., 1997). As such, the parameters of contiguity between stress and drug exposure may represent an important variable in these models.

The current studies examined the effects of restraint stress on the locomotor stimulant (Experiment 1) or discriminative stimulus (Experiment 2) effects of nicotine. Experiment 2 also examined the ability of stress to produce nicotine-like discriminative stimulus effects itself. Stress-nicotine interactions were examined using either a novel approach in which the onset of stress and nicotine exposure occurred concurrently (i.e., simultaneous exposure) or a more traditional approach in which a short delay was imposed between offset of stress exposure and nicotine administration (i.e., sequential exposure).

2. Materials and methods

2.1. Animals

Male Holtzman Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing 275–325 g at arrival were individually housed in temperature- and humidity-controlled colony rooms with unlimited access to water. Rats in Experiment 1 were housed under a regular 12-h light/dark cycle and tested for locomotor activity during the light (inactive) phase. Rats in Experiment 2 were housed under a reversed 12-h light/dark cycle so that discrimination testing would occur during the dark (active) phase. Locomotor activity and nicotine discrimination are typically tested during these phases of the light/dark cycle in our lab and several others (Bevins and Besheer, 2001; Forget et al., 2010; Harris et al., 2012; LeSage et al., 2012). We used the same lighting conditions in the present studies to examine how stress might affect these behavioral measures in our standard models. Beginning 1 week after arrival, all rats were food-restricted to ≈18 g/day rat chow to maintain good health and to prevent rats from becoming too large to fit in the restraint bottles (described below). This mild degree of food restriction (approximately 90%–95% of free access intake) does not itself represent a significant stressor (see Garcia-Belenguer et al., 1993; Heiderstadt et al., 2000). Protocols were approved by the Minneapolis Medical Research Foundation Animal Care and Use Committee and were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Drugs

Nicotine bitartrate (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile saline. The pH of all nicotine solutions was adjusted to 7.4 with dilute NaOH. Nicotine doses are expressed as the base. All injections were administered s.c. in a volume of 1.0 ml/kg.

2.3. Restraint stress

The stress condition involved immobilization of animals in eight glass restraint bottles (interior volume = 800 ml) attached to an 8-port cylinder (TSE systems, Bad Homburg, Germany) that provided nose-only exposure to fresh air (for further details, see Harris et al., 2010). For the no stress condition, animals remained undisturbed in their transport chambers rather than being exposed to restraint stress.

2.4. Nicotine discrimination

The general apparatus and training procedure used here have been described in detail elsewhere (LeSage et al., 2009). Briefly, animals ($N = 15$) were trained to discriminate nicotine (0.1 mg/kg) from saline using a 2-lever discrimination procedure. This training dose was used because it produces more clinically relevant nicotine serum levels and greater sensitivity to certain experimental manipulations than higher training doses (e.g., 0.4 mg/kg) (see Stolerman et al., 1984; Stolerman and Smith, 2009). Lever pressing was reinforced under a terminal variable interval 15-s schedule using 45-mg food pellets. Discrimination was assessed twice weekly (Tues and Fri) during 2-min extinction test sessions. Discrimination was considered stable when (a) >80% responding occurred on the injection-appropriate lever during two consecutive saline and nicotine test sessions, (b) >95% injection-appropriate responding occurred on six consecutive training sessions, and (c) response rates (total responses/session) were stable (no trend across these four test sessions and six training sessions). Animals that acquired stable discrimination under these conditions ($n = 10$) were tested in Experiment 2a. For the remaining animals ($n = 5$), the nicotine training dose was increased from 0.1 to 0.2 mg/kg. All of these animals acquired stable discrimination with this nicotine dose and were tested in Experiment 2b.

2.5. Experimental protocols

2.5.1. Experiment 1a: effects of simultaneous exposure to stress and nicotine on nicotine's locomotor stimulant effects

On each of two consecutive habituation days, rats ($N = 56$) were tested for locomotor activity in open field activity chambers (described in Cornish et al., 2011; Harris et al., 2010; Roiko et al., 2008) for 30 min (pre-test). Five minutes after the pre-test, rats were injected with s.c. saline and immediately exposed to either restraint stress ($n = 30$) or no stress ($n = 26$) for 10 min. Five minutes later, rats were again tested for activity for 30 min (post-test). Within each stress condition (stress or no stress), total distance traveled during the post-test on the second day of habituation was used to match animals into groups (see below) with similar baseline activity levels.

The test phase began 2 days after completion of habituation. On each test day, rats in the Sal + Stress group (negative control for stress condition, $n = 11$) continued to be treated as during habituation (i.e., 30 min pre-test, s.c. saline injection, 10 min restraint stress, 30 min post-test). The 0.1 Nic + Stress ($n = 10$) and 0.4 Nic + Stress ($n = 9$) groups were treated identically with the exception that rats were injected with 0.1 or 0.4 mg/kg nicotine immediately prior to stress exposure. Both of these nicotine doses have been shown to induce locomotor sensitization (Clarke and Kumar, 1983; Domino, 2001). The 0.4-mg/kg dose has also been used in several studies examining effects of stress on nicotine's locomotor stimulant effects (e.g., Cruz et al., 2008; Leao et al., 2012). The Sal + No Stress, 0.1 Nic + No Stress, and 0.4 Nic + No Stress groups ($n = 8$–10/group) were treated identically, except that animals were not exposed to restraint stress. Rats were treated in this manner 5 days a week for 3 weeks (15 test days total). Drug administration and activity testing were then suspended for 10 days, after which all rats were tested as described above (challenge test).

2.5.2. Experiment 1b: effects of sequential exposure to stress and nicotine on nicotine's locomotor stimulant effects

Two groups of rats ($n = 8$ each) were treated identically to the 0.4 Nic + Stress and 0.4 Nic + No Stress groups described above with the exception that rats were injected with 0.4 mg/kg nicotine immediately prior to the post-test (i.e., 5 min *after* exposure to stress/no stress).

2.5.3. Experiment 2a: effects of simultaneous and sequential exposure to stress and nicotine on nicotine discrimination (0.1 mg/kg nicotine training dose)

2.5.3.1. General design. Rats underwent a total of 4 test phases (each preceded by a habituation phase) using a 2 (stress or no stress) × 2 (simultaneous or sequential exposure) within-subjects design, with the order of test phases counterbalanced across subjects.

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