Pharmacology, Biochemistry and Behavior 165 (2018) 14–24

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Testing environment shape differentially modulates baseline and nicotine-induced changes in behavior: Sex differences, hypoactivity, and behavioral sensitization

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ARTICLE INFO

Keywords:
Nicotine
Behavioral sensitization
Locomotor activity
Intravenous
Rats
Biological sex
Thigmotaxis

ABSTRACT

In those who use nicotine, the likelihood of dependence, negative health consequences, and failed treatment outcomes differ as a function of gender. Women may be more sensitive to learning processes driven by repeated nicotine exposure that influence conditioned approach and craving. Sex differences in nicotine's influence over overt behaviors (i.e. hypoactivity or behavioral sensitization) can be examined using passive drug administration models in male and female rats. Following repeated intravenous (IV) nicotine injections, behavioral sensitization is enhanced in female rats compared to males. Nonetheless, characteristics of the testing environment also mediate rodent behavior following drug administration. The current experiment used a within-subjects design to determine if nicotine-induced changes in horizontal activity, center entries, and rearing displayed by male and female rats is detected when behavior was recorded in round vs. square chambers. Behaviors were recorded from each group (males-round: $n = 19$; males-square: $n = 18$; females-square: $n = 19$; and females-round: $n = 19$) immediately following IV injection of saline, acute nicotine, and repeated nicotine (0.05 mg/kg/injection). Prior to nicotine treatment, sex differences were apparent only in round chambers. Following nicotine administration, the order of magnitude for the chamber that provided enhanced detection of hypoactivity or sensitization was contingent upon both the dependent measure under examination and the animal's biological sex. As such, round and square testing chambers provide different, and sometimes contradictory, accounts of how male and female rats respond to nicotine treatment. It is possible that a central mechanism such as stress or cue sensitivity is impacted by both drug exposure and environment to drive the sex differences observed in the current experiment. Until these complex relations are better understood, experiments considering sex differences in drug responses should balance characteristics of the testing environment to provide a complete interpretation of drug-induced changes to behavior.

1. Introduction

Over the 50-year period since the original Surgeon General's report, the disease risk for tobacco smoking by women has increased despite reported decreases in rates of smoking by both genders (United States Department of Health and Human Services, 2014). Although more men report smoking, women smokers are now equal to men in the risk for lung cancers, cardiovascular disease, and chronic pulmonary obstructive disorder (Syamlal et al., 2014). Women now surpass men in the number of COPD deaths, and continue to experience greater mortality from lung cancer compared to breast cancer (USDHHS, 2014).

In individuals who abuse nicotine and other drugs, gender is an important predictor of dependence (Perkins, 1999; Lynch et al., 2002; Becker and Koob, 2016; Torres & O'Dell, 2016). Self-report studies indicate that women progress toward nicotine dependence at a faster rate (Westermeyer and Boedicker, 2000) and are less likely to benefit from treatment, resulting in lower rates of smoking cessation than men (Perkins, 1999). In the laboratory, women exhibited a greater attenuation of responding for cigarette smoke upon removal of conditional cues, indicating that tobacco smoking is maintained by non-nicotine cues to a greater extent in women relative to men (as reviewed in Perkins, 1999). Stress is also an important mediator of tobacco dependence and relapse following abstinence. Torres and O'Dell (2016) posit that the greater vulnerability to tobacco dependence for women is in part mediated by gender differences in the response to stress, particularly stress that occurs during withdrawal.

Animal models of nicotine exposure have played an important role in demonstrating differences in responses to nicotine as a function of

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https://doi.org/10.1016/j.pbb.2017.12.003
Received 29 August 2017; Received in revised form 22 November 2017; Accepted 13 December 2017
Available online 19 December 2017
0091-3057/

biological sex. Models of reinforcement which allow the animal to self-administer drug reveal that female rats acquire nicotine-reinforced responding at a lower dose (Donny et al., 2000; Chaudhri et al., 2005; Lynch, 2009) than age-matched males (also see Swalve et al., 2016) and exhibit higher break points on progressive ratio schedules of reinforcement (Donny et al., 2000). Compared to males, female rats also show a greater magnitude of responding for intravenous (IV) nicotine in the presence of a weakly reinforcing visual stimulus (Chaudhri et al., 2005); supporting enhanced cue-dependent responding in females. Furthermore, estradiol mediates dopamine function in the nucleus accumbens (Peterson et al., 2015; Becker, 2016; Calipari et al., 2017) and may drive sex differences observed following nicotine administration and likewise, under nicotine withdrawal in conjunction with stress responses (O'Dell and Torres, 2014).

Models that passively administer nicotine to male and female rats have also been important in demonstrating sex differences in nicotine-induced behavioral responses. In an early study, daily subcutaneous infusions of nicotine (6.0 or 12.0 mg/kg/day) decreased body weight in female and male rats during the drug exposure period. For four months after nicotine cessation (12.0 mg/kg/day), the male's body weights were reduced below that of vehicle controls, but females showed no differences from controls during the cessation period (Grunberg et al., 1987). Rats chronically treated with nicotine for seven days (3.16 mg/kg/day) also exhibited sex differences in nicotine withdrawal-related behaviors, and interestingly, detection of this sex difference was modulated by the testing environment. Females showed more withdrawal than males in a dimly-lit environment, but not in a brightly-lit environment (Hamilton et al., 2009).

Systemic injection of nicotine has also produced sex differences in rodent locomotor activity following acute and repeated administration (Booze et al., 1999; Kanyt et al., 1999; Elliott et al., 2004; Harrod et al., 2004; Ericson et al., 2010; Hamilton et al., 2014). First, studies that investigated the stimulus properties of nicotine on locomotor activity in a single sex showed that it modulates overt behavior in a complex manner (Stolerman et al., 1995). Acute injection induces a transient locomotor depressant effect (Morrison and Stephenson, 1972) and repeated administration produces a progressive increase in activity, termed behavioral sensitization (Morrison and Stephenson, 1972; Post, 1980; Clarke and Kumar, 1983; Ksir, 1994). Behavioral sensitization is descriptive of a learning process that occurs following repeated presentation of a single stimulus (Groves and Thompson, 1970), but is not a measure of reinforcement, as drug is administered non-contingently to the animal (DiFranza and Wellman, 2005). Instead, drug-induced sensitization is theorized to play an important role in addiction by increasing the intensity of a signal that is associated with reward which, in turn, is suggested to influence drug craving and approach behavior (Robinson and Berridge, 1993; DiFranza and Wellman, 2005). Several studies report that female rats exhibit a greater magnitude of nicotine-induced behavioral sensitization than males (Booze et al., 1999; Harrod et al., 2004; Hamilton et al., 2014). Sex differences in behavioral sensitization are thus suggested to indicate differential vulnerabilities to the conditional aspects of repeated nicotine exposure, e.g. conditional approach and drug craving (Robinson and Berridge, 1993).

Our laboratory has used the behavioral sensitization model to investigate sex differences in the stimulus properties of nicotine by administering it through the IV route of administration. The IV route mimics the absorption characteristics of inhaled nicotine (Benowitz, 1988) and the dose of nicotine used in the current model, 0.05 mg/kg/injection, produces peak arterial levels of ~25 ng/ml of nicotine with distribution and elimination half-lives of five and 50 min, respectively. Additionally, the 0.05 mg/kg/injection dose is within the range of doses that function as a reinforcer in operant studies of rodent self-administration (Donny et al., 2000; Chaudhri et al., 2005). Repeated, once/daily IV injections of this dose (e.g., 14 or 21 consecutive days) have been shown to produce behavioral sensitization in male and female rats without altering vaginal cytology, estrous cyclicity, or body weights (Booze et al., 1999). Booze et al. (1999) and Harrod et al. (2004), respectively, describe the influence of gonadal hormones as well as the association of dopamine transporter and D3 receptor levels with sex differences in nicotine-induced behaviors. Importantly, both studies describe enhanced sensitization in female rats compared to males when treated with repeated, IV nicotine. Booze et al. (1999) showed that females exhibited a greater sensitization of horizontal activity, rearing, and grooming incidence. The Harrod et al. (2004) study extended these findings, reporting that females exhibited greater sensitization of centrally directed behavior (i.e., entries and distance traveled), rearing duration, and rearing incidence. No locomotor depressant effects following acute injection were reported in either study, although, there was a sex difference in horizontal activity on this day, with females showing more activity than males (Booze et al., 1999). Interestingly, both experiments employed similar experimental designs and the same IV nicotine injection procedure, with the major difference being that the earlier study tested animals in square activity chambers and the latter study used round activity chambers. Although the sex difference in behavioral sensitization is reported in both studies, it is of interest in the current experiment to determine if one style of chamber is more or less sensitive to measure IV nicotine-induced locomotor depression and sensitization. Indeed, evidence from Hamilton et al. (2009), using passive nicotine administration in rats, showed that the testing environment influences expression of nicotine-induced changes in withdrawal behavior. The current experiment determined if round and square chambers differentially modulate the response to acute and repeated IV injection in adult female and male rats in an effort to increase rigor and reproducibility in studies of nicotine-induced behavioral sensitization. We reasoned that because square chambers provide corners as potential places of rest, round chambers promote a general increase in activity therefore increasing sensitivity to measure nicotine-induced changes in behavior. To our knowledge, no systematic comparison of activity produced by two different chamber shapes has been reported. Based on the previous literature, it was predicted that males and females would exhibit different amounts of activity, with females generally more active than males. Additionally, it was hypothesized that acute IV nicotine would produce locomotor depression, which will be more prominent in square chambers, and that sensitization elicited by repeated IV nicotine would therefore be greater in round chambers.

2. Materials and methods

2.1. Animals

Eighty-two adult male (n = 41) and female (n = 41) Sprague-Dawley rats were purchased from Harlan Laboratories, Inc. (Indianapolis, IN) and arrived to the animal care facilities at 90 days of age. Rats were quarantined for seven days and then transferred to the colony room. Rodent food (Pro-Lab Rat, Mouse Hamster Chow #3000) and water were available ad libitum through the experiment and conditions of the colony room were maintained at 21 ± 2 °C and 50 ± 10% relative humidity with a 12-h light: 12-h dark cycle with lights on at 0700 h (EST) daily. Once transferred to the colony room located in the Department of Psychology, each animal was weighed and received an IV saline injection. Surgical implantation of intracath IV catheters was conducted at Harlan Laboratories, Inc. according to the procedures described in Mactutus et al. (1994) prior to arrival. Briefly, subcutaneous ports for IV catheters were implanted dorsally and the distal end of the catheter was secured into each animal's jugular vein to ease daily administration procedures. The completely internalized nature of the IV catheter prevents cage-mates in the pair-housed environment from damaging the catheter. Seven animals were excluded from the final analyses due to insufficient catheter patency during the course of the experiment. The Institutional Animal Care and Use Committee (IACUC) of the University of South Carolina approved the animal protocol for this research.