Pharmacology, Biochemistry and Behavior 99 (2011) 519–523

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Nicotine-induced conditioned place preference in adolescent rats

Reka Natarajan [a], John W. Wright [b,c,d,*], Joseph W. Harding [b,c,d]

[a] Department of Neurosciences, University of Toledo College of Medicine, Toledo, OH 43614, USA
[b] Department of Veterinary and Comparative Anatomy, Pharmacology, and Physiology, Washington State University, Pullman, WA 99164-6520, USA
[c] Department of Psychology, Washington State University, Pullman, WA 99164-4820, USA
[d] Programs in Neuroscience and Biotechnology, Washington State University, Pullman, WA 99164-6520, USA

ARTICLE INFO

Article history:
Received 14 September 2010
Received in revised form 3 May 2011
Accepted 5 May 2011
Available online 11 May 2011

Keywords:
Nicotine
Conditioned place preference
Conditioned place aversion
Adolescent rats

ABSTRACT

A number of clinical reports have noted that women are more vulnerable to tobacco abuse than men, and adolescent females are especially vulnerable to nicotine addiction. Conditioned place preference (CPP) is a widely used technique for determining the rewarding effects of drugs with abuse potential in animal models. Several studies have reported that nicotine was ineffective in eliciting CPP in rats; while others have observed conditioned place aversion (CPA) rather than preference for nicotine. One recent investigation established CPP in adolescent female rats, however at a reasonably high dose; while a second reported dose dependence of nicotine-induced CPP in male but not female rats. The present study was designed to determine the lowest dose necessary to induce CPP to nicotine in adolescent female rats. Nicotine-induced CPP was obtained at a subcutaneous dose of 0.03 mg/kg (salt content) using a biased conditioning paradigm. Higher doses produced aversion and lower doses provided no rewarding or aversive effects. CPP persisted for at least 3 weeks following conditioning in the absence of further nicotine treatment. In contrast with results from adolescent human females and males, age-matched male rats also evidenced CPP at this very low dose of nicotine. These results indicate that even a low dose of nicotine is reinforcing and addicting in both adolescent male and female rats and brings into question the suggestion that nicotine induces greater addicting capacity in adolescent girls than boys.

1. Introduction

Conditioned place preference (CPP) is a commonly used method for determining the reward and addiction potential of a substance (O'Dell and Khroyan, 2009). Most drugs of abuse elicit robust CPP (e.g. Brown et al., 2007; Cruz et al., 2010), but there have been conflicting results concerning the ability of nicotine to induce CPP. A number of studies have reported either an aversion or no effect following nicotine administration. Several factors such as rat strain, the dosage and route of administration, the type of CPP paradigm, and length of training all potentially affect the ability of nicotine to induce CPP. Studies conducted using the hooded strain of rats show no reinforcing or aversive effects to nicotine indicating diminished sensitivity to this drug (Clarke and Fibiger, 1987; Shoaib et al., 1994). Other studies have shown a wide range of nicotine doses capable of producing either preference or aversion (Harvey et al., 2004; Laviolette et al., 2002; Laviolette and van der Kooy, 2003a,b; Torres et al., 2009). Nicotine-induced conditioned place aversion (CPA) was absent at doses lower than 0.1 and higher than 1 mg/kg across several rat strains (Ashby et al., 2002; Dewey et al., 1999; Fudala and Iwamoto, 1986; Fudala et al., 1985; Horan et al., 1997; Jorenby et al., 1990; Papp et al., 2002; Rogers et al., 2004). Further, there is a recent report (Yararbas et al., 2010) indicating nicotine-induced CPP in both male and female sexually mature Sprague–Dawley rats; however, CPP dose-dependency was seen only in male rats (doses: 0.1, 0.2, 0.4, 0.6 mg/kg, s.c.).Taken together these observations suggest that the induction of CPP by nicotine is a complex process and when successful requires a dose at or above 0.1 mg/kg, s.c.

The present study initially determined the stimulus context and lower range of nicotine doses necessary to elicit CPP in adolescent female rats. Our interest in this particular group stems from the observation that human adolescent females appear to show a particular vulnerability to nicotine (Collins and Izenwasser, 2004; Levin et al., 2003). Adolescent females utilize more tobacco products and have greater difficulty stopping nicotine use as compared with age-matched males (Pauly, 2008; Perkins and Scott, 2008; Pogun and Yararbas, 2009). If females begin smoking during adolescence they have greater difficulty quitting as compared with males (Chen and Millar, 1998). We hypothesized that adolescent girls could be susceptible to nicotine addiction because they find it rewarding at a very low dose. If this is so then adolescent female rats would be expected to show CPP to a much lower dose of nicotine than previously tested, and at a lower dose than adolescent male rats.

* Corresponding author at: Department of Psychology, P.O. Box 644820, Washington State University, Pullman, WA 99164-4820, USA. Tel.: +1 509 335 2329; fax: +1 509 335 5043.
E-mail address: wrightjw@wsu.edu (J.W. Wright).

0091-3057/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2011.05.004

2. Materials and methods

All experiments adhered to the Guidelines for the Care and Use of Laboratory Animals as required by the National Institutes of Health (NIH Publication No. 80-23), and the protocols were approved by the Washington State University Institutional Animal Care and Use Committee.

2.1. Animal housing

Sprague–Dawley female and male rats (breeding stock derived from Taconic, Germantown NY) were housed in groups of 4–5 per cage in a temperature/humidity controlled room and adapted to a 12 h light–dark cycle initiated at 0600 h in an American Association for the Accreditation of Laboratory Animal Care approved vivarium at a temperature of 21 ± 1 °C. Animals had free access to Harlan Teklad F6 rodent diet (Madison, WI) and water. All experiments were started when the animals were at postnatal day 28.

2.2. Drug

Nicotine hydrogen tartrate salt was obtained from Sigma-Aldrich (N 5260, St. Louis MO). The salt was dissolved in sterile PBS to obtain concentrations of 0.01, 0.03, 0.08, and 0.1 mg/ml nicotine solution with pH adjusted to 7.4.

2.3. Conditioned place preference protocol

The CPP apparatus consisted of a wooden box 64 (L) × 20.5 (W) × 40 (H) cm with two main compartments (28 × 20.5 cm) separated by a smaller compartment (8 × 20.5 cm). One of the main compartments was painted black and the other white. The black compartment had wire mesh flooring (1.2 cm squares) and the white had parallel metal rods (dia. = 4.8 mm) spaced 1 cm apart. The central compartment had a black wooden floor. A 15 W lamp was placed over the black compartment to compensate for high initial preference. A video camera was placed directly over the apparatus to record the activity of the rat. The camera was connected to a computer which recorded the activity interpreted by video tracking software that provided quantifiable information on locomotor activity, time spent in each compartment, and number of entries into a compartment. A biased paradigm was used in which the animal was assigned to the non-preferred compartment following nicotine administration. This protocol is thought to be more effective at producing CPP than the unbiased procedure in which the animal is randomly assigned to a chamber after nicotine injection (Acquas et al., 1989; Brielmaier et al., 2008; Calcagnetti and Schechter, 1994).

2.3.1. Preconditioning

During preconditioning, each rat was placed in the middle compartment and allowed free access to the entire box for 15 min. The animal was considered to be in a compartment if its forelimbs were inside the compartment. The time spent in each compartment was measured on 2 consecutive days and the mean of the two sessions was calculated for each compartment.

2.3.2. Conditioning

The conditioning phase began the day after preconditioning and at the same time of day for each animal. The animals received two conditioning sessions per day—one with an injection of nicotine (in sterile PBS, 1 ml/kg, at a dose of 0.01, 0.03, 0.08, or 0.1 mg/kg, s.c.) and the other with an injection of PBS (1 ml/kg, s.c.). Following drug or vehicle administration, the animals were confined to the non-preferred or preferred compartment respectively, for 15 min. The order of the drug injection was randomized each day and the sessions were conducted 4 h apart. Members of a control group received saline injections during both daily sessions. The animals underwent CPP acquisition trials for 5 consecutive days.

2.3.3. Post-conditioning

The day following the conditioning phase (day 6) each rat was tested for conditioning in a drug-free state. The rat was placed in the central compartment and allowed free access to both compartments for 15 min. The time spent in each compartment was measured.

2.3.4. Re-exposure

To test for continued drug preference the rats were maintained in their home cages for 5 additional days without drug injection. On the test day (day12) each rat was re-introduced to the CPP apparatus in a drug-free state and times spent in the nicotine-paired and saline-paired compartments were measured.

2.4. Data analysis

The degree of apparatus bias by female rats was evaluated by the use of a paired *t*-test ($p<0.05$) comparing time spent in the dark and white compartments. A paired *t*-test was also used to compare time spent in each compartment once a 15 W lamp was placed over the dark compartment. The data concerned with establishing an effective dose of nicotine were analyzed using a one-way ANOVA followed by Newman–Keuls post-hoc tests ($p<0.05$). The data sets concerned with time spent in the non-preferred compartment (drug associated compartment) following nicotine or saline injection during 1 to 5 days of conditioning trials were analyzed by one-way ANOVAs. And finally, the data set concerned with time spent in the non-preferred compartment following either nicotine or saline injection during a post-conditioning trial and at 2, 5, 11, and 21 days re-exposure using male rats was analyzed by a 2 × 5 repeated measures ANOVA, followed by Newman–Keuls post-hoc tests.

3. Results

3.1. Apparatus bias

Since preference bias introduced by the CPP apparatus can interfere with interpretation of the results, 6 female rats were first tested to determine time spent in each compartment absent of drug (Cunningham et al., 2003; Roma and Riley, 2005). These preconditioning preference trials were initially performed in the absence of a light source over the black compartment. Our results are in agreement with Roma and Riley (2005) in that the animals showed a strong preference for the black compartment ($t_5 = 20.23$, $p<0.001$; Fig. 1A). We then introduced a 15 W lamp above the black compartment and this neutralized the bias (Fig. 1B).

3.2. Effective nicotine dose determination

In order to establish the susceptibility of adolescent female rats to nicotine we first determined the dose range of nicotine that elicited CPP. We employed four groups of female rats (N = 4 per group) with nicotine doses of 0.01, 0.03, 0.08, and 0.1 mg/kg as shown in Fig. 2, and determined that the 0.03 dose elicited robust CPP ($F_{3,12} = 3.95$, $p<0.05$). Both the 0.08 and 0.1 mg/kg doses produced aversion to the chamber in which the drug was administered (post-hoc tests, $p<0.05$). The 0.01 mg/kg dose failed to elicit any behavioral change in the animals.

3.3. Development of CPP for nicotine

Next, the temporal characteristics concerning the acquisition of nicotine preference were determined. We were particularly interested in finding the minimal number of training days required for adolescent