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Single trial nicotine conditioned place preference in pre-adolescent male and female rats

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ABSTRACT

The mean age of first voluntary tobacco inhalation is 12.3 years (DiFranza et al., 2004). 60% of smokers start smoking before the age of 14 and 90% are dependent before reaching the age of 19. Females are typically more sensitive to nicotine than males yet few studies examine the effects of nicotine on the reward systems in pre-adolescent female subjects. This study utilized the single trial conditioned place preference (CPP) test in very young (postnatal day 25–27) rats of both sexes. Latent effects on anxiety and amphetamine response were determined 5 and 7 days following a second nicotine exposure. Results show that 0.05 mg/kg nicotine induced CPP in females following a single trial while both sexes showed CPP following the 0.5 mg/kg dose. Five days later, rats dosed with 0.05 mg/kg show increased time on the open arm of the elevated plus maze, an anxiolytic response. While baseline activity was increased in nicotine-exposed males 7 days following dosing, amphetamine response was not affected by the treatments in either sex. Therefore, our data suggest that young females are more sensitive to nicotine reward than males supporting a heightened sensitivity of the mesolimbic dopamine system in very young females. However, alterations in baseline activity were only seen in males suggesting that different components of the system are affected by nicotine in each sex. An anxiolytic response to nicotine 5 days after dosing may suggest that this very young age group is uniquely affected by this very low nicotine dose. Clearly, nicotine has substantial acute and lasting effects during pre-adolescence at doses substantially lower than seen at older ages as reported by others. These effects, which could potentially result from cigarette or e-cigarette smoking by 11–12 year old children , focus attention on the vulnerability of this age group to nicotine.

1. Introduction

Smoking is the single most preventable cause of death and disease in the US and throughout the world. The costs to our society include over 400,000 lives lost every year in the U.S. — over 1200 each day — and $50 billion annually in lost productivity and increased health care costs (Foundation for Smoke Free America). Sixty percent of smokers start by the age of 14 and 90% of smokers are dependent before reaching the age of 19. Stated another way, only one in ten smokers becomes addicted *after* the age of 19 (FSFA). In a study of 7th graders in Massachusetts, DiFranza et al. (2004) found that the mean age of first voluntary tobacco inhalation was 12.3 years and that 61% of subjects who inhaled had progressed to monthly smoking. Those with the greatest sensitivity to cigarette smoke (i.e. exhibit the greatest degree of relaxation, dizziness, nausea) were more likely to develop nicotine dependence (DiFranza et al., 2004). A myriad of studies have documented the increased sensitivity of adolescents to nicotine, the major psychoactive component of cigarette smoke. For example, rodent studies demonstrate that adolescents are more sensitive to the rewarding effects of nicotine and less sensitive to the aversive effects of nicotine (Doremus-Fitzwater et al., 2010; Shram et al., 2006; Torres et al., 2008, 2009). Adolescents also show greater conditioned place preference (CPP) [a test which measures the association of a location with the rewarding (or aversive) effects of experimenter administered drug] at lower nicotine doses than adults (Torres et al., 2008, 2009; Shram et al., 2006; Belluzzi et al., 2004). Adolescents show increased sensitivity to nicotine and exposure to nicotine during adolescence increases the responses to other psychostimulants (cross-sensitization) (Collins and Izenwasser, 2004). All in all, adolescent exposure to nicotine results in altered maturation of limbic circuitry which in turn increases the vulnerability to nicotine and other addictions, increased impulsivity and mood disorders (Dwyer et al., 2009).

Several studies have investigated whether there were sex-differences in response to nicotine-induced behavior or self-administration. In a

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recent comprehensive analysis of self-administration of nicotine across specific adolescent periods in male and female rats, Levin et al. (2011) found that early exposure (beginning at 4 and 5 weeks of age) resulted in significantly greater administration compared to later initiation and that this effect was more robust in males than females. When nicotine was withheld, on the other hand, females, not males, that initiated self-administration at 4 or 6 weeks of age, administered more than if administration began in adulthood (Levin et al., 2011). Female adolescent rats more readily begin to self-administer nicotine compared to adults and compared to males (Chen et al., 2007). On the other hand, some authors examining CPP which relies on experimenter-administered drug, find no CPP in adult females and robust CPP in adult males following several pairings of a range of nicotine doses in the non-preferred side of the chamber (Yararbas et al., 2010). Others report that adult females show increased CPP to nicotine compared to males but this was not observed in adolescence since adolescent males show more robust CPP than adolescent females (Torres et al., 2008, 2009). However, these studies examine rats in mid adolescence after several exposures to nicotine. Some studies that have examined single trial CPP in early adolescent rats found that a single exposure to nicotine produced CPP in P 28 male rats (Brielmaier et al., 2007, 2012; Belluzzi et al., 2004) although not all studies found this (Pastor et al., 2013). To date, no studies have been published examining single trial CPP in females during the pre-adolescent period.

Since drug–environment interactions are important in relapse, the use of conditioned place preference, a test which relies on the association of a location with interoceptive cues produced by a drug injected in that location, is a suitable test to examine the reward potency of nicotine. The goal of our study was to see if nicotine-induced place preference could potentially be established in pre-adolescent male and female rats after a single injection. Also, since the consensus is that nicotine must be administered on the non-preferred side of the test chamber in order to obtain CPP (Le Foll and Goldberg, 2005), a biased design must be utilized. We also wanted to determine whether brief exposures (2 injections) might alter behavior on the elevated plus maze, baseline locomotor behavior and the behavioral responses to low-dose amphetamine injection during the week following the nicotine exposures.

2. Methods

Subjects were VAF Sprague–Dawley rats (Charles River, Wilmington, MA) and were kept under a 12 h light–dark cycle (lights on at 7:00 h) and temperature of 20–22 °C. All rats had access to food and water ad lib. All procedures were approved by the IACUC in accord with the recommendations of the American Academy of Laboratory Animal Science. Pups (either vivarium reared or shipped) were randomly assigned to one of three nicotine training doses (0, 0.05 and 0.5 mg/kg.)

For vivarium reared subjects, females in proestrus were placed with males of the same strain at 4:00 PM. The next morning, rats were checked for sperm by vaginal smear. If sperm was present, that day was designated as gestational day 1 (G1). Pregnant dams were individually housed in plastic cages with bedding. On the day of birth (usually G23), designated as postnatal day 1 (P 1), all pups were sexed, toe-clipped, and weighed; litters were culled to 10 pups (5 males, 5 females.) At P 21 animals were ear punched and separated into same sex cages containing 2–3 pups.

For shipped subjects, rats were shipped from the vendor in groups of 5 males and 5 females on P 21 and housed in conditions identical to the vivarium reared subjects. Shipped rats were ear punched on P 22.

2.1. Dosing procedure

Nicotine bitartrate (Sigma Chemical Company) was dissolved in saline (weight of freebase) and pH adjusted to 7.4. Intraperitoneal (i.p.) injections occurred immediately prior to being placed in non-preferred chamber.

2.2. CPP method

2.2.1. Equipment

The apparatus consisted of Plexiglas boxes (42 × 42 × 30 cm) with removable opaque center doors. On one side, the walls and lid were black and white striped, and the floor was rough. On the other side, the walls and lid were white, and the floor was smooth. There was approximately the same amount of light on both sides of the testing chamber. However, since most of the rats preferred the rough side of the chamber on the preconditioning day (see Supplemental Fig. 1), the apparatus itself was inherently biased.

2.2.2. Procedure

Subjects were 25 days old at the start of testing. Since we wanted to confine the CPP training and testing to a well-defined window within early adolescence, we utilized the twice daily training procedure as described by Zakharova et al. (2009) and Badanich et al. (2006). On the first day, each rat was placed in the testing chamber with door removed to allow free movement from one side to the other. The 15 minute session was observed for the amount of time that the rat spent on each side of the chamber (in seconds) to determine the side preference for subsequent conditioning days. If two or more paws were on a side, the rat was considered on that side. A biased design was then utilized; i.e., pairing a nicotine dose with the "non-preferred side" and saline with the "preferred side" on day 1. On day 2 (the conditioning phase, run with the center door closed) rats were trained in the morning with saline (1 ml/kg) on one side of the box (initially the preferred side) and in the afternoon with a dose of nicotine on the other side of the box (initially the non-preferred side). Drug and saline sessions were not counterbalanced since we were concerned that lasting effects of nicotine from the morning injection could influence the response to the chamber paired with saline in the afternoon. Training doses were 0 (saline) .05 or .5 mg/kg nicotine free base. Each rat received only a single training dose of nicotine. Each training session lasted 15 min and the AM and PM sessions were separated by 3 h. On the 3rd day, CPP testing occurred around midday. Each rat was injected with saline (1 ml/kg) and randomly placed in the apparatus with the center door open and allowed to freely explore for 15 min. The session was analyzed for the amount of time spent on each side. The dependent measure was calculated by subtracting the time spent on the drug side of the chamber on day 1 (prior to conditioning) from the time spent on that side of the chamber on day 3 (post conditioning). On day 4, the rats underwent the same conditioning procedure as on day 2. On day 5 CPP testing was again conducted the same way as on day 3. The sessions were again analyzed for the amount of time spent on each side.

2.3. Elevated plus maze

On P 33, five days following the last dose of nicotine, all rats were tested on the elevated plus maze for time on the open arm, a measure of anxiety.

2.3.1. Apparatus

The elevated plus maze (EPM) was a black Plexiglas plus-shaped maze with arms 50.8 cm long that was elevated 55.9 cm off the floor. At the intersection of the 4 arms was the center of the maze. Two of the arms of the plus sign were fully enclosed on three sides with black Plexiglas walls 43.2 cm high and open toward the center of the maze. The other two arms were partially enclosed for 22.9 cm from the center with a 6.4 cm high rail. The remaining 27.9 cm of each open arm had no rail. Each arm of the maze was marked with 3 lines which were used to determine entries into the arms as well as overall activity level. An entry into an arm was counted if the animal crossed the first grid line with all four paws, while an entrance out of an arm was counted if the animal crossed the grid line with more than 2/3 of the body. Before each