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Self-administration of nicotine and cigarette smoke extract in adolescent and adult rats

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ABSTRACT

Although smoking initiation typically occurs during adolescence, most preclinical studies of tobacco use involve adult animals. Furthermore, their focus is largely on nicotine alone, even though cigarette smoke contains thousands of constituents. The present study therefore aimed to determine whether aqueous constituents in cigarette smoke affect acquisition of nicotine self-administration during adolescence in rats. Adolescent and adult male rats, aged postnatal day (P) 25 and 85, respectively, were food trained on a fixed ratio 1 (FR1) schedule, then allowed to self-administer one of 5 doses of nicotine (0, 3.75, 7.5, 15, or 30 μg/kg) or aqueous cigarette smoke extract (CSE) with equivalent nicotine content. Three progressively more difficult schedules of reinforcement, FR1, FR2, and FR5, were used. Both adolescent and adult rats acquired self-administration of nicotine and CSE. Nicotine and CSE similarly increased non-reinforced responding in adolescents, leading to enhanced overall drug intake as compared to adults. When data were corrected for age-dependent alterations in non-reinforced responding, adolescents responded more for low doses of nicotine and CSE than adults at the FR1 reinforcement schedule. No differences in adolescent responding for the two drugs were seen at this schedule, whereas adults had fewer responses for CSE than for nicotine. However, when the reinforcement schedule was increased to FR5, animals dose-dependently self-administered both nicotine and CSE, but no drug or age differences were observed. These data suggest that non-nicotine tobacco smoke constituents do not influence the reinforcing effect of nicotine in adolescents.

1. Introduction

Tobacco use is the leading preventable cause of death worldwide, killing more than 6 million people a year (World Health Organization, 2015). In the United States, 1 of every 5 deaths is attributed to cigarette smoking (Center for Disease Control, 2014). Smoking is an adolescent-onset disorder, with almost 90% of smokers trying their first cigarette by the age of 18 (Center for Disease Control, 2014). Although current rates of conventional cigarette use have markedly declined, the use of electronic nicotine delivery systems (e-cigarettes) among school-age children has tripled in the last year (Arrazola, 2015). E-cigarettes, which are marketed as safer alternatives and smoking cessation aids, may actually increase the likelihood of continuing and increasing tobacco use among adolescents (Dutra and Glantz, 2014).

Adolescence is characterized as a period of development when individuals demonstrate risk-taking and novelty seeking behaviors (Spear, 2000). Both clinical (Chen and Millar, 1998; Everett et al., 1999) and preclinical (Belluzzi et al., 2004; Brielmaier et al., 2008; Vastola et al., 2002) studies have found adolescents to be more sensitive to the rewarding properties of nicotine. Adolescent rats have been shown to acquire nicotine self-administration more readily, and to take more nicotine, than adults (Chen et al., 2007; Levin et al., 2007, 2003). In conditioned place preference, rats in early adolescence display enhanced sensitivity to the rewarding effects (Belluzzi et al., 2004; Brielmaier et al., 2008; Vastola et al., 2002), and reduced sensitivity to the aversive effects of nicotine (Shram et al., 2006; Torres et al., 2008; Wilmouth and Spear, 2004).

Cigarette smoke contains more than 7,000 constituents; hundreds of which are harmful, and about 60 are known to cause cancer (National Toxicology Program, 2014). However, animal models of tobacco dependence have traditionally examined only the effects of nicotine (Donny et al., 1995), the main psychoactive

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component of tobacco (Stolerman and Jarvis, 1995). Some studies have begun to look at the non-nicotine constituents found in cigarette smoke to understand how they may affect nicotine self-administration. Biologically active components such as monoamine oxidase inhibitors have been shown to increase nicotine self-administration (Arnold et al., 2014; Guillem et al., 2005; Villégier et al., 2007, 2006). Acetaldehyde, a combustion product of tobacco, also enhances nicotine self-administration in adolescent, but not adult, rats (Belluzzi et al., 2005). Although these findings show that single constituents interact with nicotine, they exclude most tobacco smoke constituents and ignore the possible interactions that may occur between them. In order to study these interactions, we have created a model in which the behavioral effects of aqueous cigarette smoke extract (CSE) are examined. Previous work by our group has shown that CSE is more potent than nicotine alone in adult male rats during the acquisition and maintenance phases of self-administration, and yields sensitized reinstatement to stressors (Costello et al., 2014).

Using a modified method from Costello et al. (2014), in order to assess the influence of age, we have now compared the acquisition of self-administration of nicotine or CSE at varying doses in adolescent and adult male rats. Since initiation of smoking typically occurs during adolescence, it is important to study this period of development in animal models of tobacco dependence.

2. Materials and methods

2.1. Drugs

Nicotine hydrogen tartrate (Sigma, St Louis, MO) was dissolved in sterile saline and adjusted to pH 7.2–7.4. All nicotine doses were calculated as free base. CSE was created by bubbling smoke from commercial cigarettes (Camel unfiltered, RJ Reynolds) through sterile saline, using a method described in Costello et al., 2014. Briefly, eight cigarettes were smoked through 35 ml of saline solution (35 ml puffs over 2 s, repeated every 30 s) and the final solution was adjusted to pH 7.2–7.4. The CSE solution was prepared fresh each day immediately before experimental testing in order to minimize differences resulting from differential stability of the constituents. All CSE doses were defined by the solutions nicotine content, which was analyzed by an outside facility (UCSF Clinical Pharmacology Laboratory).

2.2. Subjects

Male Sprague–Dawley rats were obtained from Charles River at postnatal (P) days 17 and 81. Adolescent rats remained with dam until weaning (P21). Animals, both adolescents and adults, were group-housed throughout the experiment. All rats were maintained on a 12-h light/dark cycle (lights on at 07:00 a.m.) with food and water available *ad libitum*. No more than one animal per litter per experimental group was used to avoid potential confounds. All experimental procedures were in compliance with NIH guidelines and were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

Rats were minimally food-restricted beginning two days prior to operant conditioning to promote exploration of the operant chamber and aid in acquisition of the operant task. Adolescent and adult rats were fed 15–25 or 20–25 g of food, respectively, to maintain normal growth during self-administration testing. Food was given 15 min after each experimental session, and any remaining chow was removed an hour before the following day test session. Food maintenance continued until the end of the experiment. Growth curves for both adolescents and adults followed normal trajectories (data not shown).

2.3. Behavioral studies

2.3.1. Apparatus

Animals were tested in plexiglass operant chambers (Med Associates, St Albans, VT), equipped with two levers. Responses at the reinforced (R) lever resulted in illumination of a cue light over the lever and activation of an externally mounted syringe pump that infused drug. During the infusion (5.6 s yielding 100 μl of solution) and timeout period (20 s) the cue light remained illuminated and the house light was turned off. Responses on the non-reinforced (NR) lever were recorded but had no consequences.

2.3.2. Food training

Adolescent and adult rats, aged P25 and 85, respectively, were first trained to lever-press for food pellets (45 mg rodent purified diet; Bio-Serv, Frenchtown, NJ) under a fixed ratio 1 schedule with a 1 s timeout period (FR1TO1), followed by FR1TO10, and completed with FR1TO20. Rats progressed to the next timeout period when they earned at least 35 or 50 reinforcers (adolescents and adults, respectively) in the daily 30-min session.

2.3.3. Surgery

Following successful acquisition of food responding, rats were anesthetized with equithesin (0.0035 ml/g body weight) and implanted with indwelling jugular vein catheters (Belluzzi et al., 2005). During the 3-day recovery period, catheters were flushed daily with a heparinized saline solution to maintain patency. The day before initiation of self-administration, and at intervals thereafter, catheter patency was verified for rapid (5–10 s) anesthesia by infusing propofol (5 mg/kg, i.v.). Patency was tested at the end of each schedule and only animals showing rapid anesthesia were included in analyses.

2.3.4. Self-administration

After recovery, adolescents and adults, aged P37 and 97, respectively, were allowed to self-administer a single dose of nicotine or CSE (0, 3.75, 7.5, 15, or 30 μg/kg/infusion nicotine content). Rats self-administered nicotine or CSE for 7 days at the FR1TO20 schedule, before transitioning to the FR2TO20 schedule for 2 days, and finishing with 3 days at the FR5TO20 schedule during daily 1-h sessions.

2.4. Statistical analyses

The average of the last 3 days of self-administration at the FR1 schedule (Day 5–7) and the FR5 schedule (Day 10–12) were analyzed separately with a four-way ANOVA on Age x Drug x Dose x Lever with repeated measures on Lever. Any significant main effects or interactions were further analyzed by three- or two-way ANOVAs with Dunnett's, Bonferroni-corrected paired (levers) or unpaired (drug) *t*-test post hoc comparisons. Drug intake, calculated as the number of infusions per session multiplied by the dose of drug self-administered, was analyzed with a three-way ANOVA on Age x Drug x Dose. Any significant main effects were further analyzed by two-way ANOVAs with Bonferroni-corrected unpaired *t*-test post hoc comparisons. Non-reinforced (NR) responding data was analyzed with a three-way ANOVA on Age x Drug x Dose. Any significant main effects were further analyzed by a two-way ANOVA with Dunnett's or Bonferroni-corrected unpaired *t*-test post hoc comparisons. Corrected reinforced responding data was analyzed with a three-way ANOVA on Age x Drug x Dose. Any significant main effects were further analyzed by two-way ANOVAs with Bonferroni-corrected unpaired *t*-test post hoc comparisons.