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Research report

Repeated nicotine exposure during adolescence alters reward-related learning in male and female rats

Stacey L. Quick[1], Peter Olausson[1], Nii A. Addy, Jane R. Taylor*

Department of Psychiatry Laboratory of Molecular Psychiatry, Yale University Ribicoff Research Facilities CMHC, 34 Park St New Haven, CT 06519, United States

HIGHLIGHTS

- Repeated nicotine exposure during adolescence enhances reward-associated learning in males.
- Repeated nicotine exposure during adolescence reduces reward-associated learning in females.
- Female animals display enhanced acquisition of appetitive Pavlovian approach learning at baseline.
- Repeated nicotine exposure during adolescence augments responding with conditioned reinforement.
- The ability of nicotine to enhance responding with conditioned reinforcement is not influenced by sex.

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ABSTRACT

Rationale: Repeated nicotine exposure causes neuroadaptations in limbic cortico-striatal circuits involved in learning and motivation. Such alterations are relevant to addiction because they are suggested to mediate the ability of smoking-associated stimuli to control behavior and to enhance nicotine-seeking and -taking behaviors. Female smokers report higher cue reactivity relative to their male counter parts, yet little is known about putative gender-specific effects of adolescent nicotine exposure on reward-related learning. Prior repeated nicotine exposure in adult male rats enhances Pavlovian approach behavior and conditioned reinforcement.

Objective: Given that smoking is typically initiated during adolescence, here we assessed the extent to which adolescent nicotine exposure impacts Pavlovian approach and conditioned reinforcement in male and female rats.

Methods: Rats were injected with nicotine on postnatal days 31–45 prior to training on Pavlovian approach behavior starting on day 51. They were trained to associate a conditioned stimulus (CS), illumination of a magazine light, and tone, with an unconditioned stimulus (US), the delivery of water, for 10-daily sessions, and then were tested on the acquisition of responding with conditioned reinforcement.

Results: Adolescent nicotine exposure selectively increased approach to the magazine during the CS in males but decreased approach to the magazine during the CS in female rats. Vehicle-exposed female rats, however, showed greater magazine approach during the CS than did male control rats. Prior nicotine exposure also enhanced conditioned reinforcement in both male and female rats.

Conclusions: Repeated exposure to nicotine during adolescence had opposite effects on Pavlovian approach behavior in male and female rats but enhanced acquisition of a new response with conditioned reinforcement. Novel information on how nicotine exposure influences reward-related learning during adolescence may increase our understanding of neurobiological mechanisms involved in the initiation of smoking behavior.

1. Introduction

Reward-related learning plays an important role in drug addiction because stimuli and events associated with drugs can come to support and elicit drug-seeking and -taking behavior [1]. The external and sensory cues associated with tobacco regulate smoking behavior in multiple ways, and indeed, compulsive drug use is commonly associated with cue-dependent drug-seeking and–taking behaviors [2,3]. In human smokers, nicotine-associated cues can elicit craving [4–7], and region-specific activation of limbic cortico-striatal regions [4–7]. In animals, drug-associated stimuli support nicotine self-administration [3,8], produce reinstatement of drug-seeking [9–11], and elicit conditioned responses, such as approach

* Corresponding author. Tel.: +1 203 974 7727; fax: +1 203 974 7897.
E-mail addresses: jane.taylor@yale.edu, staceylquick@gmail.com (J.R. Taylor).
[1] Co-first authors.

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to a location associated with delivery of an unconditioned stimulus (US) following onset of a conditioned stimulus (CS) [12,13], which parallels aspects of smoking behaviors in humans [8]. Recent work also suggests that pharmacological treatments, such as Olanzapine, a dopamine, and serotonin antagonist, may exert therapeutic actions by reducing both cue-elicited craving and the neurobiological responses to smoking cues [14] Therefore, it is necessary to further investigate the mechanisms by which nicotine impacts reactivity to cues.

Reward-associated stimuli acquire their reinforcing properties through Pavlovian learning. The acquisition of appetitive Pavlovian approach behavior reflects the ability of a neutral stimulus to gain salience by virtue of its association with a reinforcer. Prior repeated exposure to nicotine or psychostimulants in adult rats facilitates the subsequent acquisition of cue-elicited Pavlovian approach behavior [15–18] effects attributed to persistent alterations in neural systems involved in incentive learning, and behavioral control [19–23]. Nicotine has been shown to establish and enhance the incentive motivational properties of other reinforcers, including reward-associated cues [3,18,24,25]. Significantly, responding with conditioned reinforcement is potently enhanced following prior repeated nicotine exposure [25], as are other psychostimulants given acutely or chronically [26–28]. Furthermore, enhancement of reward occurs following repeated exposure to nicotine and not in response to acute exposure to nicotine alone [29]. Together these observations argue that nicotine, like other drugs, can facilitate the incentive salience of reward-associated stimuli and that nicotine-induced alterations in cue-elicited behaviors and incentive motivational processes may be relevant to clinical aspects of smoking.

Importantly, the emotional and neuronal responses to smoking cues may differ in males and females. For example, female smokers experienced greater craving in response to nicotine cues than male smokers [30] and gender differences in cue reactivity have been correlated with differential activation of craving- and reward-related regions of the brain [31]. Sex differences in the ability of nicotine-paired stimuli to enhance instrumental behavior also have been reported in rats. Female rats respond more for nicotine infusions when the infusion is accompanied by a visual stimulus than male rats [32]. This difference does not appear to be dependent on baseline levels of nicotine self-administration or cue-induced reinstatement [11]. Thus, it is necessary to further investigate sex-differences in nicotine modulated cue reactivity.

The initiation of smoking and other compulsive forms of drug use typically occurs during adolescence. Individuals that initiate smoking during adolescence have a high probability of developing a pattern of regular smoking a in adulthood [33]. Indeed, adolescence has been argued to be a predisposing factor for addiction [34]. Here, we examined the impact of daily nicotine administration for 15 days during adolescence (postnatal days 31–45) on cue reactivity measured by Pavlovian discriminative approach behavior and conditioned reinforcement.

We hypothesized that the behavioral consequences of adolescent nicotine exposure would be similar to our studies with adult-exposed animals but that this exposure would enhance cue reactivity to a greater extent in female rats than in male rats.

2. Materials and methods

2.1. Animals

Male and female Sprague-Dawley rats ($n = 40$; male $n = 20$, female $n = 20$), aged 31 days at the start of the experiments, were supplied by Charles River (Portage, ME, motivation USA). The rats were housed in pairs under constant cage temperature (20–21 °C), humidity (40–50%) and a controlled 12/12 h light-dark cycle (light on at 7 a.m. and off at 7 p.m.) and were initially allowed 7 days to adjust to the housing facilities. The rats had free access to food at all times. Water was available *ad libitum* until three days prior to the first day of training, and immediately after the 15 days training phase was completed. During the three days prior to the start of training, animals were restricted to 30 min access to water per day. During the testing period, water was intermittently available in the operant chambers according to the behavioral task protocol (see below) as well as in the home cage for 30 min, beginning 30 min after the daily testing session. The experiments in the present study were approved by the Yale University Animal Care and Use Committee and followed the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

(-)-Nicotine ditartrate (Sigma, USA) was dissolved in a sterile 0.9% sodium chloride solution, and the pH of the nicotine solution was neutralized with sodium bicarbonate. Nicotine was injected subcutaneously (s.c.) at 2 ml/kg. The dose of nicotine is expressed as the weight of the free-base of nicotine.

2.3. Experimental techniques

2.3.1. Locomotor activity

Locomotor activity was measured using automated activity meters (Digiscan Animal Activity Monitor, Omnitech Electronics, USA). The activity meters were equipped with two parallel rows of infrared photosensors, each row consisting of 16 sensors placed 2.5 cm apart. The activity meters were controlled by and data from the activity meters collected by a PC using the Micropro software (Omnitech Electronics, USA).

Rats were placed in transparent plastic boxes that were fitted into the activity meters. The rats were initially allowed to habituate to the locomotor activity recording equipment for 30 min, after which they were taken out, injected with nicotine or vehicle, and placed back into the boxes. Locomotor activity was then recorded for 60 min starting 5 min after drug injection. All experiments were performed between 8 a.m. and 6 p.m.

2.3.2. Pavlovian discriminative approach behavior

Standard aluminum operant chambers with grid floors (MedAssociates Inc., USA) were used to study the acquisition of Pavlovian discriminative approach behavior and responding with conditioned reinforcement. Each operant chamber was housed in a sound attenuating outer box equipped with a white noise generator and a fan to reduce external noise. A liquid dipper (0.06 ml) delivered water as the reinforcer into the magazine. Head entries were detected by a photocell mounted within the magazine, above the reinforcer receptacle. Above the magazine was a 2.5 W, 24 V light. The operant chamber was illuminated by house light mounted on the back wall. A Sonalert tone (10 kHz) generator was mounted above the magazine. A PC with interface and the MedPC software (MedAssociates Inc., USA) controlled the boxes.

On the first day, rats were familiarized with water availability (the unconditioned stimulus [US]). Water dippers (0.06 ml) were presented for 5 s on a fixed time 15 s (FT-15) schedule and the session ended after the delivery of 100 USs. Pavlovian discrimination training sessions began on the second day. Rats received 30 pairings of a 5 s compound conditioned stimulus (CS; light + tone) followed immediately by 5 s access to 0.06 ml of water. The CS + US pairings were delivered on a random time 30 s (RT-30) schedule. Head entries during the RT-30 interval resulted in a 3 s delay during which time no reinforcement was given, and the RT-30 schedule was restarted. Training on this schedule results in a discriminated