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Nicotine Withdrawal in Adolescent and Adult Rats

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ABSTRACT: Previous research with animal models has demonstrated that adolescent rats display heightened sensitivity to the reinforcing and stimulant effects of nicotine relative to adult rats. Little work has focused on the response of adolescent rats to measures of nicotine withdrawal. To test the hypothesis that adolescent rats may be differentially sensitive to withdrawal relative to their adult counterparts, the present study was designed to compare precipitated withdrawal in adolescent and adult rats following chronic nicotine administration. Adult and adolescent rats were prepared with subcutaneous osmotic minipumps that delivered either saline or nicotine (9 mg/kg per day, salt; *N* =12 per group). All rats were challenged with the nicotinic receptor antagonist mecamylamine (1.5 mg/kg) on day 7 of chronic nicotine treatment. Twenty minutes after the injection, overt somatic signs of withdrawal (i.e., eye blinks, writhes, body shakes, teeth chatter, gasps, and ptosis) were recorded for 10 min. Adult rats were observed on postnatal day 73–77, and adolescent rats were tested on postnatal day 36–40. The results revealed a robust increase in mecamylamine-induced withdrawal signs in adult rats receiving chronic nicotine relative to adult rats receiving saline. In contrast, mecamylamine did not precipitate withdrawal signs in adolescent rats receiving chronic nicotine. These results indicate that there is decreased sensitivity to the somatic aspects of nicotine withdrawal in adolescent rats that may maximize the reinforcing effects of nicotine during adolescence by minimizing the aversive effects of abstinence.

KEYWORDS: adolescence; adult; rats; nicotine; withdrawal; mecamylamine

INTRODUCTION

Chronic nicotine use produces a withdrawal syndrome in humans that is of motivational significance in maintaining nicotine dependence. The syndrome is comprised of "physical" or somatic and affective components. The most common somatic symptoms include bradycardia, gastrointestinal discomfort, increased appe-

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tite, and the affective symptoms primarily include craving, depression, irritability, anxiety, difficulty concentrating, and restlessness. It is well documented that these negative effects contribute to relapse.[1,2] A better understanding of the neurobiological mechanisms that contribute to nicotine withdrawal may lead to pharmacological treatments that alleviate withdrawal, and thus, facilitate abstinence.

Chronic nicotine administration in rats produces overt somatic symptoms of withdrawal that have been widely characterized in several laboratories.[3–6] Somatic signs of nicotine withdrawal in rats include abdominal constrictions, facial fasciculation, eye blinks, and ptosis. The emergence of the nicotine withdrawal syndrome in rats is observed after the cessation of nicotine administration (spontaneous withdrawal) or nicotinic receptor antagonist administration (precipitated withdrawal). Interestingly, the somatic signs of the nicotine withdrawal syndrome resemble those seen in opiate withdrawal, and can also be precipitated in nicotine-dependent rats using opiate antagonists as reported by Malin *et al.*[7] (however, see Watkins[8]). Support for the predictive validity of this animal model of nicotine withdrawal is provided by the finding that both the somatic and affective aspects of nicotine withdrawal are reversed by bupropion,[9] a compound that displays clinical efficacy in the treatment of nicotine dependence.[10] In addition, while the somatic signs of withdrawal are not a direct measure of changes in the brain motivational systems that drive nicotine dependence, they are sensitive to many of the same neuropharmacological agents as the affective aspects of withdrawal.[9–11] As such, somatic withdrawal syndrome provides an important index for exploring mechanisms associated with the development of dependence.

Nicotine is one of the most widely used drugs during adolescence. Although it has been argued that nicotine dependence in humans develops only after years of heavy daily smoking, recent clinical studies have observed high levels of nicotine dependence in adolescent smokers despite low cigarette exposure.[12,13] There are several factors that lead to adolescent smoking, including, but not limited to, increased risk taking, novelty seeking, and emphasis on peer interactions.[14] However, the mechanisms that lead to nicotine dependence in adolescent smokers are presently unclear.

A rat model of somatic signs of nicotine withdrawal can be used to compare physiological and behavioral differences at various stages of mammalian development. Most behavioral and physiological systems reach maximal maturation by postnatal day (PND) 60 in rats. There is general agreement that the prototypic age range for adolescence in rats conservatively ranges from PND 28–42.[14] This age range reflects a period during which age-specific behavioral discontinuities from younger and older animals are most evident.

The goal of the present study was to compare nicotine withdrawal in adult and adolescent rats. We used a model of precipitated nicotine withdrawal based on previous observations that reliable and high levels of overt somatic signs of withdrawal are observed after nicotinic receptor antagonist administration in adult rats treated chronically with nicotine. Adolescent and adult rats were challenged with the nicotinic receptor antagonist mecamylamine after 7 days of nicotine or saline administration via subcutaneous osmotic minipumps. Overt somatic signs of nicotine withdrawal were examined 20 min later during a 10-min observation period.

MATERIALS AND METHODS

Animals

Male Wistar rats (Charles River) were housed in a humidity- and temperature-controlled (22°C) vivarium on a 12-h light/dark cycle (lights off at 8 AM). This experiment was conducted in two replications with 6 animals per group for each replication (final N=12 per group). The adolescent rats arrived in the laboratory on PND 23 or 26, and the adult rats arrived on PND 60 or 64. The animals were group housed with 3–4 rats of the same developmental age group per cage. After arrival in the laboratory, the rats were handled and weighed each day during the 6-day acclimation period. Animals had *ad libitum* access to food and water throughout the course of the study except during the 10-min observation periods. All procedures were conducted in strict adherence to the *National Institutes of Health Guide for the Care and Use of Laboratory Animals.* Animal facilities and experimental protocols were in accordance with the Association for the Assessment and Accreditation of Laboratory Animal Care, and were approved by the Institute's Animal Welfare Committee.

Drugs

Nicotine tartrate and the noncompetitive nicotinic receptor antagonist mecamylamine hydrochloride were used (both purchased from Research Biochemicals International, Natick, MA). Drug doses refer to the salt form of the compound with the base in parentheses. All intraperitoneal mecamylamine injections were administered in a volume of 1 ml/kg according to body weight.

Surgical Preparation with Subcutaneous Osmotic Minipumps

Rats were anesthetized with an isoflurane/oxygen mixture (1–2% isoflurane) and prepared with Alzet osmotic minipumps (model 2ML2, 14-day; Durect Corporation, Palo Alto, CA) placed subcutaneously. The adolescent rats were surgically prepared with minipumps on PND 29 or 32, and the adults were prepared on PND 66 or 70. The minipumps were placed on the back of the animal parallel to the spine. The pumps were filled with either physiological saline or nicotine tartrate solution. The concentration of the nicotine tartrate salt solution was adjusted according to animal weight, resulting in ~9 mg/kg per day (3.16 mg/kg per day free base). There were four groups with 12 rats in each group (adolescent nicotine, adolescent saline, adult nicotine, and adult saline). The surgical wound was closed with 9-mm stainless steel wound clips (Beckton Dickson Primary Care Diagnostics, Sparks, MD) and treated with an antibiotic ointment.

Ratings of Mecamylamine-Induced Somatic Signs

The animals were tested for mecamylamine-induced precipitated withdrawal 7 days after minipump preparation. Subjects were habituated to the observation room and containers 3 times for 10 min during 3 days prior to the antagonist administration. The observation containers consisted of clear plastic cylindrical containers (30 × 29 cm) in which the animal could move freely. The adolescent rats were tested on PND 36 or 40, and the adult rats were tested on PND 73 or 77. On the test day, each

rat received an injection of mecamylamine (1.5 mg/kg, ip) and was placed in the observation container for 20 min. After 20 min elapsed, the frequency of precipitated signs of nicotine withdrawal was recorded during the subsequent 10-min observation period. The signs recorded were eye blinks, body shakes, chews, cheek tremors, escape attempts, foot licks, gasps, writhes, headshakes, ptosis, teeth chattering, and yawns. These signs were derived from a standard checklist of opiate withdrawal signs developed by Malin *et al.*[5] and used widely in our laboratory.[4,8] Multiple successive counts of any sign required a distinct pause between episodes. If ptosis was present continuously, then it was only counted once per minute. The total number of somatic signs per 10-min observation period was defined as the sum of the number of individual occurrences of the above-mentioned withdrawal signs. One observer was used that was unaware of whether the animal was treated with nicotine or saline, and reliable measures were observed between the two replication studies.

Statistical Analyses

A two-way analysis of variance (ANOVA) was performed on the total number of withdrawal signs with group (adolescent versus adult) and treatment (saline versus nicotine) as between subjects factors. Subsequent post hoc analyses were performed using the Fisher's PLSD Test. The total number of eye blinks was also analyzed separately to verify the pattern of results observed with the overall number of somatic signs measures.

RESULTS

Mecamylamine administration produced a significant increase in overt somatic signs of withdrawal in adult rats treated with nicotine relative to all other groups (see FIG. 1). A significant age X treatment interaction effect [F (1,44) = 11.1, $P < .002$] was observed, with adult rats receiving nicotine displaying significantly more precipitated signs relative to all other groups (Fisher's Test, $P < .001$). After mecamylamine administration, there was no difference in the signs exhibited by adolescent rats treated chronically with nicotine or saline. A significant age X treatment interaction effect also was observed with the analyses on eye blinks [F(1,44) = 6.1, $P < .02$], with adult rats receiving nicotine displaying significantly more eye blinks relative to all other groups (Fisher's Test, $P < .001$).

DISCUSSION

This study is consistent with previous work demonstrating that somatic signs of nicotine withdrawal are precipitated in adult rats chronically treated with nicotine when injected with a nicotinic receptor antagonist.[3,6,15,16] The present study extends these previous findings by demonstrating that a nicotinic receptor antagonist does not precipitate somatic signs of nicotine withdrawal in adolescent rats chronically treated with nicotine using the same nicotine and antagonist doses and duration of exposure to nicotine used in previous studies with adult rats. This conclusion is based on both the measure of total signs of withdrawal, and on the eye blink measure.

Previous developmental studies in rats have found that adolescent rats are more sensitive to the behavioral effects of nicotine. For example, adolescent rats exhibit nearly twice as much nicotine intake in self-administration procedures relative to their adult counterparts.[17] Adolescent rats also display place preference at a low dose of nicotine that is ineffective in adult rats.[18] Enhanced sensitivity to nicotine in adolescent rats has also been observed using behavioral measures of activity.[19,20]

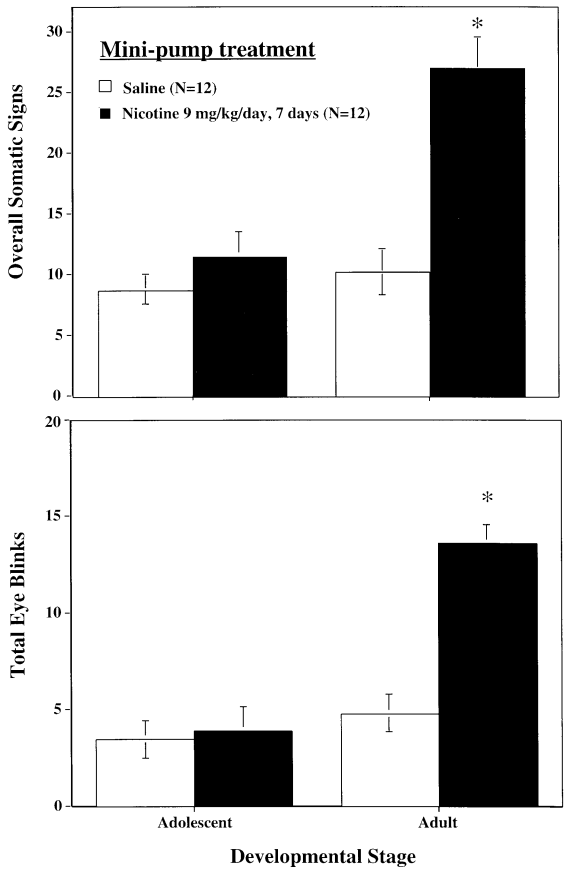

FIGURE 1. Mean total (±SEM) somatic signs of precipitated nicotine withdrawal (**top panel**) and eye blinks (**bottom panel**) in adolescent and adult rats receiving nicotine (*solid bars*) or saline (*open bars*) via subcutaneous osmotic minipumps for 7 days. All rats received mecamylamine (1.5 mg/kg, ip), and 20 min later overt somatic signs of nicotine withdrawal were recorded during a 10-min observation period. Mecamylamine precipitated robust somatic signs of nicotine withdrawal in adult rats; however, this effect was not observed in adolescent rats. The eye blinks measurement confirmed the pattern of results observed with total somatic signs of nicotine withdrawal. *Asterisks* (*) indicate a significant difference relative to all other groups ($P < .001$).

Nicotine exposure during adolescence also appears to lead to enhanced anxiolytic effects, as observed in the open field test,[21] and there is evidence that the adolescent brain is particularly sensitive to nicotine-induced alterations in cell signaling and synaptic function.[22,23] Collectively, these studies demonstrate that adolescence represents a period of enhanced sensitivity to the reinforcing and stimulant effects of nicotine.

The findings from this study demonstrate that adolescent rats do not exhibit overt somatic signs of nicotine withdrawal, at least under the conditions described. Based on the existing literature that adolescent rats appear to be more sensitive to the reinforcing and stimulant effects of nicotine and the present findings, it appears that adolescent rats are more sensitive to the positive effects of nicotine exposure and less sensitive to the negative effects of nicotine withdrawal. Support for this notion is also provided by the results from alcohol studies demonstrating that adolescent rats display place preference for doses of alcohol that are ineffective or aversive in older rats.[24] Additionally, adolescent rats display reduced sensitivity to the motor-impairing and sedative consequences of alcohol.[25] Dampened sensitivity to the negative consequences of drug use may contribute to heightened drug intake, a notion supported by the finding that alcohol-preferring rats are less sensitive to the sedative–hypnotic effects of alcohol and develop tolerance more quickly to high-dose alcohol compared to nonpreferring animals.[26]

There are well-documented changes in nicotinic receptors that are believed to contribute to precipitated withdrawal following chronic nicotine administration in adult rats (see review articles[27,28]). Developmental differences in nicotinic receptors, drug metabolism, and metabolic tolerance might contribute to the decreased nicotine withdrawal signs exhibited by adolescent rats compared to adult rats. It does not appear that a simple hypothesis of decreased nicotinic-receptor function explains our findings, because adolescent nicotine exposure elicits lasting alterations in synaptic signaling that intensify and persist during withdrawal.[22] Decreased nicotinic-receptor binding has been observed over the course of ontogeny in the rat.[29] Enhanced metabolism of nicotine may also contribute to the lack of withdrawal following chronic nicotine in adolescent rats, a notion supported by the finding that alcohol metabolism and tolerance are heightened in adolescent rats.[14] Future studies will be needed to assess whether changes in specific subtypes of nicotinic receptors and/or function contribute to the developmental effect observed in the present study.

Clinical studies have found that adolescents from age 12–18 years display a number of behaviors that may contribute to drug-seeking behavior at an early age. For example, adolescents display heightened emphasis on peer interactions, novelty seeking, and risk-taking behavior.[14] Although these behaviors can be seen as adaptive for establishing independence from the family unit, they often translate into experimentation with drugs of abuse. The present study suggests that the negative effects of withdrawal are less evident during adolescence. This finding in rats is supported widely by clinical and anecdotal evidence that negative effects of drugs of abuse, such as "hangover," are less prominent during adolescence. Taken together, the observation of enhanced sensitivity to the reinforcing effects of nicotine and resistance to the negative effects of nicotine withdrawal suggest that both of these factors contribute to the development of tobacco-smoking behavior in adolescents.

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