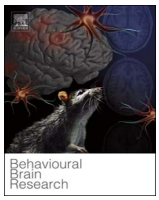
Behavioural Brain Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Behavioural Brain Research

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



Original research article

Alteration of adolescent aversive nicotine response and anxiety-like behavior in nicotine-exposed rats during late lactation period

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ARTICLE INFO

Keywords:
Psychostimulant
Rodent
Mice
Reward
Substance abuse
Conditioned preference

ABSTRACT

Early nicotine exposure is an important cause of further habitual tobacco smoking. Although nicotine has not only rewarding but also aversive properties, the effects of early nicotine exposure on the distinct properties of nicotine are not well known. To reveal the effects of early adolescent nicotine exposure on further persistent tobacco smoking, we demonstrated developmental changes in nicotine-related appetitive and aversive behaviors of rats exposed to nicotine during the late lactation period. Sprague–Dawley rats were injected with saline or nicotine (2, 6 and 12 mg/kg). We performed a two bottle free-choice test using escalating doses of nicotine (25, 50 and 100 μg/ml), saccharin and quinine and the open field test in both adolescent and adult rats. The rats' aversive response to nicotine was increased according to the increase in nicotine concentration. Adolescent rats showed higher nicotine preference and consumption behaviors than did adult rats at an aversive dose of nicotine. Nicotine-exposed rats increased adolescent nicotine consumption when the nicotine concentration was 12 mg/kg. We observed significant increases in anxious behaviors in adolescent nicotine-injected rats compared to saline-injected rats, but there were no alterations in adult rats. In both adolescent and adult rats, saccharin and quinine intake were not significantly different between groups. Taken together, it suggests that repeated nicotine exposure in late lactation period affect changes in aversive nicotine responses and anxious behaviors during adolescence but there is no difference in adults.

1. Introduction

Nicotine is a globally pervasive addicting substance in tobacco smoke that makes a heavy smoker into a compulsive nicotine-seeking addict [1]. Although the rewarding properties of nicotine are known to be weaker than those of other psychostimulants and diverse nicotine replacement therapies are used by smokers in an attempt to stop smoking, most smokers who decide to stop smoking fail to do so and tobacco use causes nearly 6 million deaths annually [2–4]. Moreover, extensive scientific knowledge emphasizes that parents creating smoking/non-smoking environments affect the future smoking habits of their offspring, though considerable numbers of child-bearing women continue to smoke throughout their pregnancies and when breast-feeding [5–10].

Early adolescence is important to rats as it is not only the critical period during which they begin to hear and open their eyes with explosive changes in brain structure, but also the peak period of expression of diverse types of nicotinic acetylcholine receptors, which then decline in adults [11,12]. In the period of transiently heightened plasticity during adolescent brain development, cholinergic regulation plays a crucial role and can be shaped by external environmental stimuli such as nicotine [13]. For instance, nicotine exposure in the prenatal, postnatal and adolescent periods can cause abnormal changes in cellular differentiation and proliferation [13,14]. An abundant amount of previous evidence has shown that developmental nicotine exposure can cause irrevocable brain and behavioral changes in [13,15–17].

Nicotine has both rewarding and noxious properties in that it not only stimulates a reward system but also leads to an unpleasant response called "nicotine aversion" [18–22]. While the strong aversive response to the noxious properties of initial nicotine exposure reduces the incidence of continued smoking, a weak response to nicotine increases the incidence of continued smoking [23]. For these reasons, changes in nicotine aversion might play a crucial role in nicotine addiction. However, although distinct roles of appetitive and aversive response to nicotine are important, experimental methods that separate appetitive and aversive responses to nicotine in animals are not well developed.

Adolescent rats in previous studies exhibited enhanced nicotine self-administration as compared with adult rats [24–26]. However, few studies have attempted to bridge the gap between the developmental

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http://dx.doi.org/10.1016/j.bbr.2017.09.034
Received 7 June 2017; Received in revised form 15 September 2017; Accepted 20 September 2017

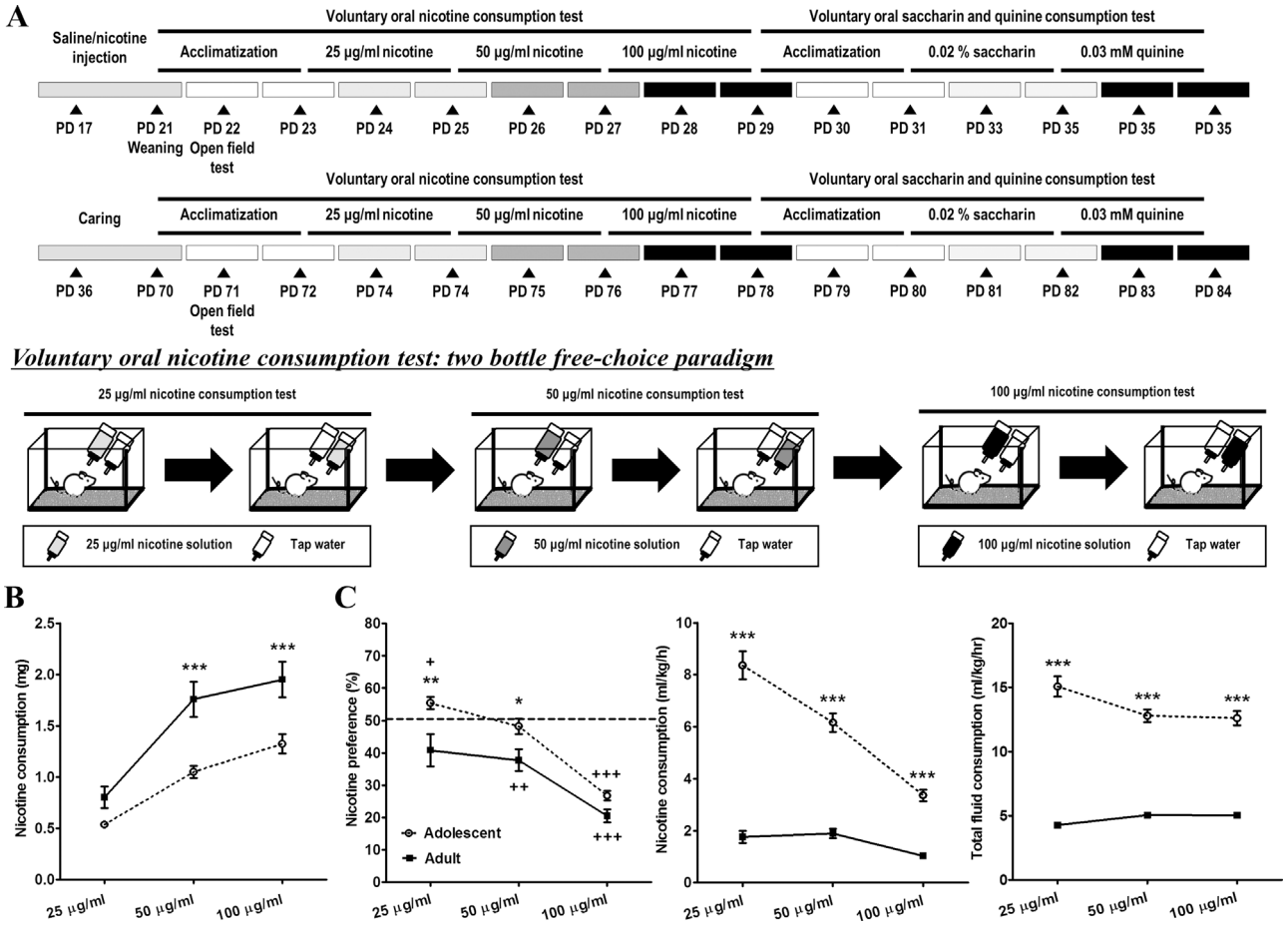

Fig. 1. Experimental procedures and nicotine consumption of rats. (A) Schematic timeline showing the schedule of experimental procedures on postnatal days (PD). (B) Both adolescent and adult rats consumed a higher amount of nicotine when the tested nicotine concentration was increased. The amount of consumed nicotine was increased in the adult rats ($^{***}P < 0.001$, Bonferroni post-hoc test). (C) Comparison of nicotine consumption between adolescent and adult control rats. *Left*, nicotine preference and consumption decreased when nicotine dose increased ($^{*}P < 0.05$, $^{**}P < 0.01$, Bonferroni post-hoc test). Adolescent nicotine preference was more than the indifferent ratio of 50% in the group provided 25 μg/ml nicotine solution ($^{+}P < 0.05$, one sample *t*-tests vs. 50). Adult rats provided with 50 μg/ml nicotine solution showed a preference of under 50% ($^{++}P < 0.05$, one sample *t*-tests vs. 50). Both adolescent and adult rats showed significantly decreased nicotine preference compared to the indifferent ratio in the 100 μg/ml nicotine consumption test ($^{+++}P < 0.05$, one sample *t*-tests vs. 50). *Middle*, nicotine consumption per body weight was less in adult than in adolescent rats ($^{***}P < 0.001$, Bonferroni post-hoc test). *Right*, total fluid consumption per body weight was decreased in the adult rats ($^{***}P < 0.001$, Bonferroni post-hoc test). Data are expressed as mean ± S.E.M.

differences in nicotine consumption and the effects of nicotine exposure in rats. Adolescent rats show a greater efficacy of nicotine-stimulated striatal dopamine release than do adults [11]. In an *in vivo* microdialysis study, nicotine exposure caused a greater increase in extracellular dopamine and serotonin levels in the nucleus accumbens of adolescent versus adult rats [27]. Dopamine and serotonin-releasing brain areas such as the ventral tegmental area, substantia nigra pars compacta and dorsal and median raphe nucleus are controlled by the habenula via gamma-aminobutyric acid neurons in the interpeduncular and rostromedial tegmental nucleus [28]. Importantly, the nicotine intake behavior of rats is known to be modulated by the unique nicotinic acetylcholine receptors found in the medial habenula [18–20]. In our previous study, we observed that repeated nicotine exposure enhances nicotine consumption and the medial habenula plays a pivotal role in nicotine consumption in rats [21]. To establish an animal model that will allow us to investigate the neurobiological role of nicotine aversion as a potent crucial factor for developing habitual nicotine use, we investigated the long-term developmental effects of early nicotine exposure in adolescent and adult rats. Moreover, we performed additional voluntary oral consumption tests using saccharin and quinine to reveal the differences between natural appetitive/aversive and nicotine-induced appetitive/aversive responses.

In the present study, we identified changes in nicotine aversion in rats with an eye towards preventing nicotine addiction. We demonstrate that changes in the aversive response to nicotine in rats depend on repeated nicotine exposure during the late lactation period. The first two weeks after birth in rodents is the critical period during which the brain growth rate is at a peak and neurogenesis of a majority of granule cells and most myelination in diverse brain areas takes place; altering these processes can cause death classified as sudden infant death syndrome [29,30]. For these reasons, we exposed early adolescent rats to nicotine during the third week after birth to avoid harsh developmental and fatal results.

2. Materials and methods

2.1. Animals

All animal experiments were conducted in accordance with the Dankook University ethics committee's guidelines for the care and use of laboratory animals (DKU-15-015). Male Sprague–Dawley rats were obtained from Samtako Bio Korea (Osan, Korea). Rats were housed in Plexiglas cages (45.72 cm × 22.86 cm × 20.32 cm) with wood bedding at a constant temperature (23 ± 1 °C) and humidity (45 ± 5%). Caring and experiments were performed in a sound-attenuating isolated room. A 12:12-h regular light-dark cycle was maintained (lights on 09:00–21:00), and the rats were provided food and water ad libitum. During the time between the adolescence (postnatal days 21–35) and