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Cigarette smoke exposure during adolescence but not adulthood induces anxiety-like behavior and locomotor stimulation in rats during withdrawal

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ABSTRACT

Adolescence is a critical period for cigarette smoking. Studies have shown that adolescent smokers are more likely to become addicted, are less likely to quit, and are more prone to relapse. In the present study, we examined the affective symptoms experienced by adolescents during withdrawal from cigarette smoke exposure. Towards this goal, adolescent male rats were repeatedly exposed to cigarette smoke, through an automated smoking machine, for 14 days. Then, cigarette smoke exposure was discontinued to induce spontaneous withdrawal. During the withdrawal period, anxiety-like behavior (elevated plus-maze test), locomotor activity (open-field test), and learning and memory (passive-avoidance test) were evaluated. These behavioral evaluations were conducted during the first, third, seventh, and fourteenth day of withdrawal. For comparison, parallel experiments were performed in adult rats. We found that adolescent rats exposed to cigarette smoke experiences increased anxiety-like behavior and locomotor activity during withdrawal relative to control rats. Learning and memory processes were undisturbed. On the other hand, adult rats exposed to cigarette smoke did not show any statistically significant behavioral alteration during withdrawal. These results are consistent with the notion that adolescents are differentially sensitive to the withdrawal effects of cigarette smoking. This sensitivity might be a factor why adolescent smokers have difficulty quitting and are more prone to relapse.

1. Introduction

Cigarette smoking typically starts at a young age. In fact, more than 80% of smokers started when they were adolescents (USDHHS, 2012). Alarmingly, this number continues to rise as thousands more adolescents start smoking every day. Adolescence has been implicated to be a critical period for cigarette smoking. Epidemiological and clinical studies have indicated that cigarette smoking during adolescence will more likely lead to long-lasting or even lifelong addiction or dependence (Buchmann et al., 2013; Grimshaw et al., 2006; Taioli and Wynder, 1991; USDHHS, 2012). Early cigarette smoking is correlated with reduced probability of quitting and higher rates of unsuccessful quit attempts or relapse (Abdolahinia et al., 2012; Khuder et al., 1999; Taioli and Wynder, 1991). Aside from these psychological aberrations, adolescent-onset cigarette smoking is also associated with increased prevalence of serious smoking-related diseases such as pulmonary disorders and cancer (USDHHS, 2012).

There is substantial preclinical and clinical evidence that adolescents are sensitive to the rewarding or positive reinforcing effects of cigarette smoking (Adriani et al., 2003; Buchmann et al., 2013; de la Pena et al., 2015; Dwyer et al., 2009). This sensitivity is implicated in the high rate of smoking initiation during this period. Likewise, there is a notion that adolescents are also sensitive to the negative consequences of smoking cessation (*i.e.* withdrawal effects). Clinical studies have shown that, despite relatively low average cigarette consumption and even before the onset of regular smoking, adolescents rapidly develop dependence towards cigarette smoking and experience withdrawal effects upon smoking cessation (Corrigall

Abbreviations: ANOVA, analysis of variance; CO, carbon monoxide; cm, centimeters; EPM, elevated plus-maze; nAChR, nicotinic acetylcholine receptor; OFT, open-field test; PND, post-natal day; SD, Sprague-Dawley; S.E.M., standard error of the mean; USDHHS, United States Department of Health and Human Services; W1, withdrawal day 1; W3, withdrawal day 3; W7, withdrawal day 7; W14, withdrawal day 14.

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et al., 2001; DiFranza et al., 2000, 2007; O'Loughlin et al., 2003). This sensitivity to the withdrawal effects of cigarette smoking is believed to be a major contributing factor why adolescent smokers have a harder time quitting and are more prone to relapse. Cigarette smoking withdrawal is a collection of somatic and affective symptoms that changes one's mood or behavior and manifests within hours or days after cigarette smoking cessation. Although varying among individuals, the most common withdrawal symptoms are anxiety, irritability, restlessness, and difficulty concentrating or cognitive disturbances (De Biasi and Dani, 2011). These affective withdrawal symptoms bring about a negative emotional state that can promote further cigarette smoking, probably through negative reinforcement mechanisms, and subsequently lead to relapse. Due to the high prevalence of adolescent cigarette smoking and the important role of withdrawal symptoms in relapse, efforts are being made to better understand cigarette smoking withdrawal effects in adolescents.

Animal models are beneficial in understanding the effects of cigarette smoking because it bypasses various limitations associated with human studies. It could help us better understand the mechanisms underlying cigarette smoking withdrawal, which could then lead to the development of measures that would benefit individuals who are experiencing it. However, animal studies that assessed smoking-related withdrawal effects in adolescent subjects have yielded inconsistent results. Some of these studies support the notion that adolescents are sensitive to smoking-related withdrawal effects (Faraday et al., 2001; Manhaes et al., 2008), but others reported somewhat conflicting results (O'Dell et al., 2004, 2006, 2007; Wilmouth and Spear, 2006). These animal studies may be limited in modeling human cigarette smoking due to the fact that they tested the effects of nicotine alone, disregarding the thousand other constituents or compounds present in cigarette smoke. Some of these constituents have been shown to produce psychopharmacological effects and/or can significantly influence the effects of nicotine (Belluzzi et al., 2005; Hoffman and Evans, 2013). Thus, to more accurately model the human condition, animal studies must utilize methods that more closely resembles cigarette smoking (*e.g.* cigarette smoke exposure).

In the present study, our goal was to evaluate the withdrawal effects induced by cigarette smoke exposure during adolescence. To simulate cigarette smoking, adolescent rats were repeatedly exposed to cigarette smoke for 14 days. Then, cigarette smoke exposures were discontinued to induce spontaneous withdrawal. Affective withdrawal symptoms were assessed through the elevated plus-maze (EPM) test, open-field test (OFT), and the passive-avoidance task. The EPM is a widely used and accepted method to assess anxiety-like behavior in rodents (Carobrez and Bertoglio, 2005; Ennaceur and Chazot, 2016). Previous studies have shown that rats undergoing nicotine withdrawal demonstrate anxiety-like behavior in this test (Irvine et al., 2001; Manhaes et al., 2008; Wilmouth and Spear, 2006). The OFT is a behavioral tool that measures the general locomotor or psychomotor activity of rodents. It is also used to assess restlessness and irritability in subjects undergoing withdrawal (Faraday et al., 2001). The passive-avoidance task is a test used to evaluate learning and memory in rodents (King et al., 2003). It was used to detect cognitive disturbances associated with smoking withdrawal. For comparison, parallel experiments were performed in adult rats.

2. Materials and methods

2.1. Animals

The subjects of this study were male Sprague-Dawley (SD) rats obtained from Hanlim Animal Corporation (Hwasung, Korea). Rats were considered adolescent from post-natal day (PND) 21 until PND 60 (maximal maturation); beyond this age, they were considered adults (O'Dell et al., 2006; Spear, 2011). A total of 44 rats (22 adolescents and 22 adults) were used in this study. They were randomly assigned to either the control (10 rats/group) or smoking group (12 rats/group) in their respective age group. They were housed in a standard animal room, with controlled temperature ($22 \pm 2\,^{\circ}C$) and humidity ($50 \pm 5\%$), and a 12-h light/dark cycle. Food and water were available *ad libitum*. The rats were acclimatized to the laboratory setting for five days before the commencement of any experiments. Behavioral tests were conducted one day (W1), three days (W3), seven days (W7) and fourteen days (W14) after the cigarette smoke exposure period (Fig. 1). All procedures were performed in adherence with the Principles of Laboratory Animal Care (NIH publication No. 85-23 revised 1985) and the Animal Care and Use Guideline of Sahmyook University, Korea.

2.2. Cigarette smoke exposure

Cigarette smoke was generated by burning 3R4F reference cigarettes (University of Kentucky, College of Agriculture, Reference Cigarette Program, Lexington, KY) through an automated cigarette smoking machine (SciTech Korea, Seoul, South Korea). A standardized smoking procedure was followed: 35 cm^3 puff volume, one puff per minute, 2 s per puff (de la Pena et al., 2014; Small et al., 2010), such that one cigarette approximately takes 10 min to consume. The smoke produced by the smoking machine, both mainstream and sidestream, was transported to the exposure chambers. Rats were transferred to new cages (4 rats/cage) and placed inside the exposure chamber (whole body exposure). They were unrestrained with freely available food and water. They were exposed to cigarette smoke for 2 h (12 cigarettes), two times a day (morning and afternoon), for 14 days [adolescent (PND 28–41), adult (PND 63–77)]. In our previous study, we have shown that this cigarette smoke exposure paradigm produces stable blood nicotine levels and induces long-lasting behavioral changes in both adolescent and adult rats (de la Pena et al., 2014, 2015). Carbon monoxide (CO) levels in the exposure chambers were assessed using a real-time CO analyzer (COH-9902SD, Lutron Electronic, Taipei, Taiwan). Air quality was monitored for particulate matters by MetOne 831 Aerosol Mass Monitor (Grant Pass, Oregon, USA). The average CO and total particulate matter levels were 97 mg/m^3 and 382 ppm, respectively. Control rats were subjected to the same animal handling but were placed in a chamber free from cigarette smoke.

2.3. Elevated-plus maze test

The EPM apparatus was made of polyvinyl plastic and consisted of two open-arms (30 cm × 6 cm) and two closed-arms (30 cm × 6 cm) surrounded by 20 cm high walls. At the junction of these four arms was a central platform measuring 10 × 10 cm. The apparatus was setup 50 cm above the floor. To start a test, a rat was placed on the central platform facing one of the open arms. It was allowed to explore the maze freely for 5 min. A computerized system (Ethovision System; Noldus Information Technology, Wageningen, Netherlands) recorded and analyzed the number of entries and the time spent (seconds) on each arm. An arm entry was defined as all four paws of the rat crossing the line marking of an arm.

2.4. Open-field test

The open-field test was performed in open-field boxes made of polyvinyl plastic measuring 42 × 42 × 42 cm. Each rat was placed into the center area (15 × 15 cm) of the open-field and allowed to explore freely for 10 min. The Ethovision system measured the total