



## Maternal Nicotine Exposure During Late Gestation and Lactation Increases Anxiety-Like and Impulsive Decision-Making Behavior in Adolescent Offspring of Rat

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Prenatal nicotine exposure over an entire pregnancy has been associated with an increased prevalence of hyperactivity, anxiety-like behavior and depression-like behavior in mature rats. However, the effects of maternal nicotine exposure in late gestation and lactation on the psychology and behavior of adolescent rat offspring are unclear. Thus, we investigated the effect of nicotine exposure during late gestation and lactation on anxiety-like and impulsive decision-making behavior in adolescent offspring of rat. Female rats were orally exposed to nicotine which is within range of plasma level of human chronic smokers during the period of third last period of gestation and lactation. When the offspring were weaned, we observed alterations in the anxiety-like behavior and decision-making ability of adolescent rat offspring using light/dark box test and T-maze delay-based cost-benefit decision-making task. The maternal consumption of nicotine reduced both the time spent in the light compartment and the number of transitions compared to nicotine-free rats. Moreover, such nicotine exposed adolescent offspring rats showed impulsive decision making which chose the instant reward in a decision-making situation. We found that nicotine exposure during late gestation and lactation induces an increase in anxiety-like and impulsive decision-making behavior at this developmental stage. These findings suggest that maternal nicotine-exposed offspring are at an increased risk of developing anxious and impulsive behavior.

**Key words:** Anxiety, Decision-making, Gestation, Impulsivity, Lactation, Nicotine

### INTRODUCTION

Poor and impulsive decision-making during adolescence has resulted in fatal automobile crashes, violence, unprotected sex, and substance abuse (1-3). In particular, children exposed to tobacco gestationally show an increased risk for impulsive disorders, such as attention deficit hyperactivity disorder (ADHD) and substance abuse (4-6). This is possibly due to perturbed development of the decision-making parts of the brain, including imbalances in the impulsive, amygdala system and the reflective, prefrontal cortex system (7). Because exposure of the prefrontal cor-

tex to drugs before maturity could be harmful to decision-making, it is crucial to protect offspring from the influence of drugs taken by the mother during pregnancy.

Nicotine can cause cardiovascular toxicity and changes of neuropeptides levels as well as interferes with critical developmental events such as neurogenesis, in most brain regions, and with early synaptogenesis (8-10). Although prenatal nicotine exposure over the entire pregnancy has been shown to increase the risk of ADHD and drug abuse, few studies have focused on examining the effects of nicotine exposure during late pregnancy and lactation on behavioral abnormalities in the offspring. Moreover, most studies have examined the effects of nicotine administered subcutaneously, which could lead to artifacts due to significant fetal hypoxia and stress caused to pregnant dams (11,12). Here, we examined alterations in the decision-making ability and anxiety-like behavior of adolescent rat offspring after maternal nicotine exposure, administered orally, to determine whether nicotine consumption during late gestation and lactation disrupted development of decision-making behavior in offspring.

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## MATERIALS AND METHODS

**Animals.** All animal studies were conducted in accordance with the Dankook University ethics committee's guidelines for the care and use of laboratory animals. Sprague-Dawley rats were obtained from Samtako Bio Korea (Osan, Korea). Rats were housed in plexiglass cages with wood-shaving bedding in a room that was air conditioned at  $23 \pm 1^\circ\text{C}$  ( $45\% \pm 5\%$  humidity) with a standard 12-hr light/dark cycle (lights on 09:00–21:00), with food and water available ad libitum.

**Nicotine-exposed rat model.** During the period of late gestation and lactation (gestational day 15 to postnatal day 18), female rats in the nicotine group were orally exposed to nicotine hydrogen tartrate (Sigma-Aldrich Co. LLC, St. Louis, MO, USA) at a concentration of 0.1 mg/mL, which is within range of plasma level of human chronic smokers, in 2% of saccharine-dissolved water to make the nicotine less aversive (13–15). Oral administration is one of prevalent experimental method in rodent nicotine and toxicology studies (16–18). Nursing female rats in the nicotine-unexposed group were given 2% saccharine solution without the presence of nicotine. All rats used in the following tests had equal gender ratio in adolescent offspring.

**Light/dark box test.** For the light/dark box test, rats were transferred to the testing room 20 min before the test, for acclimation. The light/dark box measured  $40 \times 20 \times 31$  cm. Rats were transferred through the front door ( $8 \times 8$  cm) and placed in a dark compartment illuminated at approximately 1 lux. The middle door between the dark compartment and the light compartment (illuminated at 110–150 lux) was opened 3 s after the front door was closed. To reduce noise from the outside, the light/dark box was covered with a transparent acrylic seal. Rats were allowed to explore the box for 15 min, and their behavioral activities were recorded with a camcorder (HMX-H304BD, Samsung Electronics Co., Ltd., Suwon, Korea) through a window in the transparent ceiling of the light compartment.

**T-maze delay-based cost-benefit decision-making task.** A T-maze delay-based cost-benefit decision-making task was modified from the study of Salamone *et al.* (19). The apparatus consisted of 3 arms, including a start arm ( $22 \times 6 \times 30$  cm) and 2 goal arms ( $27 \times 15 \times 30$  cm). During the test period, the food quantity supplied to the rats daily was limited to 80% of the ad libitum value. In the training phase of the experiment, rats were introduced to the T-maze. The sliding door adjacent to the rats was opened after 10 s and the rats were given free access to the maze, which contained a large reward (2 g, food pellet) in one arm and a small reward (one-quarter of the size of the large one) in the other. Training was repeated 3 times daily at 5 min inter-

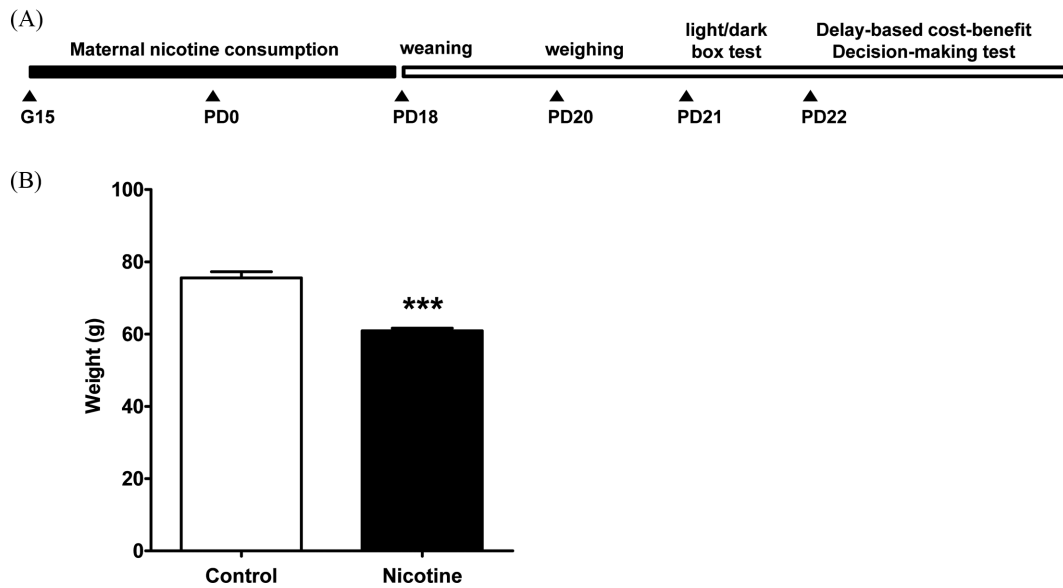
vals for 3 consecutive days. During the test period, the locations of the large and small food rewards remained unchanged to ensure that the rats learnt their locations. Next, a delay task was imposed for three days. In the delay task, all the test procedures were same as in the training phase, but immediately after the start, a sliding door adjacent to the larger food pellet was closed, blocking access to it. The opening of this door was progressively delayed by 5, 15, and 25 s after start of the test.

**Statistical analysis.** The data, presented as the mean  $\pm$  standard error of the mean, were analyzed with Prism5 software (GraphPad Software, Inc., La Jolla, CA, USA). A Student's *t*-test was employed to determine whether the differences between the two experimental groups were significant. Preference of large food pellet was analyzed by two-way analyses of variance (ANOVA) and Bonferroni's multiple comparison test for post hoc comparison.

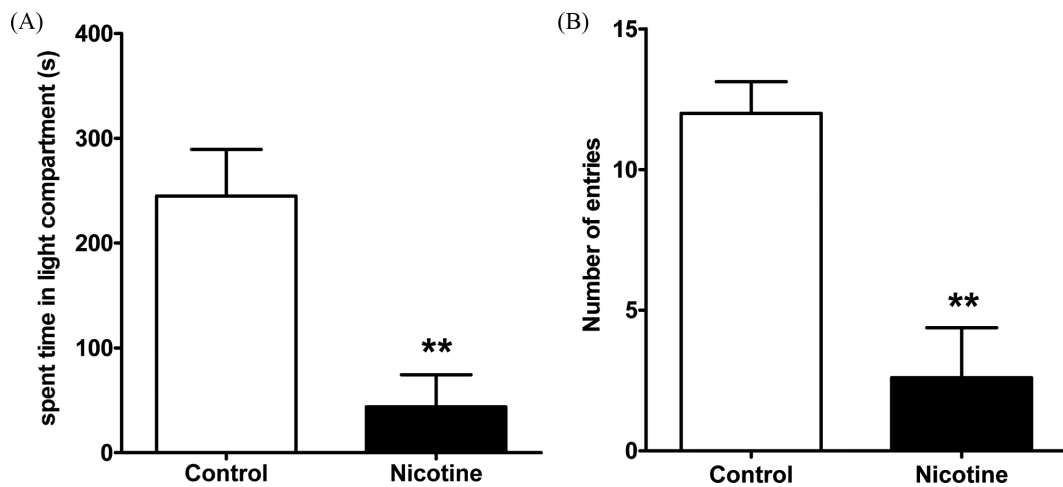
## RESULTS

The rats were administered nicotine as described during the third last period of gestation, and it was ended when the offspring were weaned (Fig. 1A). By avoiding nicotine exposure in the early trimesters of pregnancy, we were able to prevent the rats from experiencing fatal pregnancy damage, such as miscarriages, that is induced by maternal smoking (20). In our study, adolescent offspring that were exposed to maternal nicotine consumption during the last period of gestation and during lactation showed an approximately 19% decrease in body weight compared to the nicotine-unexposed offspring (Fig. 1B). This result was in concordance with previous findings which showed that maternal nicotine consumption lowers the weight of rats, suggesting that gestational nicotine reduces body weight (21). There was no difference of birthrate between control and nicotine exposure rats.

To determine whether the anxiety-like behavior of adolescent offspring was altered by maternal oral nicotine consumption, we performed a light/dark box test. In this test, anxiety-like behavior is measured by the decrease in time spent in the light compartment of the test box and decrease in the number of transitions between the two compartments (22). Both time spent in the light compartment and transition numbers showed no significant differences between naïve rats and control (data not shown). Maternal consumption of nicotine decreased the time spent by offspring in the light compartment to 18% and reduced the number of transitions to 22% compared to nicotine-unexposed rats (Fig. 2). These are speculated to be a result of nicotine-mediated anxiety-like behavior in accordance with previous studies that have shown that nicotine administration induces anxiety (23–26). These findings support the hypothesis that maternal exposure to nicotine poses a threat to normal brain



**Fig. 1.** Experimental procedures and body weight alterations in rats. (A) Schematic time line showing the different stages of each experiment (G, gestational day; PD, postnatal day). (B) The body weight data of the nicotine-unexposed and maternally nicotine-exposed 3 weeks old offspring. The nicotine group shows a significant decrease in body weight compared to the control group (Control,  $75.57 \pm 1.69$  g,  $n = 6$ ; Nicotine,  $60.92 \pm 0.69$  g,  $n = 5$ ;  $***P < 0.001$ ).

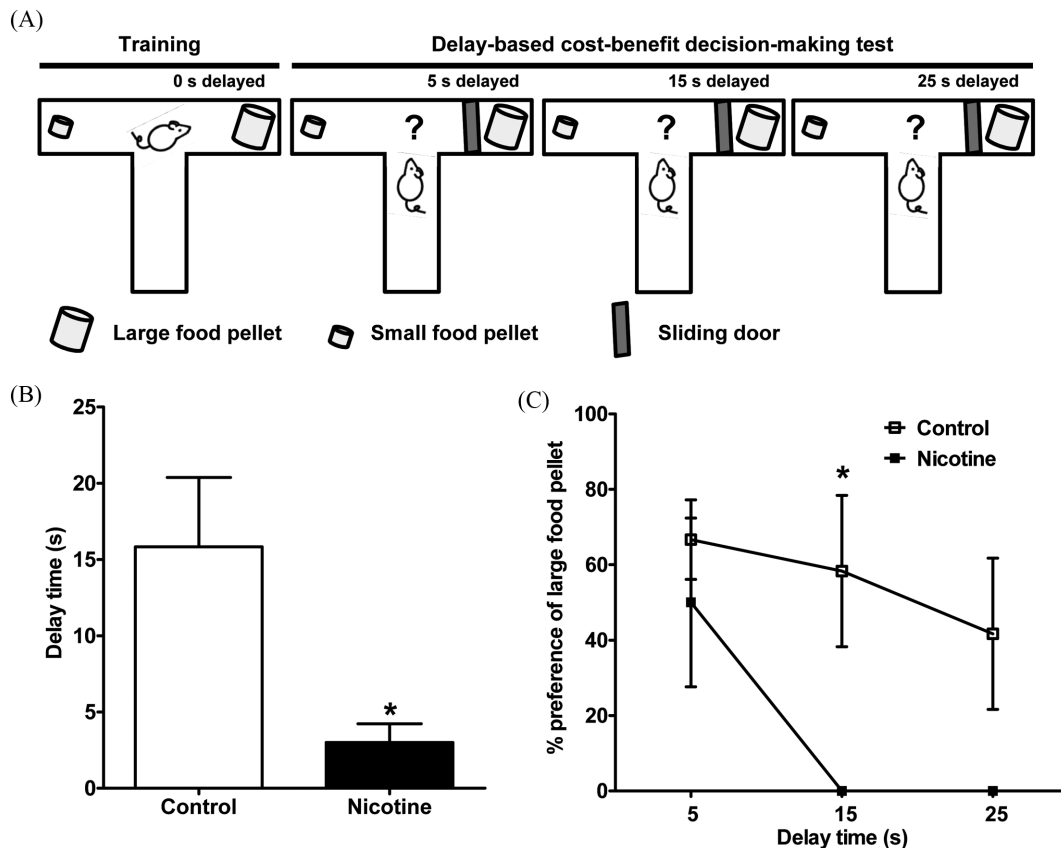


**Fig. 2.** The nicotine-exposed offspring exhibit increased anxiety-like behavior. (A) The time spent in the light and dark compartments was recorded during a 15-min session in the light/dark box. The anxiety-like behavior of nicotine-exposed offspring is more pronounced than that of the nicotine-unexposed offspring (Control,  $245.00 \pm 44.31$  s,  $n = 6$ ; Nicotine,  $43.79 \pm 30.58$  s,  $n = 5$ ;  $**P < 0.01$ ). (B) Total number of transitions into the light compartment. Nicotine-exposed offspring show a significant decrease in the number of such transitions compared to nicotine-unexposed offspring (Control,  $12.00 \pm 1.13$ ,  $n = 6$ ; Nicotine,  $2.60 \pm 1.78$ ,  $n = 5$ ;  $**P < 0.01$ ).

and behavior development in the offspring.

To examine whether the decision-making behavior of rats was changed by maternal oral nicotine exposure, we performed a delay-based cost-benefit decision-making test. Under diet restriction, nicotine-unexposed and maternally nicotine-exposed offspring were allowed to choose between immediately available small food pellets and progressively delayed large food pellets (Fig. 3A). In experimental runs in

which the rat chose the large pellet, the maternal nicotine-exposed offspring exhibited a significant decrease in delay tolerance compared to nicotine-unexposed rats (Fig. 3B, 3C). Furthermore, the number of large food pellets chosen despite unexpected delays was reduced in maternal nicotine-exposed offspring. Taken together, the maternal oral nicotine-exposed offspring showed impulsive decision-making behavior. This was reflected in their tendency to choose the instant rewards



**Fig. 3.** The nicotine-exposed offspring exhibit increased impulsive decision-making. (A) A schematic diagram illustrating the cost-benefit decision-making test. In the task, the rat is able to choose an immediate small food pellet or a larger food pellet, the latter associated with delays of 5, 15, or 25 s. (B) The delay time is presented as the maximum tolerated delay of the nicotine-unexposed and nicotine-exposed offspring. The nicotine-exposed offspring show a significant decrease in the amount of delay tolerance to gain a larger food pellet, compared to the nicotine-unexposed offspring (Control,  $15.83 \pm 4.55$  s,  $n = 6$ ; Nicotine,  $3.00 \pm 1.23$  s,  $n = 5$ ;  $*P < 0.05$ ). (C) The data are presented as the percentage of trials exhibiting a preference for the large food pellet against progressively increasing delay time. There were significant effects in preference of large food pellet among the control and nicotine groups as determined by two-way repeated measures ANOVA (groups,  $F(1, 18) = 5.28$ ,  $P < 0.05$ ; delay time,  $F(2, 18) = 4.65$ ,  $P < 0.05$ ), which revealed no significant interaction between groups and delay time ( $F(2, 18) = 1.32$ ,  $P > 0.05$ ). In both group, all rats preferred large food pellet at 0 delay time. The nicotine-exposed offspring show a decrease in the preference for the large food pellet compared to the nicotine-unexposed rats in 15 seconds delay time (Control,  $58.33 \pm 20.07\%$ ,  $n = 6$ ; Nicotine,  $0.00 \pm 0.00\%$ ,  $n = 5$ ;  $*P < 0.05$ ).

in a decision-making situation. This suggests a loss of the ability to decide based on the value of the reward, and an increase in the offspring's impulsive decision-making behavior.

## DISCUSSION

Maternal cigarette smoking during pregnancy can result in behavioral problems of the offspring. Maternal exposure of pollutants or maternal protein restriction in the rat during pregnancy and lactation alters anxiety-like behaviors of offspring (27,28). In this study, we focused on alterations in offspring behavior that were caused by exposure to nicotine administered to the mother orally during the last period of gestation and in the lactation period. Because the levels of urinary cotinine, which is a metabolite of nicotine, in

infants who are breastfed by smoking mothers are similar to those found in adult smokers, nicotine that is consumed through lactation would also lead to disturbed neurodevelopment and the acceleration of the risk for a range of psychiatric problems, even though the breastfeeding mothers are exposed by passive smoking (29).

When nicotine is orally administered, the blood levels do not rise as quickly as intravenous application, subsequently the effects are relatively more subtle. Compared to intravenous self-administration in rodents or inhalation in humans, the rate of entry to the brain is slower and the quantities are lower and more variable (11). The  $200 \mu\text{g/mL}$  nicotine dose generally is selected in an oral administration as it has been shown previously to have both behavioral and biochemical efficacy in rodents (11). Rowell *et al.* demonstrated that

mice which were forced to consume nicotine (60 µg/mL for 5~6 weeks) in the drinking water had steady-state nicotine plasma levels of 34.4 ng/mL (18). Based on these rodent studies, we used 100 µg/mL of nicotine in drinking water for approximately 4 weeks. Because our nicotine concentration by the calculation is within range of plasma level of human chronic smokers (10~60 ng/mL), we suggest that our nicotine dose is enough to have physiological meaning (13,14).

We found that the offspring in the maternal nicotine-exposed group had relatively poorer and more impulsive decision-making behavior and higher anxiety-like behavior, suggesting that nicotine-exposed offspring are susceptible to addiction due to their poor decision-making ability and have an increased incidence of ADHD and addictive disorders. Since impulsive decision-making and high anxiety are related to an imbalance between the amygdala and the reflective, prefrontal cortex, strengthening the reflective system through methods such as social caregiving and bonding may be necessary for nicotine-exposed adolescent offspring. This would probably reduce their impulsivity-related antisocial behavior and/or group exclusion, and help them cope with stress better (30,31).

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