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Assessment of nicotine withdrawal-induced changes in sucrose preference in mice

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ABSTRACT

Anhedonia, induced by nicotine withdrawal, may serve as an important affective sign that reinforces tobacco use and smoking relapse rates in humans. Animal models provide a way to investigate the underlying neurobiological factors involved in the decrease in responding for positive affective stimuli during nicotine withdrawal and may aid in drug development for nicotine dependence. Thus, we explored the use of the sucrose preference test to measure nicotine withdrawal-induced reduction in response for positive affective stimuli in mice. C57BL/6J and knockout (KO) mice were chronically exposed to different doses of nicotine through surgically implanted subcutaneous osmotic minipumps for 14 days and underwent spontaneous nicotine withdrawal on day 15. A sucrose preference time course was performed and the results were compared to another well-established affective sign of nicotine withdrawal, the reduction in time spent in light side, using the Light Dark Box test. Subsequently, our results demonstrated a time-dependent and dose-related reduction in sucrose preference in nicotine withdrawn male C57BL/6J mice, indicative of a decrease in responding for positive affective stimuli. Furthermore, the sucrose preference reduction during nicotine withdrawal was consistent with decrease in time spent in the light side of the Light Dark Box test. We also found the reduction for positive affective stimuli and time spent in the light side was not present in nicotine withdrawn β2 and α6 KO mice, suggesting that these nicotinic subunits are involved in the affective signs of nicotine withdrawal. Thus, this report highlights the potential utility of the sucrose preference test as a useful measure of the decrease in responding for positive affective stimuli during spontaneous nicotine withdrawal.

1. Introduction

Nicotine dependence is not only based on the positive reinforcing and hedonic effects of nicotine, but it is also associated with a withdrawal syndrome that results from smoking cessation (George et al., 2007; Kenny and Markou, 2001). Indeed, the nicotine withdrawal syndrome in humans is represented by a variety of signs such as somatic signs that include gastrointestinal disturbances, weight gain, decreased heart rate (American Psychiatric Association 2000), sweating, dizziness (Hughes and Hatsukami 1986), fatigue, nausea, constipation, and diarrhea (Shiffman, 1979). In addition to these physical signs, there are other unpleasant negative mood symptoms that occur during smoking cessation in humans. Anxiety and anhedonia are affective features of nicotine withdrawal that are thought to contribute to continued tobacco use (Dawkins et al., 2007; Cook et al., 2015; American Psychiatric Association, 2000; Hughes, 2007). It has been reported that tobacco smokers may continue smoking to escape the loss of pleasure induced by nicotine withdrawal (Dawkins et al., 2007; Perkins & Karelitz, 2013; Cook et al., 2015). Also, laboratory human studies suggest that nicotine deprivation results in anhedonia (Al-Adawi and Powell, 1997; Dawkins et al., 2006; Powell et al., 2002; Powell et al., 2004). Therefore, assessment of anhedonia induced by the nicotine withdrawal syndrome may have clinical implications in terms of treatment of tobacco addiction.

Similarly, in animal studies, it has also been reported that nicotine withdrawal following chronic nicotine administration leads to reduction of operant responding for rewarding electrical brain stimulation using intracranial self-stimulation (ICSS) in rats and mice, a measure of reduction in responding for positive affective stimuli (Epping–Jordan et al., 1998; Hilario et al., 2012; Johnson et al., 2009; Stoker et al., 2015). However, the ICSS procedure is an operant conditioning method that requires surgery, which can be labor-intensive. It also involves a special training schedule and a distinctive apparatus (Carlezon and Chartoff, 2007). The two-bottle choice procedure for assessing sucrose preference is another useful test to investigate reduction in positive affective stimuli in laboratory rodents (Thompson and Grant, 1971).

Abbreviations: KO, knockout; WT, wild-type; ICSS, intracranial self-stimulation; LDB, light dark box

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Compared to the ICSS procedure, the sucrose preference test is simple, fast, does not require surgery, or long term testing and training sessions.

Accordingly, the current study aimed to establish a mouse model of spontaneous nicotine withdrawal-induced decrease in affect using the sucrose preference test. We assessed the time-course and dose-dependency of sucrose preference during spontaneous nicotine withdrawal in C57BL/6J mice. In addition, we investigated the role of the β2 and α6 nicotinic subunits in this behavior by utilizing β2 and α6 nicotinic knockout (KO) and wild-type (WT) mice. It has been previously reported that the affective signs of nicotine withdrawal including reduction in responding for positive affective stimuli were absent in these KO mice (Jackson et al. 2008, 2009; Stoker et al., 2008). Finally, we compared the results of the sucrose preference test to another well-established affective sign of nicotine withdrawal, the reduction in time spent in light side by using the Light Dark Box (LDB) test.

2. Methods

2.1. Animals

8-week-old male C57BL/6J mice were obtained from the Jackson Laboratory (Bar Harbor, ME). C57BL/6J provided the background strain for our α6 and β2 KO and WT mice. The β2 KO mice (Institut Pasteur, Paris, France) and their WT littermates were bred in an animal care facility at Virginia Commonwealth University. Healthy, viable mice null for the α6 nicotinic subunit were provided by Dr. Uwe Maskos at Institut Pasteur (Paris, France) (Champtiaux et al., 2002). All mice used in each experiment were backcrossed at least 10 to 12 generations. Mutant and wild types were obtained from crossing heterozygote mice. This breeding scheme controlled for any irregularities that might occur with crossing solely mutant animals. Animals were 8–10 weeks of age at the beginning of the experiments and were group-housed (four animals per group with ad libitum access to food and water under a 12 h light/dark cycle in a 21 °C). Experiments were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University and followed the National Institutes of Health Guidelines for the Care and Use of laboratory animals.

2.2. Drugs

(−)-Nicotine hydrogen tartrate [(-)-1-methyl-2-(3-pyridyl) pyrrolidine (þ)-bitartrate], sucrose and saccharine were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Nicotine was dissolved in sterile physiological saline (0.9% sodium chloride). Nicotine (12 and 24 mg/kg/day) was infused through 14-day subcutaneous osmotic minipumps (model 2002, Alzet, Palo Alto, CA, USA). The doses were expressed as the free base of the drug. The doses were used according to the previous literature (Damaj et al., 2003; Salas et al., 2004). Sucrose (2%) and saccharine (0.4%) were dissolved in water and given orally in the two-bottle choice procedure.

2.3. Induction of nicotine withdrawal

Mice were anesthetized by inhaling isoflurane/oxygen vapor mixture (1–3%). Alzet osmotic minipumps were then implanted subcutaneously within the mice for 14 days. The Alzet minipumps were filled with either nicotine or saline solutions and inserted by making an incision parallel to the spine at shoulder level of the mice. The wound was closed using wound clips and the mice were placed in a surgery room for recovery before using them in experiments. Post-operative care was done for 14 days by observing the mice daily. For all of the procedures, the doses of nicotine were 12 and 24 mg/kg/day, calculated according to body weight. On day 15 spontaneous nicotine withdrawal was induced by removing the minipumps under isoflurane anesthesia in aseptic surgical conditions. The experiment was adapted as previously described (Damaj et al., 2003; Jackson et al., 2008). On day 16, mice were tested for sucrose preference, saccharin preference and LDB for several days. Summary of time and duration of each behavioral test and the mice that used in each experiment was shown in Supplementary Table 1.

2.4. Sucrose preference test

Sucrose preference test was used to investigate the reduction in responding for positive affective stimuli in rodents after the induction of nicotine withdrawal (Thompson and Grant, 1971). In this experiment, mice were individually housed and acclimated to cages with food and water. Mice had free access to two 30 ml sipper tubes containing tap water for 3 days as a baseline. Animals then were exposed to two 30 ml sipper tubes, one with tap water and the other with 2% sucrose solution. Measurements of consumed water and sucrose solution were taken every 24 h. To prevent any bias, tube placement was switched daily. 24 h following saline and nicotine minipump removal, the same cohort of C57BL/6J mice were tested for sucrose preference for 9 consecutive days. For the experiments using KO mice, the measurements of sucrose preference were taken at day 2 after removal of osmotic minipumps. Sucrose preference was determined as the percentage of 2% sucrose volume consumed over the total fluid intake volume. Sucrose preference (percentage) was calculated as follows: preference = [sucrose solution intake (ml)/total fluid intake (ml)] × 100. The experiment was adapted as previously described (Toma et al., 2017; Pothion et al., 2004). Same cohorts of C57BL/6J male mice (n = 11 per group) were tested for 11 consecutive days during chronic nicotine exposure and 9 consecutive days after nicotine minipumps removal. For KO mice, we tested (n = 8 per group) at day 2 (48 h) after removal of minipumps.

2.5. Saccharine preference test

Male C57BL/6J mice were individually housed and acclimated to cages with free access to food and water for 3 days. Same cohort of mice was tested for 24 h with access to two 30 ml sipper tubes. One tube was filled with tap water and the other with 0.4% saccharin solution. The measurements were taken at 24, 48 and 96 h after removal of nicotine or saline minipumps. Saccharin preference (percentage) was calculated as follows: preference = [saccharine solution intake (ml)/total fluid intake (ml)] × 100. The experiment was conducted as previously described (Jastrzębska et al., 2016). We used same cohorts of C57BL/6J male mice (n = 8) for 3 days after removal of minipumps.

2.6. Light-Dark Box (LDB) test

The LDB procedure depends on the innate aversive behavior of rodents to bright areas as well as their stress induced-natural exploratory response (Crawley and Goodwin, 1980). The test was slightly modified as previously reported (Wilkerson et al., 2016). The LDB apparatus is composed of a small, enclosed dark or black compartment (36 × 10 × 34 cm) with a passageway (6 × 6 cm) extending to a larger, light or white compartment (36 × 21 × 34 cm). The mice were habituated to the experiment room for 30 min before testing. First, mice were placed in the light chamber and allowed to freely explore the apparatus for 5 min. Then the number of entries into the light compartment, the number of transitions and the total time spent in the light compartment were recorded for 5 min by a video monitoring technique and ANY-MAZE software (Stoelting Co., Wood Dale, IL). Same cohorts of C57BL/6J male mice (n = 10 per group) were tested at days 1, 2 and 5 after nicotine minipumps removal. For KO mice, we tested same cohort of male mice (n = 8 per group) at day 2 (48 h) after removal of minipumps.