Pharmacology, Biochemistry and Behavior 131 (2015) 91–97

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Nicotine withdrawal in selectively bred high and low nicotine preferring rat lines

Tanseli Nesil [a,b], Lutfiye Kanit [a,c], Muzeyyen Ugur [d], Sakire Pogun [a,*]

[a] Ege University, Center for Brain Research, Bornova, Izmir, Turkey
[b] University of Virginia, Department of Psychiatry and Neurobehavioral Sciences, Charlottesville, VA, USA
[c] Ege University, School of Medicine, Department of Physiology, Bornova, Izmir, Turkey
[d] Ege University, Institute of Health Sciences, Department of Neuroscience, Bornova, Izmir, Turkey

ARTICLE INFO

Article history:
Received 11 December 2014
Received in revised form 6 February 2015
Accepted 7 February 2015
Available online 14 February 2015

Keywords:
Nicotine
Selective breeding
Sex differences
Withdrawal symptoms
Oral nicotine intake
Addiction

ABSTRACT

Background: We have generated high- and low-nicotine preferring (high-NP, low-NP) rat lines using voluntary oral nicotine intake as the selection criterion. After nine generations, the estimated realized heritability for high intake was 0.26. The aim of the current study is to compare how nicotine withdrawal varies between these two lines. This new analysis would help elucidate if nicotine withdrawal and intake share common genetic mechanisms.
Methods: After exposing male and female Sprague Dawley rats (F_8 generation) to six weeks of nicotine exposure, nicotine was withdrawn. Somatic signs of withdrawal, locomotor activity, and weight were measured at 16 and 40 h. One week after withdrawal, resumption of nicotine intake was determined.
Results: The High-NP line had higher nicotine intake before and after withdrawal than the Low-NP line. High-NP rats were more active than Low-NP rats, and locomotor activity decreased during withdrawal; this decrease was more pronounced in the High-NP line. High-NP rats gained more weight during withdrawal than Low-NP rats. Escape attempts decreased during withdrawal in all groups, but overall females demonstrated more escape attempts than males. The other somatic signs of withdrawal were higher during withdrawal compared to baseline and more pronounced in females.
Conclusions: Selection for nicotine preference affected nicotine intake, locomotion and weight, suggesting the heritability of these traits. However, despite differences in nicotine preference and intake, high-NP and low-NP rats showed similar withdrawal responses: escape attempts decreased and somatic signs increased. Withdrawal responses of females were more pronounced than males suggesting sex differences in the negative affect induced by nicotine withdrawal. The major finding of this novel analysis is showing that nicotine preference does not predict withdrawal symptoms. This finding, together with sex differences observed during withdrawal, may contribute to a better understanding of nicotine dependence and have translational value in developing more effective strategies for smoking cessation.

1. Introduction

Several studies involving human subjects point to the heritability of various smoking-related phenotypes including initiation, dependence, failed smoking cessation and nicotine withdrawal (Berger et al., 2010; Biondolillo et al., 2009; Hui et al., 2009; Lessov et al., 2004; Rezvani et al., 2010). Nonetheless, the genetic mechanisms of the inheritance of nicotine addiction related behaviors have not been fully elucidated due to the genetic and phenotypic heterogeneity in the human population. The variability in smoking initiation and persistence, withdrawal symptoms, and quitting success are attributed to heritable factors. The reported impact ratios related to the heritable factors range between 29 and 84% (reviewed in Maisonneuve and Glick, 2003).

Animal models provide an opportunity to determine the impact of genetic factors involved in the development of addiction related behaviors and help to identify addiction-relevant genes. Selective breeding has been widely used to generate genetic models of complex traits. We reported the first nicotine preference lines from outbred Sprague Dawley rats by using bidirectional mass selection for voluntary oral nicotine intake behavior. Our results indicate that selective breeding of rats for high voluntary nicotine intake behavior resulted in increased nicotine consumption with a realized heritability of 0.26 after nine generations of selection (Nesil et al., 2013). Selection of a nicotine preference trait over the generations can increase vulnerability to the reinforcing effects of nicotine, but it can also increase sensitivity to the negative effects of nicotine withdrawal, which contributes to nicotine addiction

* Corresponding author at: Ege University, Center for Brain Research, Bornova, 35100 Izmir, Turkey. Tel.: +90 532 584 6272; fax: +90 232 374 6597.
E-mail addresses: sakire.pogun@ege.edu.tr, sakirepogun@gmail.com (S. Pogun).

http://dx.doi.org/10.1016/j.pbb.2015.02.009
0091-3057/

and the maintenance of nicotine intake. The novelty of the current study is to study withdrawal responses in rat lines that demonstrate high or low preference for nicotine, measured by high or low voluntary oral nicotine intake.

Selectively-bred rat and mice lines have been widely used in drug addiction studies to reveal the genetic influences on response to drugs such as methamphetamine, opiates, and alcohol (Carlson and Perez, 1997; de Fiebre et al., 1989; Gubner et al., 2013; He et al., 2008; Milner and Buck, 2010; Wheeler et al., 2009). However, animal models of nicotine dependence, using selectively-bred rodent lines are limited. More than two decades ago out-bred mice were selected for nicotine sensitivity based on activity alterations in the Y-maze after a single dose of nicotine injection (Smolen and Marks, 1991; Smolen et al., 1994). Results demonstrate that, the heritability of nicotine-induced activity was estimated to be 0.12 by the F_6 generation. This mice-line was not carried further and information on nicotine preference, intake or withdrawal was not reported.

Genetic background is one of the major factors underlying individual differences in nicotine dependence, success in quitting, and relapse. Elucidating the effect of inherited genetic factors on the development of the nicotine dependence phenotype is critically important. Genetic models of addiction promise to provide a detailed causal explanation of the genetic vulnerability to nicotine addiction and developing effective strategies for smoking cessation. Sex differences observed in smoking behavior is an important issue that deserves further analyses. Despite lower rates of smoking, females experience greater reward from nicotine, and more stress during withdrawal compared to males (O'Dell and Torres, 2014; Pogun and Yararbas, 2009). Findings from animal studies have substantial translational value regarding withdrawal symptom severity, and sex differences observed in smoking cessation.

The goal of the study was to assess if rats selectively outbred for nicotine preference and intake also display different withdrawal responses; in other words, will high-NP rats also show greater withdrawal symptoms. Since sex differences in nicotine reward and withdrawal have been demonstrated in both clinical and experimental studies, we included sex as a factor. Consequently, we evaluated the somatic signs of withdrawal, locomotor activity, weight gain and re-establishment of nicotine intake in male and female rats, selectively outbred for different nicotine preferences. Here we report the results of withdrawal and resumption of nicotine intake studies from the F_8 generation of high and low nicotine-preferring (high-NP and low-NP) rats.

2. Methods

2.1. Subjects

Adult male and female Sprague Dawley rats (F_0, n = 77), obtained from Ege University Animal Breeding Facility, were subjected to oral nicotine self-administration, as the founding population for the selective breeding of the high-NP and low-NP lines (Collins et al., 2012; Nesil et al., 2011). Rats were kept in same-sex groups with ad libitum access to food and water and were housed under standard laboratory conditions (20–22 °C, 12–12 h light–dark cycle) when they were not being used for breeding or testing. When being tested for oral nicotine intake, each rat was housed individually. After the termination of oral nicotine self-administration, rats (n = 48) that consumed the highest and lowest nicotine were selected [high-NP Female (n = 14), Male (n = 13); low-NP Female (n = 7), Male (n = 14)], using the Ward Cluster Method (Nesil et al., 2011), as breeders for the next generation. In the following generations (F_1–F_8) the highest nicotine consuming of the high-NP and the lowest of the low-NP were selected and outbred. The two groups were always significantly different from each other ($p < 0.001$, t-test); the number of selected rats was variable and depended on the total number of offspring. At the 8th generation (n = 98), the number of animals in each group [high-NP Female (n = 41), Male (n = 30); low-NP Female (n = 16), Male (n = 11)] was sufficient to carry out withdrawal studies.

The animals were treated under the prescriptions for animal care and experimentation of the pertinent EU Directive 2010/63/EU; the Institutional Animal Ethics Committee of Ege University approved all the procedures.

2.2. Measurement of voluntary nicotine intake behavior

Oral nicotine self-administration was performed as described earlier (Nesil et al., 2011). Briefly, rats were given access to either nicotine in water [Sigma, (−) nicotine hydrogen tartrate] or plain water using a two bottle free-choice method. The concentration of nicotine, calculated as base, was 10 mg/L during the initial two weeks. At the beginning of the third week, the nicotine concentration was raised to 20 mg/L and kept at this amount for the rest of the experiments. Nicotine exposure lasted six weeks. Sodium saccharine (10 mg/L) was added to both the nicotine and water bottles to mask the bitter taste of nicotine.

Nicotine consumption and body weight of each animal were monitored weekly. Nicotine consumption was calculated as milligrams per kilogram body weight, averaged over the last three weeks of drug exposure.

2.3. Withdrawal procedure

To determine the possible genetic differences (related to sex and selection) in response to nicotine withdrawal and resumption of intake in high-NP and low-NP lines by the eight generation of selection, we conducted experiments in three phases: Baseline (drug administration), withdrawal (post-drug administration, 16 and 40 h) and resumption of nicotine intake (re-exposure to drug).

A total of 98 animals from both lines were exposed to oral nicotine administration for six weeks in the F_8 generation. During the last week of oral nicotine exposure, locomotor activity and somatic signs of withdrawal were measured and taken as baseline recordings, before withdrawal. Following six weeks of nicotine exposure, the bottle containing the nicotine + saccharine solution was replaced with water + saccharine. Somatic signs of withdrawal, locomotor activity, and weight were measured at 16 and 40 h. In the assessment of somatic signs of withdrawal (Malin et al., 1992) rats were placed in Plexiglas cylinders (ø: 30 cm, h: 50 cm, pine shaving bedding) and the activity of each subject was recorded by the video for 10 min. Two observers, blind to the experimental conditions watched the recordings and counted the number of body shakes, cheek tremors, escape attempts (two hind paws on the floor, trying to climb the sides of the cylinder), eye blinks, foot licks, gasps, writhes, genital licks, head shakes, ptosis, teeth chattering and yawns. Ptosis was counted no more frequently than once per minute. An observer assessed locomotor activity (four paws on the floor, moving) during the same period using a stopwatch; since we wanted to have all recordings during the same time period and in the same context, activity chambers were not employed. A different observer assessed locomotor activity from video recordings at a later date. The difference between the recordings of the two observers was not significant in all cases. All somatic signs were merged for analyses; escape attempts were analyzed separately.

2.4. Resumption of nicotine intake (re-exposure to drug)

One week after the withdrawal phase, nicotine was provided again using the two-bottle free choice procedure. The amount of liquid consumed from nicotine and water bottles and weights of the animals were recorded one day after resumption of intake.