

Genetic Background Influences Nicotine-induced Conditioned Place Preference and Place Aversion in Mice

Yuya Ise^{1,2}, Tomohisa Mori², Shirou Katayama¹,
Tsutomu Suzuki² and Tzu-Chueh Wang³

¹Section of Pharmaceutical Services, Nippon Medical School Hospital

²Department of Toxicology, Hoshi University, School of Pharmacy and Pharmaceutical Science

³Department of Pharmacy, Chia Nan University of Pharmacy and Science, Taiwan

Abstract

This study was designed to determine whether genetic differences influence the rewarding effects of nicotine in 4 inbred strains of mice (DBA/2, BALB/c, C3H, and C57BL/6). Nicotine (subcutaneous) induced a place preference in DBA/2 and BALB/c mice but a place aversion in C57BL/6 mice. A low dose of nicotine produced a significant place preference, whereas a high dose of nicotine produced place aversion in C3H mice. These effects were completely reversed by the nicotinic receptor antagonist mecamylamine. These results strongly suggest that a conditioned state, such as rewarding effects or aversive effects, can be influenced by genetic background.

(J Nippon Med Sch 2014; 81: 53–56)

Key words: conditioned place preference paradigm, nicotine, mecamylamine, genetic difference, mice

Introduction

The greater concurrence of smoking behavior in monozygotic twins than in dizygotic twins¹ suggests that nicotine dependence is regulated by genetic factors. Differential effects of nicotine on open-field behavior, the Y-maze cross, and conditioned taste aversion have been observed in 4 inbred strains of mice (DBA/2, BALB/c, C3H, and C57BL/6). Furthermore, 6 strains of mice (A, BUB, C3H, C57BL/6, DBA/2, and ST/B) differ markedly in oral self-selection of nicotine, with C57BL/6 exhibiting the highest intake of nicotine solution, and ST/B the

lowest². These results suggest that genetic factors play an important role in the behavioral effects induced by nicotine.

Previous studies have shown that conditioned states, such as reinforcing/rewarding effects of abused drugs, are defined by genetic factors. Aversive effects might also be regulated by genetic factors. Therefore, the present study was designed to investigate differences in the acquisition of rewarding or aversive effects induced by nicotine in 4 inbred strains of mouse (DBA/2, BALB/c, C3H, and C57BL/6) as measured with a conditioned place-preference paradigm.

Correspondence to Tsutomu Suzuki, PhD, Department of Toxicology, Hoshi University, School of Pharmacy and Pharmaceutical Science, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan

E-mail: Suzuki@hoshi.ac.jp

Journal Website (<http://www.nms.ac.jp/jnms/>)

Materials and Methods

The study was performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, as adopted by the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Male mice (body weight, 25–30 g) of the DBA/2N, BALB/c, C3H/HeN, and C57BL/6N strains were obtained from Tokyo Animal Laboratories (Tokyo, Japan). The animals were housed in a temperature-controlled ($22^{\circ}\text{C} \pm 1^{\circ}\text{C}$) room, and maintained on a 12-hour light/dark cycle (lights on from 8 : 00 a.m. to 8 : 00 p.m.). Food and water were available *ad libitum*.

Place conditioning was performed as described previously³⁴. The apparatus was a shuttle box (15 cm wide $\times$ 30 cm long $\times$ 15 cm high) made of acrylic sheets and divided into 2 compartments of equal size. To create equally preferred compartments, 1 compartment was white with a textured floor, and the other was black with a smooth floor. Conditioning sessions (3 for nicotine, 3 for saline) were held once daily for 6 days. Immediately after subcutaneous treatment with nicotine or saline, the animals were placed in the white or black compartment for 1 hour. On alternate days, the animals were given injections of saline or nicotine and then placed in the other compartment. In the combination study, mecamylamine (1 mg/kg, subcutaneous) was administered 30 minutes before subcutaneous treatment with nicotine. On day 7, tests of conditioning were performed as follows. The partition separating the 2 compartments was raised to 7 cm above the floor, and a neutral platform was inserted along the seam separating the compartments. Mice that had not been treated with either nicotine or saline were placed on the platform. The time spent in each compartment during a 900-second session was recorded automatically with an infrared beam sensor (KN-80, Natsume Seisakusho Co., Tokyo, Japan). The mean conditioning score was the time spent in the nicotine-conditioned compartment minus the time spent in the saline-

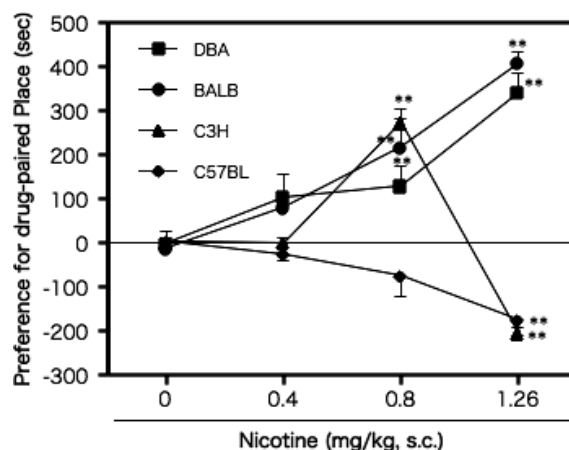


Fig. 1 Subcutaneous administration of nicotine produced a place preference or place aversion in 4 inbred strains of mice. Each point represents the mean $\pm$ S.E.M. of 8 to 11 mice. ** $P < 0.01$ vs. saline control.

conditioned compartment. All sessions were held under conditions of dim illumination and masking white noise.

The drugs used in the present study were (–)-nicotine hydrogen tartrate (Sigma-Aldrich, St. Louis, MO, USA) and mecamylamine hydrochloride (Sigma-Aldrich). Both drugs were dissolved in saline.

Conditioning scores represent the time spent in the postconditioning score of the drug-injected place minus the time spent in the preconditioning score of there and are expressed as the mean (seconds) $\pm$ standard error of the mean (S.E.M.). Behavioral data were evaluated statistically with one-way analysis of variance followed by Bonferroni's *post hoc* test. A P value of <0.05 was considered to indicate statistical significance.

Results

Nicotine produced a dose-dependent place preference in DBA/2 mice ($F_{3,36}=13.24$, $P < 0.01$) and in BALB/c mice ($F_{3,37}=24.84$, $P < 0.01$) and a place aversion in C57BL/6 mice ($F_{3,34}=7.68$, $P < 0.01$). On the other hand, nicotine produced both a significant dose-dependent place preference ($F_{2,28}=64.90$, $P < 0.01$) and a dose-dependent place aversion ($F_{2,28}=125.84$, $P < 0.01$) in C3H mice (**Fig. 1**). These effects were completely reversed by pretreatment with the nicotinic receptor antagonist mecamylamine ($P < 0.01$, **Fig. 2**).

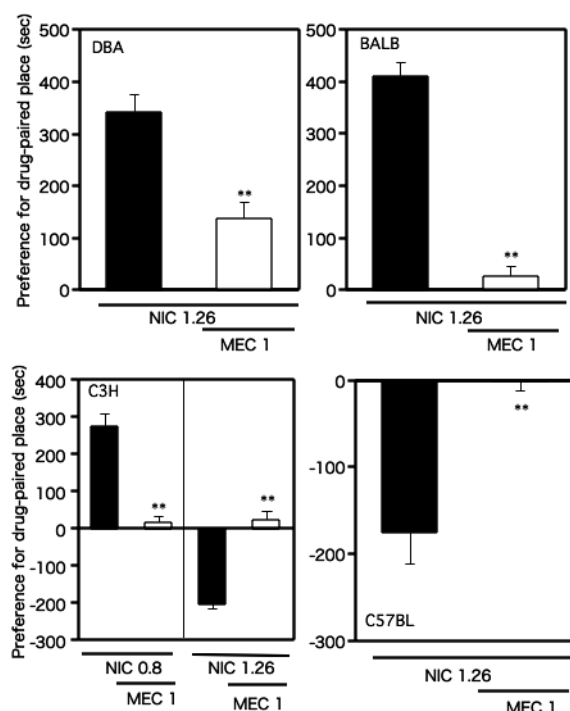


Fig. 2 Subcutaneous pretreatment with mecamylamine (MEC) completely reversed the nicotine (NIC)-induced place preference or place aversion in 4 inbred strains of mice. Each column represents the mean $\pm$ S.E.M. of 8 to 11 mice. ** $P < 0.01$ vs. nicotine alone.

Discussion

Inbred strains are produced by mating close relatives over approximately 20 consecutive generations to obtain homozygosity of virtually all genetic loci. A member of a given inbred strain can be considered a genetic replicate of all other members of the strain. Different inbred strains possess different genotypes². Therefore, inbred mice strains are useful for investigating genetic differences in the behavioral and biochemical effects of several abused drugs.

Our study has demonstrated that subcutaneous administration of nicotine produces a dose-dependent place preference in DBA/2 and BALB/c mice. These results are consistent with a previous report that DBA/2 and BALB/c mice are high responders to the reinforcing/rewarding effects induced by abused drugs². It is possible that the rewarding effect of nicotine is more likely to develop in DBA/2 and BALB/c mice than in other inbred

strains of mice. The mesolimbic dopaminergic system is a major neural substrate of the rewarding effect of nicotine. Therefore, the present findings support the idea that DBA/2 and BALB/c mice are sensitive to the activation of the mesolimbic dopaminergic system induced by nicotine.

In the present study C3H mice showed a biphasic response to nicotine-induced place preference. This result is consistent with previous findings that nicotine produces biphasic behavioral effects². However, why a high dose of nicotine did not produce a rewarding effect in C3H mice is unclear. Furthermore, nicotine produced a dose-dependent place aversion in C57BL/6 mice. Nicotine-induced toxicity may have influenced aversive effects, and the mechanism of nicotine-induced place aversion should be addressed in future research.

Genetic-engineering studies in mice have identified several nicotinic acetylcholine receptor subunits that are critical for nicotine to activate the reward system in the brain, consisting of the dopaminergic cell bodies in the ventral tegmental area and their terminals in the nucleus accumbens and other portions of the mesolimbic system⁵. The different constitution of nicotinic acetylcholine receptor subunits in the 4 inbred strains of mice might cause the different results in the present study.

Nicotine-induced place preference and place aversion were completely reversed by the nicotinic receptor antagonist mecamylamine, which passes the blood-brain barrier. These results indicate that the effects of nicotine are mediated by specific action at central nicotinic receptors.

Acknowledgement: This work was supported in part by a Research Grant (8A-5) for Nervous and Mental Disorders and a Research Grant from the Ministry of Health, Labour and Welfare to T. Suzuki. We wish to thank Mr. Takahiro Moriizumi and Ms. Maki Tsubai for their expert technical assistance.

References

1. Fischer RA: Lung cancer and cigarettes? *Nature* 1958; 182: 254.
2. Mohammed AH: Genetic dissection of nicotine-related behavior: a review of animal studies. *Behav*

- Brain Res 2000; 113: 35–41.
3. Suzuki T, Funada M, Narita M, Misawa M, Nagase H: Morphine-induced place preference in the CXBK mice: characteristics of μ opioid receptor subtypes. Brain Res 1993; 602: 45–52.
 4. Ise Y, Mori T, Katayama S, Nagase H, Suzuki T: Rewarding effects of ethanol combined with low doses of morphine through dopamine D1 receptors. J Nippon Med Sch 2013; 80: 34–41.
 5. Mineur YS, Picciotto MR: Genetics of nicotinic acetylcholine receptors: relevance to nicotine addiction. Biochem Pharmacol 2008; 75: 323–333.
- (Received, November 13, 2012)
(Accepted, December 11, 2012)
-