Pharmacology Biochemistry and Behavior, Vol. 63, No. 1, pp. 27–32, 1999

0091-3057/99/$–see front matter

PII S0091-3057(98)00262-7

Discriminative Stimulus (DS) Properties of Nicotine in the C57BL/6 Mouse

S. A. VARVEL,* J. R. JAMES,† S. BOWEN,† J. A. ROSECRANS,† AND L. D. KARAN‡

*Department of Psychology, Virginia Commonwealth University, Box 842020, Richmond, VA,
†Department of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA,
‡Department of Internal Medicine, Division of Substance Abuse Medicine, Medical College of Virginia, Virginia Commonwealth University, Box 980109 Richmond, VA 23298

Received 6 March 1998; Revised 2 September 1998; Accepted 28 September 1998

VARVEL, S. A., J. R. JAMES, S. BOWEN, J. A. ROSECRANS AND L. D. KARAN. *Discriminative stimulus properties of nicotine in the C57BL/6 mouse.* PHARMACOL BIOCHEM BEHAV **63**(1) 27–32, 1999.—Previous research conducted in this and other laboratories has examined the role of genetic factors in determining sensitivity to (−)-nicotine in a variety of behavioral and physiological measures in the rat. More recent research further indicates that genetic factors can also influence the level of sensitivity to (−)-nicotine when serving as a discriminative stimulus (DS) in different rat strains. However, there has been little work examining the influence of genotype on the discriminative stimulus (DS) properties of (−)-nicotine in mice, a species that has played a major role in understanding the relationship between genetics and (−)-nicotine pharmacological effects. To further our understanding of the role of genetics and the ability of (−)-nicotine to exert DS control of behavior in the mouse, a group of C57BL/6 mice was trained to discriminate 0.4 mg/kg (−)-nicotine from saline using a two-lever operant procedure. (−)-Nicotine's discriminative stimulus in C57BL/6 mice appears to be similar to that generated in the rat. Results from behavioral tests with other drugs indicated that *d*-amphetamine exhibited a partial generalization, while (+)-nicotine fully generalized with nicotine. Tests of antagonism with mecamylamine and scopolamine further showed the cholinergic specificity of the (−)-nicotine DS in the mouse; mecamylamine but not scopolamine completely antagonized the (−)-nicotine DS. This work lays the groundwork for future comparisons of different mouse strain's sensitivities to (−)-nicotine's discriminative stimulus as well as using this behavioral model to search for new nicotinic receptor agonists and antagonists. © 1999 Elsevier Science Inc.

Nicotine Drug discrimination C57BL/6 mice (−)-Nicotine Mecamylamine Scopolamine Amphetamine Genotype

DRUG discrimination techniques can be useful tools for characterizing the mechanisms of centrally acting drugs, and they have proven to be particularly important to the study of drugs of abuse. After all, it seems quite reasonable that a property of a drug that creates an internal state that can "set the occasion" for certain behaviors to be reinforced would be extremely relevant to the dynamics of human drug abuse. This intuitive suspicion has been confirmed over the past 3 decades by the wealth of information obtained by applying these techniques to investigate the actions of many different classes of drugs of abuse [for some recent reviews, see (1,2,4,40,42)]. (−)-Nicotine has been repeatedly shown to produce a reliable, robust discriminative stimulus (DS) in rats, and this DS has been extensively characterized pharmacologically [e.g., (30,39)] in an attempt to better understand the neurochemical basis for the behavioral effects of nicotine and ultimately to relate that understanding to smoking behavior. The (−)-nicotine cue appears to be mediated through central nicotinic–cholinergic receptors, because the ganglionic nicotinic antagonist mecamylamine has been shown to completely block the (−)-nicotine cue, while peripheral nicotinic antagonists such as hexamethonium and muscarinic antagonists such as scopolamine fail to do so (29,33,39). The (−)-nicotine cue has also been shown to be extremely specific, as many drugs with similar chemical structures fail to produce generalization (6,31). Much has been learned about the actions of (−)-nicotine in the CNS using drug discrimination procedures in rats, yet this paradigm has not been applied to investigating the actions of (−)-nicotine in mice. In fact, few investigators have examined the discriminative stimulus properties of any drug in mice, de-

Requests for reprints should be addressed to S. A. Varvel, Virginia Commonwealth University, Department of Psychology, Box 842020, Richmond, VA 23284.

spite the growing importance of mice to the field of pharmacology [however, see (3,18,38)]. The wide availability of a large variety of mouse strains with varying sensitivities to the effects of nicotine as well as recent advances in molecular biology provides an opportunity to explore the role that genotype plays in the mediation of (−)-nicotine's discriminative cue.

Numerous studies have shown a relationship between genotype and sensitivity to both the acute and chronic effects of (−)-nicotine [e.g., (8)]. Different strains of mice have shown varying degrees of sensitivity to (−)-nicotine across a variety of behavioral and physiological measures, and rank orderings of different strains' sensitivities have been obtained for many measures. Interestingly, sensitivities to the effects of (−)-nicotine are not the same across all measures. For example, while male DBA mice were shown to be more sensitive to (−)-nicotine than C3H mice in a measure of locomotor depression in a Y-maze task (13), they were the least sensitive strain tested in a measure of (−)-nicotine–induced seizures (22). A detailed comparison of the effects of nicotine in 19 different inbred mouse strains in a behavioral test battery showed that some, but not all, of the effects of nicotine were correlated with each other. A factor analysis of this data suggested that two underlying variables were influencing nicotine sensitivity: one variable influenced sensitivity to measures such as Y-maze rearings, Y-maze crossings, and body temperature, while the second variable influenced measures of nicotine induced seizures and seizure latencies (15). Thus, it is likely that there are at least two distinguishable mechanisms by which the behavioral effects of nicotine are mediated.

The differences in the sensitivities of various mouse strains to (−)-nicotine in different behavioral measures can at least in part be explained by differences in receptor characteristics, as there seems to be no differences in the rate of metabolism of (−)-nicotine across these strains (13,24). Neuronal nicotinic–cholinergic receptors are known to be composed of α and β subunits that combine to form ligand-gated ion channels, and receptor binding experiments have shown that there are at least two types of distinct nicotinic binding sites in the CNS. One type binds labeled [^{3}H]nicotine and [^{3}H]cytosine with a high affinity (14,16), and is thought to be primarily be composed of α_4 and β_2 subunits (11). Another type binds [^{127}1]α-bungarotoxin (36), and is thought to be composed of α_7 subunits (7,12,23). Additionally, the large number of functional receptors produced by combining different subunits in Xenopus oocyte expression systems suggests that more nicotine receptor subtypes are likely to be identified [for a review see (17)]. By examining a wide range of mouse strains, the relative abundance of these receptor subtypes can be correlated with the sensitivities of each strain to the different effects of nicotine. For example, studies examining (−)-nicotine–induced seizures in different mouse strains have shown a relationship between seizure sensitivity and the number of α-bungarotoxin binding sites in the brain. The most sensitive strains (i.e., lower ED_{50}s and shorter latencies to seize) had the highest number of α-bungarotoxin binding sites (19–21). Conversely, sensitivities to Y-maze crosses, Y-maze rears, and body temperature were highly correlated with overall numbers of nicotinic binding sites labeled with [^{3}H]nicotine (15).

The primary purpose of the present set of experiments is to determine whether or not C57BL/6 mice can be trained to discriminate (−)-nicotine from saline in a two-lever operant procedure. An initial characterization of the discriminative cue will lay the groundwork for future comparisons of different mouse strain's sensitivities to the (−)-nicotine DS. This should help to characterize the receptor mechanisms mediating the (−)-nicotine DS and show to what extent genotype influences the expression of those mechanisms.

METHOD

Subjects

Twenty-two male C57BL/6 mice were obtained from Jackson Laboratories, weighing 24–30 g. Mice were housed individually in an animal colony room that was temperature and humidity controlled, and maintained on a 12 L:12 D cycle (lights were on from 0600 to 1800 h PM). Mice were allowed 2 weeks to acclimate before discrimination training. Water was available ad lib in the colony room, and the animals were restricted to 3–4 g/day of Rodent Chow to facilitate operant responding for food reward.

Apparatus

All tests were conducted in four Plexiglas operant chambers, the inside of which measured 15 cm (L) × 11.5 cm (D) × 17.5 cm (H), with stainless steel grid floors [for a more complete description of the operant chambers see (3)]. On one wall of the chamber a stainless steel lever was situated on either side of a central opening that allowed access to a dipper that delivered a 0.02 ml sugar-milk reinforcement (33% milk, 33% water, and 33% sucrose). As each reinforcement requirement was met, the dipper scooped up approximately 0.1 ml of the sugar-milk solution and returned to its resting position, allowing the mouse access. The operant chambers were secured in sound- and light-attenuating boxes. Operant contingencies and data collection were controlled with a Med-PC interface and software (Med associates, St. Albans, VT).

Drugs

All drugs were dissolved in 0.9% saline, and all drug concentrations are expressed as bases. (−)-Nicotine bitartrate, (+)-nicotine (prepared by Dr. Everette May, MCV), and *d*-amphetamine sulfate (Sigma, St. Louis, MO) were injected 5 min before the start of the operant sessions. Mecamylamine (Sigma, St. Louis, MO) was injected 10 min, and scopolamine HCl (Sigma, St. Louis, MO) 15 min before testing. All injections were subcutaneous at an injection volume of 10 ml/kg.

Statistical Analysis

A one-factor repeated measures analysis of variance (ANOVA) was performed on the data with a GB-STAT for Windows statistical program. Where appropriate, Tukey's Protected *T* post hoc analysis was used to determine the differences between group means. ED_{50} calculations were performed by The Pharmacologic Calculation System (41).

Lever Pressing and Discrimination Training

On day 1, mice were placed in the operant chambers and responding was reinforced on one lever on an FR1 schedule. Initial lever pressing was facilitated by placing a drop of the sugar-milk solution on the lever. Once consistent lever pressing on the first lever was attained, the active lever (i.e., the lever that triggered reinforcement) was switched, and the mice were required to press the other lever on an FR1 reinforcement schedule. Once all mice could press both levers, one lever was assigned as the drug lever, and the other lever was assigned as the vehicle lever. Lever assignments were counterbalanced to control for side preferences and any odor cues (9). Each

mouse was then given 4 days of vehicle, followed by 4 days of 0.21 mg/kg (−)-nicotine, 4 days of vehicle, and 4 days of 0.21 mg/kg (−)-nicotine, with only the appropriate lever being active as the FR requirement was systematically increased to FR20.

Discrimination training consisted of 15-min sessions Monday through Friday on a double-alternation schedule, with a FR20 reinforcement schedule. The ratio requirement was reset if a mouse switched levers before completion of the FR20. Mice were considered ready for testing when they responded on greater than 85% on the correct lever and completed their first FR on the correct lever on 4 out of 5 consecutive days.

Testing Procedures

All tests were conducted by reinforcing lever presses on either lever (FR20) during 2-min test sessions. The percent of responding on the drug lever (%DLR), the lever on which the first FR requirement was met, and response rates were recorded. Tests were conducted on Tuesdays and Fridays, and continued training sessions were run on Mondays, Wednesdays, and Thursdays throughout the duration of the study. For the initial (−)-nicotine dose–response curve the doses 0.07, 0.14, 0.28, and 0.42 mg/kg (−)-nicotine were tested with a Latin square design. Subsequently, to obtain an adequate dose response curve, 0.1 and 0.56 mg/kg (−)-nicotine were also tested. Antagonism tests used 0.3 mg/kg mecamylamine or 0.03 mg/kg scopolamine administered 10 or 15 min (respectively) before administration of 0.42 mg/kg nicotine (the training dose, as described below). Generalization tests with *d*-amphetamine and (−)-nicotine were also conducted.

Another set of experiments were conducted to determine the time course of (−)-nicotine's discriminative cue. Injections of 0.42 mg/kg (−)-nicotine were given at various time points before the start of the 2-min test session. During the first test animals were placed in the operant chambers immediately after injection. Due to difficulty in establishing the exact time it took to place all the mice (four at a time) in their chambers and start the program, this time interval was considered simply <1 min. Tests were subsequently conducted 5, 15, 30, and 60 min after drug administration.

RESULTS

Training

Initial difficulty was experienced in determining the correct training dose to use in the present set of experiments because no published data on mice trained to discriminate (−)-nicotine was found, and due to our desire to discover the minimally effective training dose for this strain. Most rank orderings of sensitivity to (−)-nicotine ranked C57BL/6 mice as one of the most sensitive strains (15,21), so we initially attempted to train them at a relatively low dose, 0.21 mg/kg. After 24 sessions of 0.21 mg/kg (−)-nicotine as the training dose, no mice could consistently choose the correct lever at the beginning of the session, so the training dose was raised to 0.28 mg/kg (−)-nicotine. After 28 sessions at 0.28 mg/kg (−)-nicotine, the mice were still not choosing the correct lever. The most consistent 12 responders (based on %DLR) were selected and the training dose was raised to 0.42 mg/kg (−)-nicotine. After 18 sessions at 0.42 mg/kg (−)-nicotine, all but one mouse achieved our discrimination criteria.

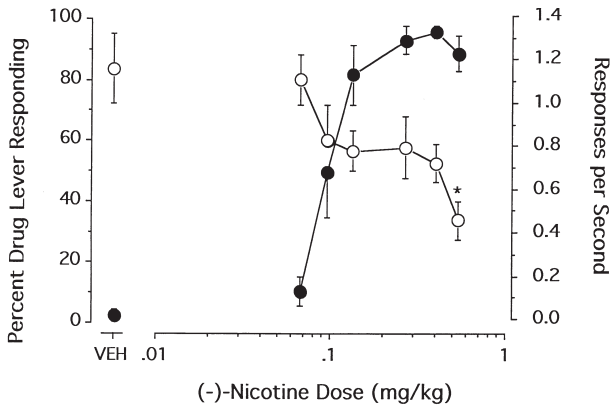

FIG. 1. Percent drug-lever responding (filled circles) and responses per minute (empty circles) as a function of dose of (−)-nicotine. Data are expressed as means ± SEM. Mice were tested 5 min after SC injection of nicotine. *$p < 0.05$ (different from vehicle), $n = 10$.

Dose–Effect of (−)-Nicotine

The effects of increasing doses of (−)-nicotine on percent drug lever responding (%DLR) and response rates are shown in Fig. 1. Percent drug lever responding increased systematically across all doses, $F(6, 54) = 29.24$, $p < 0.0001$, except for 0.56 mg/kg, which showed a slight (but nonsignificant) decrease. Tukey's Protected *T* post hoc analysis showed that every dose except for 0.07 mg/kg elicited %DLR significantly higher than vehicle ($p < 0.01$), and the maximum %DLR was observed at the training dose of 0.42 mg/kg. The ED_{50} was determined to be 0.11 mg/kg, with a 95% confidence interval of 0.09–0.14 mg/kg. Response rates were significantly affected by dose of nicotine, $F(6, 54) = 6.24$, $p < 0.0001$. Responses per minute after injection of 0.56 mg/kg nicotine were significantly less than after vehicle administration ($p < 0.01$).

Time Course Analysis

The results of the time course analysis are shown in Fig. 2. The effect of the time between drug administration and test

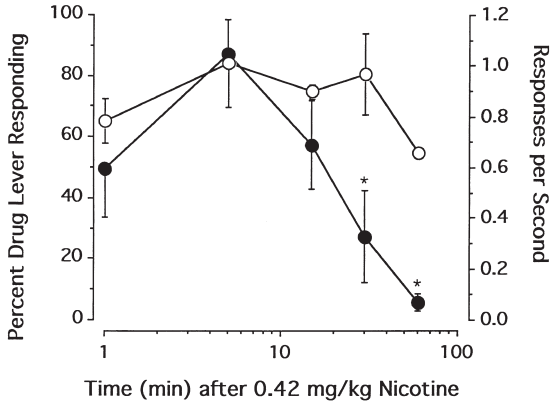

FIG. 2. Time course of the nicotine discriminative stimulus in the C57BL/6 mouse. Mice were injected with 0.42 mg/kg nicotine and tested after different time intervals. Percent drug lever responding (filled circles) and responses per minute (empty circles) are expressed as means ± SEM. *$p < 0.05$ (different from 5 min), $n = 10$.

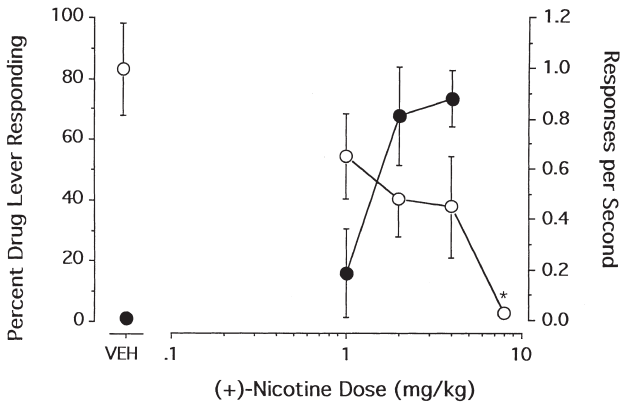

FIG. 3. Generalization to (+) nicotine. Mice were tested 5 min after SC injection of (+) nicotine. Percent drug lever responding (filled circles) and responses per minute (empty circles) are expressed as means ± SEM. *$p < 0.05$ (different from five vehicles), $n = 10$.

session on %DLR was significant, $F(4, 32) = 10.4$, $p < 0.0001$. When the mice were tested less than 1 min after administration of 0.42 mg/kg (−)-nicotine the mean %DLR was 49.6. After 5 min (the time point used with (−)-nicotine throughout the study), the mean %DLR had increased to 87.2. Post hoc analysis revealed that %DLR after 30 min (mean = 27.2) and 60 min (mean = 5.7) were significantly less than after the 5-min tests. No significant differences in rate of responding were observed at any time, $F(4, 32) = 2.29$, $p = 0.08$.

Generalization and Antagonism Tests

Results from the generalization tests with (−)-nicotine are shown in Fig. 3. (+)-Nicotine substituted for (−)-nicotine only at doses at least five times larger than the training dose of (−)-nicotine. The ED_{50} was calculated to be 1.75 mg/kg, with a 95% confidence interval of 0.75–4.05 mg/kg. Response rates were significantly affected by (+)-nicotine, $F(4, 24) = 5.28$, $p < 0.01$, with the 8.0 mg/kg dose suppressing rates entirely ($p < 0.01$).

The antagonism tests with mecamylamine and scopolamine and generalization tests with *d*-amphetamine are presented in Table 1. Mecamylamine (0.3 mg/kg) by itself did not elicit any responding on the drug lever, and when given in conjunction with 0.42 mg/kg (−)-nicotine completely blocked the (−)-nicotine cue. Response rates were not significantly different from vehicle. Scopolamine (0.03 mg/kg) given in conjunction with 0.42 mg/kg (−)-nicotine was unable to attenuate the cue properties of (−)-nicotine, even though this dose combination significantly reduced response rates ($p < 0.05$). Scopolamine (0.03 mg/kg) given alone did not elicit any responding on the drug lever, and had no significant effect on response rates. Both of the *d*-amphetamine doses that were tested (0.5 and 1.0 mg/kg) partially substituted for (−)-nicotine, and both doses suppressed response rates when compared with vehicle ($p < 0.01$).

DISCUSSION

The results obtained from the present series of experiments show that C57BL/6 mice can be trained to discriminate (−)-nicotine from saline in a two-lever operant procedure. Initial lever pressing and discrimination training took a total of 99 sessions to complete, though it is not known whether or not this many sessions will be required in future studies due to the difficulty experienced in determining an adequate training dose. It is our hope that the effort exerted here to identify the minimally effective training dose for C57BL/6 mice will help to guide future attempts to identify appropriate training doses for other mouse strains, as relative sensitivities to other effects of (−)-nicotine have been established for many strains [e.g., (21)].

Based on this initial study, the (−)-nicotine cue in C57BL/6 mice appears similar to the (−)-nicotine cue produced in rats. For example, the dose–effect curve and ED_{50} value generated ($ED_{50} = 0.11$) are comparable with those obtained from Sprague–Dawley rats trained at a similar dose in a previous study ($ED_{50} = 0.098$) (30). Also, the results from these initial generalization and antagonism tests (Table 1) are similar to those obtained from rats (29–31,34). The nicotinic antagonist mecamylamine completely blocked the (−)-nicotine cue without affecting response rates, while the muscarinic antagonist scopolamine failed to have any effect on the (−)-nicotine cue, even though response rates were significantly suppressed. (+)-Nicotine substituted for (−)-nicotine only at doses much higher than the training dose [the ED_{50} value generated for (+)-nicotine was almost 15 times higher than the ED_{50} for (−)-nicotine], suggesting a degree of stereospecificity of the

TABLE 1
OTHER GENERALIZATION AND ANTAGONISM TESTS

Drug	No. of Mice Substituting	%DLR (Mean ± SEM)	Responses/Minute (Mean ± SEM)
Vehicle	0/10	2.5 ± 1.97	1.16 ± 0.16
0.42 mg/kg (−)-Nicotine	10/10	96.0 ± 2.07	0.73 ± 0.09
0.3 mg/kg Mecamylamine	0/10	6.0 ± 4.43	1.13 ± 0.03
0.3 mg/kg Mecamylamine + 0.42 mg/kg (−)-Nicotine	0/10	0.1 ± 0.09	0.88 ± 0.14
0.03 mg/kg Scopolamine HCl	0/9	11.59 ± 4.99	1.1 ± 0.03
0.03 mg/kg Scopolamine HCl + 0.42 mg/kg (−)-Nicotine	8/9	93.5 ± 4.62	0.56 ± 0.14*
0.5 mg/kg *d*-Amphetamine	4/9	57.67 ± 14.69	0.48 ± 0.12*
1.0 mg/kg *d*-Amphetamine	4/9	53.18 ± 15.49	0.36 ± 0.13*

Number of mice substituting, percent drug lever responding (%DLR), and responses per minute from the generalization and antagonism tests with mecamylamine, scopolamine HCl, and *d*-amphetamine. For the purpose of comparison, results from control tests with vehicle and 0.42 mg/kg (−)-nicotine are also presented.

*$p < 0.05$ (different from five vehicles).

receptor mediating (−)-nicotine's DS. Partial generalization was produced by amphetamine, again mirroring results obtained from the rat (6,34). One difference between the results obtained in the present study and those generally obtained from studies with rats relates to the time course of the (−)-nicotine cue. The time course analysis revealed a rapid onset of the cue, which was completely gone after 60 min. This is in contrast to data obtained from Sprague–Dawley rats that indicate the cue can last up to twice as long (34). A probable explanation is differences in the metabolism of (−)-nicotine between mice and rats, as data suggests that mice metabolize (−)-nicotine faster than rats (32).

The ability to examine the discriminative stimulus properties of nicotine in mice will provide additional opportunities to investigate the genetic regulation of responsiveness to a behavioral effect that is likely to be relevant to the abuse liability of nicotine. A measure of relative sensitivity to the nicotine cue could be obtained by determining how many training sessions were required for each strain to learn a discrimination task using a selected dose of nicotine. Alternatively, a protocol could be developed to determine the minimally effective training dose of nicotine for each strain. Other strategies could employ new techniques in molecular biology that are particularly well suited to mice. Most notably, the availability of mouse strains that are deficient in certain receptor subunits (i.e., "knockout" mice) could help to clarify the role of different nicotine receptor subtypes. Several studies have demonstrated that mice that lack either β_2 or α_7 nicotine receptor subunits are viable and generally appear to be normal [e.g., (23,25)], and when compared to normal mice, dramatic differences in responsiveness to the effects of nicotine can be observed. For example, mice prepared with a null mutation for the β_2 subunit will not self-administer nicotine and display abnormal avoidance learning (25,26), while mice with a null mutation for the α_7 subunit express no α-bungarotoxin binding sites and lack the rapidly desensitizing nicotinic currents associated with them (23). If the absence of a particular subunit prevented mice from learning the nicotine discrimination task or appreciably altered its generalization profile, then the receptor(s) associated with that subunit would be implicated in playing a critical role in mediating the discriminative stimulus effects of nicotine.

Recent pharmacological evidence in rats suggests that the α_7 nicotinic receptor is not involved in the discriminative stimulus effects of nicotine, as the α_7 selective antagonist methyllycaconitine failed to block it (5), suggesting that it may be action at $\alpha_4\beta_2$ nicotinic receptors that is responsible for producing the nicotine cue. If mouse strains that are more sensitive to the DS effects of nicotine are shown to possess relatively higher levels of $\alpha_4\beta_2$ nicotinic receptors, they would be implicated in playing an important role in mediating the DS of nicotine. Sensitivities to nicotine of different mouse strains have already, for some behavioral and physiological measures, been strongly correlated with the maximum number of binding sites labeled by either [^{3}H]nicotine or [^{127}I] α-bungarotoxin (11,21). In fact, indirect evidence supporting the importance of $\alpha_4\beta_2$ receptors to the DS properties of (−)-nicotine comes from the finding that mice bred for sensitivity to the locomotor stimulating effects of nicotine (which are correlated with higher levels of [^{3}H]nicotine binding) (15) are also more sensitive to nicotine-induced conditioned place preference, an effect that is easily relatable to DS properties (35). Although a more complete understanding of which subunits assemble in vivo to form functional receptors and the development of more selective nicotinic agonists and antagonists will be required to fully characterize the receptor mechanisms that mediate the DS effects of nicotine, the use of inbred mouse strains to investigate the role of genotype has the potential to significantly advance this effort.

It will be important to determine whether the sensitivity of the nicotine discriminative stimulus will correlate with the expression of nicotinic receptor subunits across strains, as this research may have important implications for humans. There is a great deal of experimental evidence that genetics plays a key role in determining whether or not humans will become smokers (10,27,28,37), and it is likely that the relative expression of particular nicotinic receptor subunits (and the functional receptor subtypes they compose) contributes to this tendency. It also seems likely that the discriminative stimulus properties of nicotine are related to the abuse potential of tobacco. Therefore, a clearer understanding of the link between the genetic regulation of nicotine receptors and sensitivities to the discriminative stimulus properties of nicotine could help to explain why some people become smokers, while others do not. This would, in turn, aid efforts to develop rational pharmacological strategies for helping people to quit smoking. These initial experiments with C57BL/6 mice have shown that such an analysis of the nicotine cue in mice is possible.

ACKNOWLEDGEMENTS

This research was funded by grant #K20DA00183-03 from NIH-NIDA.

REFERENCES

1. Andrews, J. S.: Drug discrimination and anxiolytic drugs. Behav. Pharmacol. 3:527; 1992.
2. Ator, N. A.: Drugs as discriminative stimuli. Pharmacol. Biochem. Behav. 42:362; 1992.
3. Balster, R. L.; Moser, V. C.: Pentobarbital discrimination in the mouse. Alcohol and Drug Res. 7:233–242; 1987.
4. Balster, R. L.; Mansbach, R. S.; Gold, L. H.; Harris, L. S.: Preclinical methods for the development of pharmacotherapies for cocaine abuse. In: Harris, L. S., ed. Problems of drug dependence 1991. NIDA Monograph 119. Washington, DC: U.S. Government Printing Office; 1992.
5. Brioni, J. D.; Kim, D. J. B.; O'Neill, A. B.: Nicotine cue: Lack of effect of the a7 nicotinic receptor antagonist methyllycaconitine. Eur. J. Pharmacol. 301:1–5; 1996.
6. Chance, W. T.; Murfin, D.; Krynock G. M.; Rosecrans, J. A.: A description of the nicotine stimulus and tests of its generalization to amphetamine. Psychopharmacology (Berlin) 55:19–26; 1977.
7. Clarke, P. B. S.: The fall and rise of neuronal a-bungarotoxin binding proteins. Trends Pharmacol. Sci. 13:407–413; 1992.
8. Collins, A. C.; Marks, M. J.: Progress towards the development of animal models of smoking-related behaviors. J. Addict. Disord. 10:109–126; 1991.
9. Extance, K.; Goudie, A.: Inter-animal olfactory cues in operant drug discrimination procedures in rats. Psychopharmacology (Berlin) 73:363–371; 1981.
10. Fischer, R. A.: Cancer and smoking. Nature 182:596; 1958.
11. Flores, C. M.; Rogers, S. W.; Pabreza, L. A.; Wolfe, B. B.; Kellar, K. J.: A subtype of nicotinic cholinergic receptor in rat brain is composed of a4 and b2 subunits and is up-regulated by chronic nicotine treatment. Mol. Pharmacol. 41:31–37; 1992.

12. Gopalakrishnan, M.; Buisson, B.; Touma, E.; Giordano, T.; Campbell, J. E.; Hu, I. C.; Donnelly-Roberts, D.; Arneric, S. P.; Bertrand, D.; Sullivan, J. P.: Stable expression and pharmacological properties of the human alpha 7 nicotinic acetylcholine receptor. Eur. J. Pharmacol. 290:237–246; 1995.
13. Hatchel, P. C.; Collins, A. C.: The influence of genotype and sex on behavioral sensitivity to nicotine in mice. Psychopharmacology (Berlin) 71:45–49; 1980.
14. Marks, M. J.; Miner, L. L.; Cole-Harding, S.; Burch, J. B.; Collins, A. C.: A genetic analysis of nicotine effects on open field activity. Pharmacol. Biochem. Behav. 24:743–749; 1986.
15. Marks, M. J.; Romm, F.; Campbell, S. M.; Collins, A. C.: Variation of nicotinic binding sites among inbred strains. Pharmacol. Biochem. Behav. 33:679–689; 1989.
16. Martino-Barrows, A. M.; Kellar, K. J.: [^{3}H]Acetylcholine and [^{3}H](−)-nicotine label the same recognition site in rat brain. Mol. Pharmacol. 31:169–174; 1987.
17. MeGehee, D. S.; Role, L. W.: Physiological diversity of nicotinic acetylcholine receptors expressed by vertebrate neurons. Annu. Rev. Physiol. 57:521–546; 1995.
18. Middaugh, L. D.; Bao, K.; Becker, H. C.; Daniel, S. S.: Effects of Ro 15-4513 on ethanol discrimination in CS7BL/6 mice. Pharmacol. Biochem. Behav. 38:763–767; 1991.
19. Miner, L. L.; Marks, M. J.; Collins, A. C.: Classical genetic analysis of nicotine-induced seizures and hippocampal nicotinic receptors. J. Pharmacol. Exp. Ther. 231:454–464; 1984.
20. Miner, L. L.; Marks, M. J.; Collins, A. C.: Genetic analysis of nicotine-induced seizures and hippocampal nicotinic receptors in the mouse. J. Pharmacol. Exp. Ther. 239:853–860; 1986.
21. Miner, L. L.; Collins, A. C.: Strain comparison of nicotine-induced seizure sensitivity and nicotinic receptors. Pharmacol. Biochem. Behav. 33:469–475; 1989.
22. Miner, L. L.; Collins, A. C.: The effect of chronic nicotine treatment on nicotine-induced seizures. Psychopharmacology (Berlin) 95:52–55; 1988.
23. Orr-Urtreger, A.; Goldner, F. M.; Saeki, M.; Lorenzo, I.; Goldberg, L.; De Biasi, M.; Dani, J. A.; Patrick, J. W.; Beaudet, A. L.: Mice deficient in the a7 neuronal nicotinic acetylcholine receptor lack α-bungarotoxin binding sites and hippocampal fast nicotinic currents. Neurosci. 17:9165–9171; 1997.
24. Peterson, D. R.; Norris, K. J.; Thompson, J. A.: A comparative study of the disposition of nicotine and its metabolites in three inbred strains of mice. Drug Metab. Dis. 12:727–731; 1984.
25. Picciotto, M. R.; Zoli, M.; Lena, C.; Bessis, A.; Lallemand, Y.; Le Novere, N.; Vincent, P.; Pich, E. M.; Brulet, P.; Changeux, J. P.: Abnormal avoidance learning in mice lacking functional high-affinity nicotine receptor in the brain. Nature 374:65–67; 1995.
26. Picciotto, M. R.; Zoli, M.; Rimondini, R.; Lena, C.; Marubio, L. M.; Pich, E. M.; Fuxe, K.; Changeux, J. P.: Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. Nature 391:173–177; 1998.
27. Pomerleau, O. F.; Collins, A. C.; Shiffman, S.; Pomerleau, C. S.: Why some people smoke and others do not: New perspectives. J. Consult. Clin. Psychol. 61:723–731; 1993.
28. Pomerleau, O. F.: Individual differences in sensitivity to nicotine: Implications for genetic research on nicotine dependence. Behav. Genet. 27:161–177; 1995.
29. Romano, C.; Goldstein, A.; Jewell, N. P.: Characterization of the receptor mediating the nicotine discriminative stimulus. Psychopharmacology (Berlin) 74:310–315; 1981.
30. Rosecrans, J. A.; Chance, W. T.: Cholinergic and non-cholinergic aspects of the discriminative stimulus properties of nicotine. In: Lal, H., ed. Discriminative stimulus properties of drugs. New York: Plenum Press; 1977:155–185.
31. Rosecrans, J. A.; Spencer, R. M.; Krynock, G. M.; Chance, W. T.: Discriminative stimulus properties of nicotine and nicotine-related compounds. In: Battig, K., ed. Behavioral effects of nicotine. Basel: S. Karger; 1978:70–82.
32. Sastry, B. V.; Chance, M. B.; Singh, G.; Horn, J. L.; Janson, V. E.: Distribution and retention of nicotine and its metabolite, cotinine, in the rat as a function of time. Pharmacology 50:128–136; 1995.
33. Schechter, M. D.; Rosecrans, J. A.: Effect of mecamylamine on discrimination between nicotine- and arecoline-produced cues. Eur. J. Pharmacol. 17:172–182; 1972.
34. Schecter, M. D.; Meehan, S. M.: Further evidence for the mechanisms that may mediate nicotine discrimination. Pharmacol. Biochem. Behav. 41:807–812; 1992.
35. Schecter, M. D.; Meehan, S. M.; Schecter, J. B.: Genetic selection for nicotine activity in mice correlates with conditioned place preference. Eur. J. Pharmacol. 279:59–64; 1995.
36. Schwartz, R. D.; Kellar, K. J.: Nicotinic cholinergic receptor binding sites in the brain: Regulation in vivo. Science 220:214–216; 1983.
37. Shields, J.: Monozygotic twins brought up together: An investigation into the genetic & environmental causes of variation in personality. London: Oxford University Press; 1962.
38. Snoddy, A. M.; Tessel, R. E.: Nisoxetine and amphetamine share discriminative stimulus properties in mice. Pharmacol. Biochem. Behav. 19:205–210; 1983.
39. Stolerman, J. P.; Kumar, R.; Reavill, C.: Discriminative stimulus effects of cholinergic agonists and the actions of their antagonists. In: Colpaert, F. C.; Balster, R. L., eds. Transduction mechanisms of drug stimuli. Berlin: Springer Verlag; 1988:32–43.
40. Stolerman, I. P.: Drug discrimination. In: Haaren, F. V., ed. Methods in behavioral pharmacology. Amsterdam: Elsevier; 1993.
41. Tallarida, R. J.; Murray R. B.: The pharmacologic calculation system, ver. 4.0. New York: Springer Verlag; 1986.
42. Woods, J. H.; France, C. P.; Comer, S. D.: The discriminative stimulus effects of opioids. Psychopharmacology (Berlin) 101:s76; 1990.