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Nicotine-Induced Conditioned Place Preference and Conditioned Place Aversion in Mice

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RISINGER, F. O. AND R. A. OAKES. *Nicotine-induced conditioned place preference and conditioned place aversion in mice.* PHARMACOL BIOCHEM BEHAV 51(2/3) 457–461, 1995.—The motivational effects of nicotine were examined in mice using an unbiased place conditioning design. Swiss–Webster mice received four 15-min parings of a tactile stimulus with different doses of nicotine (0.25–2.0 mg/kg, IP). A different tactile stimulus was paired with saline injections. During conditioning, nicotine produced locomotor depression at the 2.0-mg/kg dose, with the greatest reduction in activity occurring during the latter part of each nicotine conditioning session. After four trials, nicotine produced increases in locomotor activity during the initial part of the nicotine sessions at doses 0.5 mg/kg or above. Upon testing, nicotine-induced conditioned place preference was noted in mice receiving 0.5 mg/kg nicotine. Conditioned place aversion was noted in mice receiving 2.0 mg/kg nicotine whereas doses of 0.25 and 1.0 mg/kg produced no conditioning. These results indicate that nicotine has dose-dependent rewarding and aversive effects measured in an unbiased place conditioning paradigm using mice.

Nicotine Mice Reward Aversion Locomotor activity Place conditioning

NICOTINE addiction, which has been likened to the addiction produced by cocaine or heroin, has become widely accepted as the mechanism supporting chronic tobacco use (27). However, this premise has been strongly criticized, in part, because demonstrations of nicotine's rewarding or reinforcing effects appear difficult to achieve (25). For example, although nicotine functions as an effective reinforcer in intravenous self-administration paradigms [e.g. (7,15,16)], the conditions under which nicotine is self-administered are more limited than other drugs of abuse (15). Thus, at present, nicotine's reinforcing effects measured in self-adminstration paradigms are often viewed as modest when compared to other drugs [e.g. (11)].

An alternative procedure used for studying the motivational properties of abused drugs is place conditioning [cf. (4)]. Studies of nicotine reward using this task have yielded mixed results. Although some reports indicate nicotine-induced conditioned place preference (1,3,12,14,17), a number of other studies indicate a lack of conditioned place preference (5,23) or, in some cases, conditioned place aversion (13,14,18). These discrepancies are also generally consistent with the notion that nicotine displays rewarding actions under a limited set of conditions (13,18,26).

Whereas previous place conditioning studies with nicotine have used rats as experimental subjects, recent studies with other drugs suggest that mice are equally suited to the study of reward (9,10,21). Moreover, the results of a preliminary study in this lab, using mice, suggest nicotine interacts with ethanol in the place conditioning design (24). These studies have encouraged the use of a similar design in examination of nicotine's motivational effects in mice. We report here, in mice, nicotine-induced conditioned place preference at a 0.5-mg/kg nicotine dose and nicotine-induced place aversion at a 2.0-mg/kg nicotine dose.

METHOD

Subjects

Male Swiss–Webster mice were obtained from Simonson (Gilroy, CA) at 7 weeks of age and were allowed to acclimate to the colony for 1 week prior to the beginning of the experiment. They were housed in polypropylene cages (33 × 16 × 13 cm) with cob-type bedding replaced twice weekly. A 12L : 12D cycle was in effect with the onset of the light portion of the cycle beginning at 0700 h. Experimental procedures were conducted during the light portion of the cycle. Food and

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water were continuously available in the home cage and the colony room temperature was maintained at 22 ± 2°C.

Drugs

Nicotine [(−)-nicotine di(+)tartrate salt, Sigma Chemical Co., St. Louis, MO] was mixed in physiological saline in a concentration corresponding to the base weight of each dose (see Procedure below). Injections were IP at a 10 ml/kg volume.

Apparatus

The place conditioning apparatus consisted of eight identical Plexiglas and aluminum chambers (30 × 15 × 15 cm) enclosed in ventilated, light- and sound-attenuating boxes (Med Associates ENV-015M; St. Albans, VT). Infrared light sources and detectors were positioned opposite each other at 5-cm intervals on the long walls of each place conditioning chamber, 2.2 cm above the floor surface. Occlusion of the infrared light beams was used both as a measure of general activity and to determine the animal's position (left or right side) in the chamber. Data were recorded each minute by computer.

The floor of each box consisted of interchangeable halves with one of two distinctive textures: "hole" floors were made from perforated stainless steel with 6.4-mm round holes on 9.5-mm staggered centers; "grid" floors were composed of 2.3-mm stainless steel rods mounted 6.4 mm apart in Plexiglas rails.

Procedure

The experiment involved three phases: habituation (one session), conditioning (eight sessions), and testing (one session). Sessions were conducted daily with a 2-day break between the first four and second four conditioning sessions.

Habituation. During habituation, all subjects received saline (10 ml/kg) and were immediately place in the conditioning apparatus for 15 min on a smooth floor covered with paper. Subjects were not exposed to the distinctive floor textures to avoid the development of latent inhibition (22). The habituation session was intended to reduce the novelty and stress associated with handling, injection, and exposure to the apparatus.

Conditioning. During the conditioning phase, mice were randomly assigned to one of four nicotine dose groups: 0.25, 0.5, 1.0, or 2.0 mg/kg nicotine. Within each group, mice were randomly assigned to one of two conditioning subgroups (n = 9–12/group) and exposed to an unbiased differential conditioning procedure. On alternate days mice received nicotine (CS+ days) prior to placement on the grid floor (grid+ subgroup) or the hole floor (grid− subgroup). Mice received saline (CS− days) prior to placement on the opposing floor type. Therefore, one complete conditioning trial consisted of a pairing of a distinctive floor after nicotine exposure and a pairing of a different distinctive floor with saline. Presentation of CS+ and CS− days was counterbalanced for order of presentation. Thus, the conditioning subgroups within each nicotine dose group were matched for exposure to nicotine and floor type, and differed only in the specific floor–nicotine relationship [cf. (8)]. To determine floor preference in the absence of nicotine–floor parings, a separate group of mice (n = 20) received saline injections paired with exposure to both floors.

Testing. For the preference test, all subjects received saline injections before placement in the apparatus for a 30-min session with half grid floor and half hole floor (left/right position counterbalanced within groups).

Data Analysis

Conditioning activity data were analyzed by unweighted means analysis of variance (ANOVA) using an alpha level of 0.05. For the preference test data, initial analysis consisted of overall ANOVA comparisons of time on the grid floor with nicotine dose and conditioning subgroup as factors. Following a significant nicotine dose × conditioning subgroup interaction, planned comparisons of conditioning subgroup at each nicotine dose were conducted using a Bonferroni correction (19) for familywise error (alpha of 0.05/four comparisons = corrected alpha of 0.0125 for each follow-up analysis). An additional analysis of the preference test data was conducted using a within-subjects comparison of the time spent on the nicotine-paired floor (i.e., time on the grid floor for the grid+ groups and time on the hole floor for the grid− groups) with time spent on the saline-paired floor. Conditioning subgroup was collapsed for each nicotine dose and the saline-only group was included in this analysis with time spent on the grid floor used as the drug-paired condition. Probability levels where $0.01 < p < 0.05$ are listed. All other significant outcomes are $p < 0.01$.

RESULTS

Conditioning

Figure 1 depicts mean ± SEM activity rate during the first 5 min (top panels) and last 5 min (bottom panels) of conditioning trial 1 (left panels) and conditioning trial 4 (right panels). In general, activity was highest during the first 5 min of the session and declined within each session. On trial 1, the 2.0-mg/kg nicotine dose caused reductions in locomotor activity that were greatest during the last 5 min of the session. Lower nicotine doses did not produce either locomotor stimulation or depression during conditioning trial 1. However, after four conditioning trials, nicotine produced relative increases in locomotor activity levels. Specifically, the 0.5, 1.0, and 2.0 mg/kg doses produced higher levels of activity during the first 5 min of CS+ trial 4, compared to activity during CS− trial 4. During the latter part of CS+ trial 4, activity levels in mice receiving 0.5 and 1.0 mg/kg nicotine declined to saline levels. The locomotor activation displayed in the first 5 min of CS+ trial 4 by mice receiving 2.0 mg/kg nicotine was replaced by locomotor depression in the last 5 min of CS+ trial 4.

Overall analysis of activity levels (nicotine dose × trial type) during the first 5 min of conditioning trial 1 yielded significant effects of nicotine dose, $F(3, 86) = 5.4$, and nicotine dose × trial type, $F(3, 86) = 6.8$. Within-dose group comparisons showed a significant trial type effect for the 2.0-mg/kg nicotine dose, $F(1, 23) = 33.5$, but not for the other nicotine dose groups (all $F < 1.6$). Similar results were seen in an analysis of the last 5-min activity levels on conditioning trial 1, with overall significant effects of nicotine dose, $F(3, 86) = 6.1$, trial type, $F(1, 86) = 20.9$, and nicotine dose × trial type, $F(3, 86) = 22.1$. Again, significant within-group trial type effects were seen in the 2.0-mg/kg dose, $F(1, 23) = 145.7$, but not in the lower dose groups (all $F < 2.1$).

Analysis of activity levels during the first 5 min of condi-

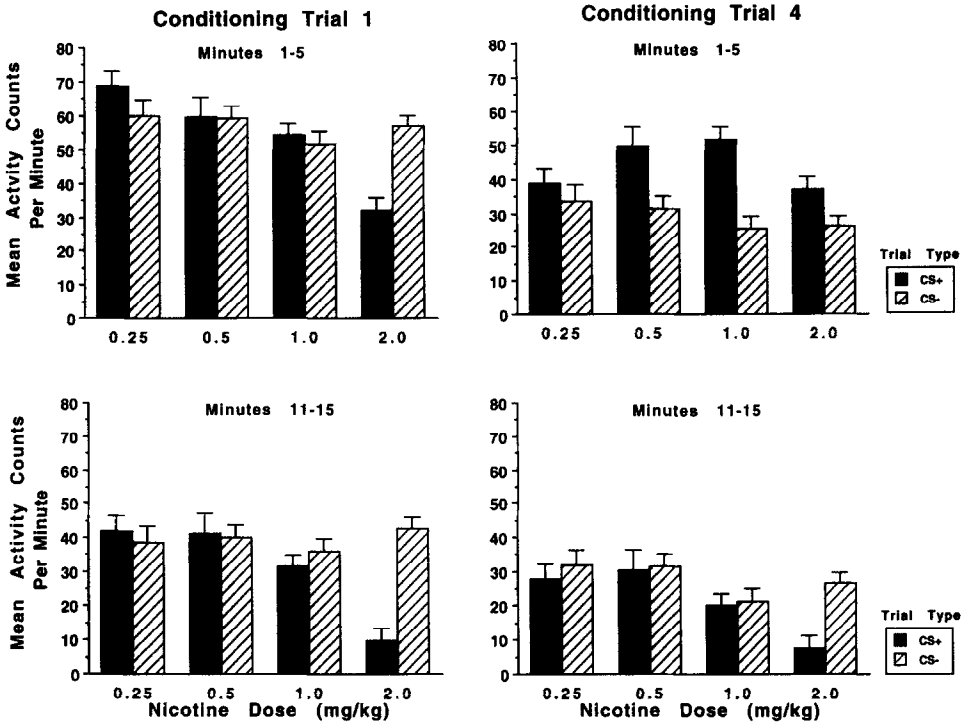

FIG. 1. Mean ± SEM activity counts per minute on the first (left panels) and last (right panels) conditioning trials for the first 5 min (top panels) and last 5 min (bottom panels) of each trial. CS+ days (nicotine treatment) are shown by the solid bars and CS− days (saline injections) are shown by the hatched bars.

tioning trial 4 yielded overall significant effects of trial type, $F(1, 86) = 44.8$, and nicotine dose × trial type, $F(3, 86) = 3.3$, $p < 0.03$. Within-group analysis showed significant trial type effects in the 0.5-, 1.0-, and 2.0-mg/kg groups [all $F(1, 23) > 7.2$, all $p < 0.02$] but not in the 0.25-mg/kg group, $F(1, 17) = 1.6$. Analysis of activity levels during the last 5 min of conditioning trial 4 showed overall effects of nicotine dose, $F(3, 86) = 3.9$, $p < 0.02$, trial type, $F(1, 86) = 14.5$, and nicotine dose × trial type, $F(3, 86) = 6.9$. Significant within-group effects of trial type were seen in the 2.0-mg/kg group, $F(1, 23) = 32.8$, but not in the lower nicotine dose groups (all $F < 1.2$).

Analysis of activity changes (nicotine dose × conditioning trial) between conditioning trial 1 and 4 for CS− trials yielded significant overall trial effects for both the first 5-min and last 5-min activity rates [both $F(3, 258) > 26.4$], reflecting a general decrease in locomotor activity levels after repeated exposure to the apparatus. Significant dose or dose × trial effects were not seen (all $F < 1.9$). Analysis of activity over CS+ trials also yielded significant trial effects for both the first 5-min and last 5-min activity rates [both $F(3, 258) > 11.3$]. Significant dose effects were seen for both the first 5-min and last 5-min activity rates [both $F(3, 86) > 3.4$, $p < 0.02$]. A significant dose × trial effect was seen in the first 5-min activity analysis, $F(9, 258) = 4.6$, but not in the last 5-min activity analysis, $F(9, 258) = 1.5$. Within-group analysis indicated that the first 5-min activity rates during CS+ trials decreased in the 0.25- and 0.5-mg/kg dose groups (both $F > 4.2$) and remained the same for the 1.0- and 2.0-mg/kg groups (both $F < 1.1$). The last 5-min activity during CS+ trials decreased over trials in the 0.25-, 0.5-, and 1.0-mg/kg groups (all $F > 4.9$) but not in the 2.0-mg/kg group, $F(3, 69) = 0.7$.

Testing

Figure 2 depicts the mean ± SEM seconds per minute on the grid floor during preference testing for both subgroups within each drug treatment condition. The dashed line represents the mean seconds per minute on the grid floor for the saline-only group, which, after saline-floor parings on each floor, spent approximately equal time on either floor type (e.g., mean seconds per minute spent on grid floor during test: 29.5 ± 4.9). As indicated by the difference between the grid+ and grid− subgroups, mice receiving 0.5 mg/kg nicotine displayed a preference for the nicotine-paired floor. In contrast, mice receiving 2.0 mg/kg nicotine displayed place aversion. Overall analysis (nicotine dose × conditioning group) produced significant effects of nicotine dose, $F(3, 82) = 2.9$, $p < 0.04$, and nicotine dose × conditioning group, $F(3, 82) = 4.6$. Subsequent comparisons of the conditioning groups within each nicotine dose showed preference in the 0.5-mg/kg group, $F(1, 22) = 7.5$, $p < 0.0125$, and aversion in the 2.0-mg/kg group, $F(1, 22) = 8.1$, $p < 0.0125$. No conditioning group effects were noted in the 0.25- and 1.0-mg/kg groups (all $F < 1.8$).

Within-subjects analysis comparing seconds per minute spent on the nicotine-paired floor with seconds per minute spent on the saline-paired floor yielded a significant overall within-subjects effect of nicotine dose, $F(4, 105) = 3.5$, $p <$

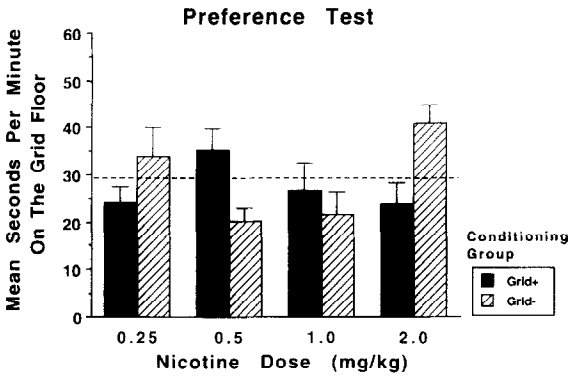

FIG. 2. Mean seconds per minute ± SEM spent on the grid floor during floor choice testing. Grid+ groups had previously received pairings of the grid floor with nicotine (and hole floor with saline), whereas grid− groups had previously received pairings of the grid floor with saline (and hole floor with nicotine). Conditioned place preference is shown when time spent on grid floor by the grid+ group exceeds time spent on the grid floor by the grid− group. Conditioned place aversion is shown when time spent on grid floor by the grid+ group is less than time spent on grid floor by the grid− group.

0.02. Follow-up comparisons (paired t-tests, two-tailed) of each nicotine dose group confirmed the observation of conditioned preference in the 0.5-mg/kg nicotine dose group, $T(23) = 2.7, p < 0.02$, and aversion in the 2.0-mg/kg nicotine dose group, $T(23) = -2.6, p < 0.02$. Within-subject differences in floor preference were not seen in the saline, 0.25-, and 1.0-mg/kg nicotine dose groups. Mean ± SEM seconds per minute spent on the nicotine-paired floor during the 30-min floor preference test for each nicotine dose group were as follows: saline, 29.5 ± 4.9; 0.25 mg/kg nicotine, 25.2 ± 3.8; 0.5 mg/kg nicotine, 37.5 ± 3.3; 1.0 mg/kg nicotine, 32.4 ± 3.3; 2.0 mg/kg nicotine, 21.4 ± 3.3.

Activity levels during the preference test did not differ across nicotine dose groups, $F(3, 86) = 1.3$. Mean ± SEM activity counts per minute for each nicotine dose group were as follows: 0.25 mg/kg, 31.9 ± 3.4; 0.5 mg/kg, 29.0 ± 2.8; 1.0 mg/kg, 27.2 ± 3.0; 2.0 mg/kg, 29.3 ± 2.5.

DISCUSSION

The present study is the first to examine nicotine's motivational effects in mice using the place conditioning paradigm. Nicotine produced conditioned place preference at a low (0.5 mg/kg) dose and conditioned place aversion at a high (2.0 mg/kg) dose. In general, these results are consistent with the view that nicotine has dose-dependent rewarding and aversive effects (14). Further, the lack of nicotine preference or aversion in mice receiving the intermediate 1-mg/kg dose could reflect a summation of nicotine's rewarding and aversive effects.

The present design used a counterbalanced place conditioning procedure in which saline-treated mice displayed equal preference for either floor type. However, most previous reports of nicotine-induced place preference in rats have used a biased design, with nicotine selectively paired with the nonpreferred compartment of a two-compartment shuttle box (1, 3,12,14,17). Procedures using unbiased designs have typically reported either no conditioning or conditioned place aversion leading to the speculation that nicotine reduces the aversiveness of a nonpreferred chamber (5,18). However, centrally administered nicotine produces conditioned place preference regardless of initial bias (17). More recently, nicotine-induced conditioned place preference has been reported using an unbiased place conditioning design, but only in rats receiving a regimen of nicotine preexposure (26), suggesting that tolerance to nicotine's aversive effects or sensitization to nicotine's rewarding effects must be developed before rats display conditioned preference. The reasons for the mixed pattern of results in rats are unknown, although strain and procedural differences have been suggested as factors (17,18). However, the present pattern of results in mice is not consistent with an interpretation based on reduced aversion, because unconditioned preference for the combination of floor cues used in this study was approximately equal. Further, the nicotine-induced conditioned place preference seen in the present study did not require extensive nicotine preexposure. Additional considerations are based on the possibility of species differences in sensitivity to nicotine's motivational effects. One possibility is that the peripheral actions of nicotine, which are avoided by central nicotine adminstration (17) or are reduced by nicotine preexposure (26), hinder the acquisition of conditioned place preference in rats, but not in mice. Alternatively, mice may be more sensitive than rats to the rewarding effects of nicotine as measured in the place conditioning design. However, additional studies using comparable procedures in both rats and mice will be needed to support these assertions. Finally, the present results suggest that nicotine-induced conditioned place preference is relatively difficult to demonstrate, given the modest magnitude of preference noted in the 0.5-mg/kg nicotine group and the failure to induce conditioned preference using lower and higher nicotine doses. Thus, the conditions under which nicotine-induced conditioned place preference is produced appear to be narrow.

During conditioning, initial exposure to 2.0 mg/kg nicotine produced locomotor depression. Lower nicotine doses had no discernable effect on activity. However, by the fourth nicotine exposure an increase in activity levels relative to saline activity was seen during the first 5 min of the conditioning trial. This change in the locomotor effects of nicotine can be interpreted in a number of ways. For example, nicotine's stimulant effects have been reported to be most apparent in rats tested in a familiar environment, when activity levels are low (2). Thus, nicotine's stimulant effect was seen on trial 4, after saline activity levels had declined. Also, nicotine's locomotor stimulant effects appear to be greatest after repeated testing with nicotine (6). In any event, these results are generally consistent with findings, in rats, of nicotine-induced locomotor stimulation and depression depending on dose and conditions of testing (20). Also, similar locomotor effects, stimulation or depression, have been noted in selectively bred mice using nicotine doses comparable to the ones used in the present study (28).

In conclusion, the present study has demonstrated dose-dependent nicotine reward and aversion using a relatively conventional place conditioning design. It remains to be determined whether the present results are due to central or peripheral nicotine effects. To address this question, a future study needs to ascertain the effects of the nicotinic receptor blocker mecamylamine on nicotine-induced place preference and aversion. However, previous demonstrations of nicotine-induced conditioned place preference have been shown to be centrally mediated (14). Provided that nicotine's motivational effects measured in the present design are central in origin, a number of intriguing questions can be addressed. For example, there are no genetic studies of nicotine's rewarding effects,

and the present design has been used successfully for the characterization of genetic differences in ethanol's and morphine's motivational effects (10). Further, recent preliminary studies in this lab have suggested ethanol and nicotine may interact in this design. Specifically, nicotine appears to reduce the acquisition of ethanol-induced conditioned place preference (24). These studies are continuing.

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