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Further Studies on Nicotine-Induced Conditioned Place Preference in the Rat[1]

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FUDALA, P. J. AND E. T. IWAMOTO. *Further studies on nicotine-induced conditioned place preference in the rat.* PHARMACOL BIOCHEM BEHAV **25**(5) 1041–1049, 1986.—Rats received subcutaneous (SC) injections of either nicotine (NIC, 0.001 to 2.0 mg/kg) or saline (SAL, 1 ml/kg) immediately prior to conditioning sessions in a conditioned place preference (CPP) paradigm. NIC was paired for 3 conditioning sessions with one environment of a 3 compartment CPP apparatus; SAL was paired with another environment. The animals were then tested for place preference by determining the proportion of time spent in each compartment during a 15 min test session. A dose-response curve was obtained for the place conditioning effect of nicotine as measured by its ability to alter baseline preferences calculated from control rats. NIC's place preference, but not place aversion, effect was linearly correlated with respect to dosage within the range of 0.1 to 0.8 mg/kg. NIC, 0.8 mg/kg, induced a place preference when it was administered immediately prior to conditioning sessions, but not when administered 20, 60 or 120 min prior to the sessions. Three repeated conditioning and testing cycles, or the daily administration of NIC for 2 weeks between conditioning and testing cycles had little or no effect on NIC place conditioning. Lobeline (2, 10 and 20 mg/kg) or cotinine (1 to 50 mg/kg) failed to condition a place preference. NIC, 0.1 or 1.2 mg/kg SC, administered to rat pups on postnatal days 5 through 8, did not alter subsequent place preference (induced by 0.8 mg/kg of NIC) measured at approximately 40 and 70 days of age. Periodic measurements of spontaneous motor activity, forelimb grip strength and negative geotaxis were unaltered by the perinatal exposure to nicotine.

Conditioned place preference Conditioned place aversion Nicotine Lobeline Cotinine Rat
Dose response Time course Tolerance Perinatal Motor activity Grip strength Negative geotaxis

IN a previous investigation [4], our laboratory reported the dose-dependent place conditioning effects of nicotine in the conditioned place preference (CPP) paradigm, and the ability of mecamylamine to antagonize the nicotine-induced place preference which suggests a central site of action. The first purpose of the present experiments was to verify our previous findings and to elaborate the nature of the nicotine response cue.

Maximal brain nicotine levels have been found in the rat within 20 min of a 400 mcg/kg subcutaneous dose [21]. Similarly, Martin *et al.* [10] found the highest levels of brain nicotine 10 min after a 1 mg/kg subcutaneous dose. The second purpose of this study was to examine the time course of the nicotine-induced response in the CPP paradigm by administering nicotine at zero, 20, 60 and 120 minutes prior to conditioning sessions in order to determine the duration of conditioning of a place preference or place aversion.

Tolerance to the subjective and physiologic effects of nicotine has been shown in human subjects [8], to the rate decreasing effect of nicotine on reinforced responding in mice [6] and rats [3] and to the depressant effect of nicotine on locomotor activity in rats [1,11]. It is not known whether tolerance develops to the place conditioning effects of nicotine as measured by the CPP paradigm. The present experiments addressed this point by assessing the effects of repeated nicotine conditioning and testing cycles and the effects of single daily nicotine injections between nicotine conditioning and testing cycles.

Lobeline is used as a smoking deterrent product [14], and cotinine is a major metabolic product of nicotine [5]. The fourth objective of this study was to determine dose-response relationships for these two compounds in the CPP paradigm.

Nicotine has been reported to increase adrenergic receptor binding [15] and nicotine binding [24] in the rat brain following prenatal exposure and to increase central noradrenaline uptake and endogenous noradrenaline after neonatal exposure [9]. Deficiencies in brightness discrimination and reduced spontaneous alternation in guinea pigs [7] and decreased horizontal locomotor activity and rearing in rats [16] have been shown in animals whose mothers were exposed to nicotine during gestation. However, little is known about

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how perinatal nicotine exposure alters subsequent behavioral responses to nicotine later in life. We examined the effects of nicotine, 0.1 and 1.2 mg/kg, administered to rat pups on postnatal days 5 through 8, on subsequent measures of nicotine-induced CPP as well as on measures of spontaneous motor activity, negative geotaxis (coordination) and forelimb grip strength (neuromotor function).

METHOD

Animals

Experimentally naive, adult male Sprague-Dawley rats (Harlan Sprague-Dawley Inc., Indianapolis, IN) were used in all experiments except the perinatal studies. The animals were initially quarantined for 10 days before being housed in groups of two. The rats were then individually housed 24 to 48 hours prior to the beginning of each experiment. Food and water were freely available in the home cages and the animals were kept on a 12 hour light/dark cycle. The animals used in the studies of the effects of perinatal nicotine exposure were derived from litters of Sprague-Dawley rats (Harlan Sprague-Dawley Inc.) received on gestational day 16 or 18. Litter sizes were kept to a maximum of 10. The pups were weaned on days 24 to 26 of age and then single housed as described above at day 34 or 35 of age.

Apparatus

Three place conditioning boxes, one constructed of plywood and the other two of Plexiglas, were used in the experiments. The place preference apparatus has previously been described in detail [4]. Briefly, it consisted of three distinctive inter-connected chambers. One chamber was cubical with black walls and a grid floor. The middle one was much smaller than the other two, with gray walls and a wood floor. The third chamber had an equilateral triangular mesh floor with white walls. Sliding removable doors separated the middle chamber from the other two. A clear Plexiglas hinged door covered the top of the apparatus. Additionally, a dilute acetic acid solution (0.06%) was applied to the mesh floor to provide another distinguishing feature between the white and black chambers. Photobeam detectors and transducers (Coulbourn Instruments, Lehigh Valley, PA) were used to monitor the position of the animals in the apparatus. The photobeams lay in a horizontal plane, one beam bisected the gray chamber and one beam each traversed the white and the black chambers. A cumulative timer (Coulbourn Instruments) was activated when the infrared beam passing through the white chamber was initially interrupted. Subsequent interrruptions did not modify the timing. Concurrent interruptions of the white and gray chamber beams, or of the gray chamber beam alone, suspended the white chamber timer. Timing was reinitiated when the white chamber beam was again broken. A cumulative timer for the black chamber operated in an analogous manner.

The measures of spontaneous motor activity were carried out in portable plastic cages (27×47×15 cm deep) or in suspended wire cages (30.5×22×20 cm deep). The measures of negative geotaxis were performed on a Plexiglas sheet (27×63.5 cm) with a black opaque underside which was angled at +26.3 degrees with respect to horizontal. Forelimb grip strength measurements were carried out using a metal T-bar (outer diameter: 1.2 mm, width: 22 mm) connected to an isometric strain gauge (Model FT 0.03, Grass Instrument Co., Quincy, MA) mounted horizontally. A U-shaped, Plexiglas alley (23.5×11×11 cm) was used to restrict the animals' movements. Recordings of the animals' performance were made on a polygraph-recorder (Model 79D, Grass Instrument Co.).

Drugs

Nicotine base was obtained from Eastman Kodak Co. (Rochester, NY), or Sigma Chemical Co. (St. Louis, MO), and l-lobeline hydrochloride and l-cotinine were obtained from Sigma Chemical Co. Cotinine and lobeline solutions were prepared weekly in sterile normal saline and stored at −70 degrees Centigrade; the doses were expressed in terms of the free base. Nicotine dose was expressed in terms of the free base assuming a density of 1 mg/microliter. All nicotine solutions were prepared fresh daily with sterile normal saline. Injection volumes of 1 ml/kg were used except in the lobeline 10 and 20 mg experiments where the volume was 1.5 ml/kg and the perinatal treatments where the volume was 0.1 ml/10 g. All injections were made SC.

PROCEDURE

The procedures used in place conditioning have been described previously [4]. Briefly, on conditioning days, one rat was injected with saline and placed in the closed (doors in place) white chamber. Another rat was injected with drug solution and placed in the closed black chamber. All injections were given immediately prior to placing the rats in the apparatus, except as noted in Experiment 2. Animals were conditioned in pairs and the conditioning time was 20 minutes per day. The conditioning chambers and treatments were reversed on the following day. Therefore, over the six consecutive conditioning days, a given rat in the experimental drug-treatment group received a total of three drug-black chamber pairings and three saline-white chamber pairings. In contrast, a rat in the vehicle-control group received three saline-black chamber pairings and three saline-white chamber pairings. One-half of the subjects within a given experimental group began their conditioning in the white chamber and the other half started in the black chamber. The daily order of conditioning a typical group of 32 rats was randomized over a six day period.

On preference testing day (day 7), one animal was placed into the closed central gray chamber of the place preference apparatus. The sliding doors were removed and the amount of time (in sec) spent in the white and black chambers was automatically recorded (Coulbourn Instruments) over the 900 sec testing period.

Experiment 1: Nicotine-Induced CPP

Vehicle control group. Over a period of nine weeks, a vehicle control group comprised of four groups of 16 rats was conditioned with saline (SAL) in both the white and black chambers and then tested for place preference. The amounts of time spent in the white and black chambers for each rat during the 900 sec test sessions were used in the calculation of a 95% confidence interval (see the Statistical Treatment of the Data section). The conditioning of this vehicle control group took place either concurrently or during alternating weeks with the following treatment groups.

Treatment groups. Over a period of nine weeks, five groups of 16 rats were conditioned with various doses of nicotine (NIC) in the black chamber and SAL in the white chamber and then tested for place preference. The doses of NIC used were 0.001, 0.025, 0.05 and 1.5 mg/kg (N=16 for all

TABLE 1

DESCRIPTION OF MOTOR ACTIVITY ASSESSMENTS MEASURED IN EXPERIMENT 6

Still:	Asleep or still for at least 3 seconds.
Loco:	Locomotion with all 4 legs moving and travelling a distance equivalent to at least one body length.
Explo:	Moving head from left to right to left or vice versa (one complete cycle).
Rear:	Rearing.
Climb:	In rearing position but with forelimb(s) against side of cage.
Sniff:	Sniffing.
Scrat:	Scratching body with hindlimb for at least 3 seconds.
Groom:	Grooming for at least 3 seconds.
Feed:	Feeding. (Food was only available for the last 3 sets of measurements).

groups except 0.025 where N=32). The amounts of time spent in the white and black chambers for each rat during the 900 sec test sessions were used to calculate % place preference or aversion (see the Statistical Treatment of the Data section). In a different experiment, two groups of 16 rats each were conditioned with either NIC (2.0 mg/kg) or SAL (controls) in the black chamber and SAL in the white. This experiment was analyzed separately. The data obtained from these experiments were analyzed with previous data for nicotine doses between 0.1 and 1.2 mg/kg [4] in order to obtain a complete dose-response assessment.

Experiment 2: Onset of the Nicotine Response Cue

Over a one week period, a vehicle-control group of 16 rats received SAL immediately prior to being conditioned in both the white and black chambers and was then tested for place preference. Over the same period, a NIC-treatment group comprised of 16 rats received NIC, 0.8 mg/kg, or SAL immediately prior to conditioning in the black or white chambers, respectively, and was subsequently tested for place preference. These animals were used to assess the effects of NIC administered at $t_{-0'}$ (immediately prior to conditioning).

A series of similar conditioning and testing sessions using NIC and SAL was completed in which the animals were injected at $t_{-20'}$, $t_{-60'}$ or $t_{-120'}$ (20, 60 or 120 min prior to the beginning of conditioning sessions). All N=32 except $t_{-20'}$ where N=64. After the animals were weighed and injected, the rats were immediately returned to their home cages until conditioning. The data for the $t_{-20'}$ experiment were analyzed separately since the experiment was performed subsequently to the others, replicated twice and was performed using a phosphate buffer (0.76% dibasic sodium phosphate + 0.18% monobasic potassium phosphate; approximate pH=7.1) instead of sterile normal saline.

Experiment 3: Effects of Single Daily Nicotine Injections Between Two Cycles of Nicotine Place Conditioning

Over a seven day period, 16 SAL and 16 NIC, 0.6 mg/kg, treated rats were conditioned and tested for place preference. On the day after preference testing (Day 8), all animals received single daily NIC injections of 0.6 mg/kg for a total of 14 consecutive days. On DAY 22, SAL and NIC conditioning sessions were resumed and the animals tested for place preference on day 28. In another experiment, 15 SAL and 16 NIC treated rats were conditioned and tested exactly as above, except that the animals received no injections for the 14 intervening days between conditioning and testing cycles.

Experiment 4: Repeated Nicotine Place Preference Conditioning and Testing Over Three Consecutive Cycles

Two groups of 16 rats were initially conditioned with either SAL or NIC, 0.8 mg/kg. After the animals were tested for place preference, they were maintained in their home cages for two days. On the third day after preference testing, another seven day conditioning and testing cycle was performed in a manner identical to the first. Again, after two days, the conditioning and testing sessions were repeated so that each rat received a total of three complete conditioning and testing cycles over a 25 day period.

Experiment 5: Effects of Lobeline and Cotinine in the Place Conditioning Paradigm

In a manner analogous to the procedure used for NIC conditioning, groups of rats were conditioned with various doses of lobeline (LOB) and cotinine (COT) as well as SAL (controls). Over one seven day period, three groups of 10 rats were conditioned with either SAL or LOB, 2 and 20 mg/kg. Over another seven day period, two groups of 16 rats were conditioned with either SAL or LOB 10 mg/kg.

Five doses of COT (1, 2, 10, 20 and 50 mg/kg) were tested for their ability to induce a place preference or place aversion. At the time of drug conditioning, an equal number of control rats was simultaneously conditioned (N=16 for all groups except for the controls associated with the 10 mg/kg dose level where N=14). The conditioning and testing of each dose level and their corresponding controls was performed on separate weeks.

Experiment 6: Effect of Perinatal Nicotine Exposure on Subsequent Measures of Spontaneous Motor Activity, Negative Geotaxis, Forelimb Grip Strength and Nicotine-Induced Conditioned Place Preference

Newborn litters of Sprague-Dawley rat pups were culled from females received on gestational day 16 or 18. The pups and dams were maintained in portable plastic cages (27×47×15 cm) with screened hardwood chip bedding and food and water available ad lib. A total of 66 pups from eight litters were obtained (32 were used for the present experiment). The sex of the pups was determined on postnatal day (PND) 2 or 3 by measuring the urethral/anal distance (PND 1 = the day of birth). The litters were divided into equal numbers of males and females with a maximum litter size of 10 pups. NIC, 0.1 mg/kg, administered in a volume of 0.1 ml/10 g, or an equivalent volume of SAL was given SC to the pups once daily on PNDs 5 through 8. An equal number of males and females received NIC or SAL (8 per sex per treatment). The pups were weaned on PND 24, 25 or 26 and single housed in suspended wire cages (30.5×22×20 cm) on PND 34 or 35.

Measures of spontaneous motor activity were performed on six separate occasions (PND 10, 14, 17, 48 or 49, 52 or 53 and 78 or 79). The first three sets of measurements were performed with the animals individually placed in the plastic portable cages with the measurements made after a five minute acclimation period. The latter three sets of measure-

ments were done with the rats in their home, suspended wire cages. Assessments of activity were made by a trained observer blind to the rats' previous treatments. Behavioral activities were measured over a 10 sec period every 5 min for 15 min. Table 1 describes the measurements.

In the assessments of negative geotaxis, which were performed at least one hour after the spontaneous motor activity measurements, one rat was placed head-down 24 cm from the top of the angled Plexiglas sheet. The time required for the rat to reorient 180 degrees was measured to the nearest 1/100 of a sec; the maximum score was 60 sec. Timing was temporarily suspended while the rat was returned to the head-down starting position after it had slid off the sheet or had climbed off the side.

Forelimb grip strength was measured on four occasions (PND 21, 24, 49 or 50 and 79 or 80). One rat was held with both hindlimbs against its body and allowed to grasp the T-bar with both forepaws. The rat was then pulled gently but firmly backwards until its grip was broken. Grip strength was measured in grams and the best performance of 5 consecutive trials was used in the analysis of the data.

Place preference conditioning and testing were carried out on two occasions beginning on PND 39 or 40 and on PND 70 or 71, periods roughly corresponding to late adolescence and early adulthood. All rats were conditioned with NIC in the black chamber and SAL in the white chamber. Due to the limitations of group size and since comparisons were desired with respect to perinatal treatments, no SAL-white chamber/SAL-black chamber conditionings were conducted.

The effects of perinatal exposure to NIC, 1.2 mg/kg, on PNDs 5 through 8 were also examined in an analogous manner to that described above. However, only the effects of this drug exposure on subsequent NIC-induced place preference were assessed in these animals.

STATISTICAL TREATMENT OF THE DATA

Calculation of Percent Place Preference and Aversion

Using the data from the SAL vehicle-control groups in Experiment 1, a quantity called the residence ratio (RR) was calculated, where RR equals (W− B)/(W + B). For each rat, W=time in sec spent in the white chamber and B=time in sec spent in the black chamber. A 95% confidence interval was obtained for the mean RR for the combined SAL-control group according to the following formula: mean RR±1.96 × the standard deviation from the mean=95% CI. Using the RRs calculated for each rat in the drug-treatment group in Experiment 1, the animals at each dose level were categorized as showing place preference, place aversion or no difference from controls according to the following assignments: Place preference, if the observed RR<lower limit of the CI; place aversion, if the observed RR>upper limit of the CI; no response, if lower limit of CI≤observed RR≤upper limit of CI. Likewise, the results from our previous experiment [4] were recalculated to the above specifications and this data was incorporated with the present data to give a complete dose-response analysis. The data are plotted as percent place preference (100 × the fraction of the rats per group at a given dose exhibiting a place preference) versus the dose of nicotine. The percent place preference values for NIC doses between 0.1 and 0.8 mg/kg were analyzed by linear regression. The regression equation and the coefficient of determination were obtained using statistical methods [13] with the aid of the Statistical Analysis System (SAS) at the University of Kentucky. Pairwise comparisons between control and treatment groups were performed using the method of multiple contrasts [22]. The data from those groups of rats which received NIC, 2.0 mg/kg, or SAL conditioning were analyzed separately using a two sample *t*-test for unpaired samples.

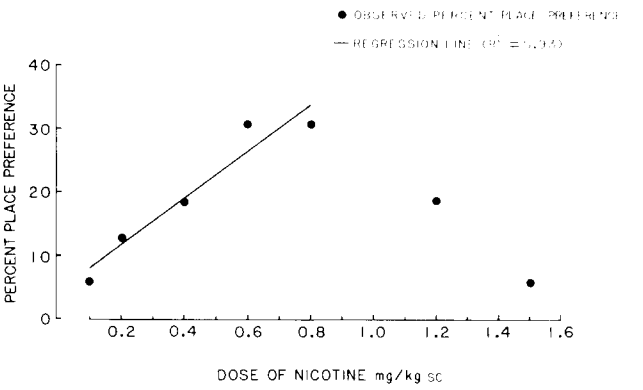

FIG. 1. Percent of rats exhibiting place preference. Each point depicts the proportion of groups of 16 rats exhibiting a place preference on test day after conditioning with nicotine in the place conditioning paradigm. The percent place preference values (percentage of rats exhibiting place preference) ranged from 6 to 31%.

Experiment 2

The RRs were calculated for each rat in the $t_{-0'}$, $t_{-60'}$ and $t_{-120'}$ NIC-treatment groups and their corresponding controls. A mean RR was obtained for each group and the means analyzed using a two-way ANOVA for the factors treatment prior to conditioning (NIC or SAL) and week of conditioning. Pairwise comparisons were subsequently used to compare the NIC-treatment groups to their corresponding controls.

Since the conditioning and testing of the $t_{-20'}$ NIC-treatment group and its corresponding controls were performed subsequently to the above analysis, the treatment and control groups were anlayzed separately using a two-way ANOVA for the factor time and treatment prior to conditioning and the factor place preference box used (the experiment was run as two replications performed in the same week using two boxes). Pairwise comparisons were then performed to assess differences between groups.

Experiments 3, 4 and 5

Individual RRs and group means were calculated for each rat and group, respectively. The data were initially analyzed using one-way (Experiment 5) or two-way repeated measures (Experiment 3 and 4) ANOVA. Subsequently, *t*-tests using standard errors adjusted for repeated measures [25] or multiple contrasts [22] were used to compare differences between selected groups.

Experiment 6

A three-way repeated measures ANOVA was used to analyze the data from each of the CPP, negative geotaxis and forelimb grip strength experiments. The dependent variables were RR, seconds to reorient and grip strength (g) per body weight (g), respectively. Individual, adjusted *t*-tests [25] were then used for pairwise comparisons.

The analysis of the spontaneous motor activity data was

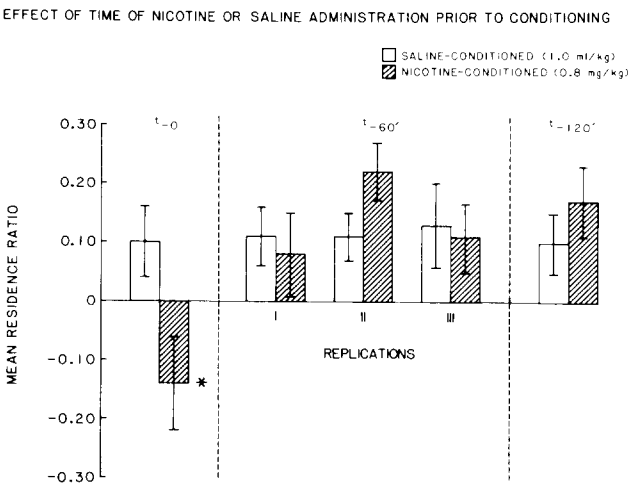

FIG. 2. The effects of time of nicotine or saline administration prior to place preference conditioning sessions. The histograms represent the mean residence ratios (±S.E.) from groups of rats (N=16) administered nicotine 0.8 mg/kg or saline at zero ($t_{-0'}$), 60 ($t_{-60'}$) or 120 ($t_{-120'}$) minutes prior to conditioning sessions. The $t_{-60'}$ study was replicated a total of three times. *Indicates significant difference from control values, $p<0.05$.

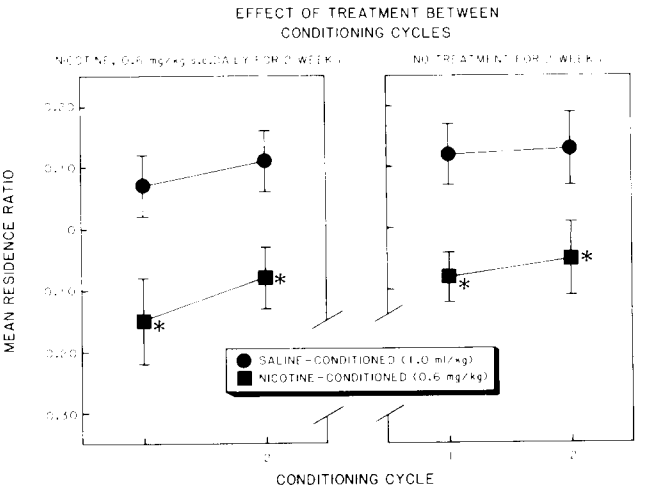

FIG. 3. The effects of single daily nicotine injections between repeated conditioning and testing cycles. The squares and circles represent the mean residence ratios (±S.E.) from groups of rats (N=15 or 16) conditioned with nicotine 0.6 mg/kg or saline, respectively, over two conditioning and testing cycles. During the 14 intervening days between cycles, rats received either single daily injections of nicotine, 0.6 mg/kg, or received no treatment. *Indicates significant difference from control values, $p<0.05$.

performed separately for each day and each behavioral measurement. The data were fit to a log linear model [22] with the factors sex and perinatal treatment (NIC or SAL). Subsequent chi-square analyses were performed to assess any differences between groups.

RESULTS

Nicotine Dose-Response

Nicotine produced a dose-dependent place preference, but not place aversion, within the dose range tested (0.001–1.5 mg/kg). In those rats which exhibited place preference, the conditioned response was a direct function of dose between 0.1 and 0.8 mg/kg (Fig. 1). The percent place preference values ranged from 6 to 31%. No animals at the 0.001, 0.025 or 0.05 mg/kg dose level were categorized as showing a place preference or aversion. The 1.2 and 1.5 mg/kg dose levels were not used to estimate the regression due to the apparent decreasing response at these doses. The regression equation for the predicted percent response is given by: % place preference = 0.04 + 0.38 (DOSE). The F test for significant regression yielded, $F(1,3)=47.4$, $p<0.01$.

Only 1 of 16 rats at the 0.6 mg/kg and 2 of 16 rats at the 1.2 mg dose levels were categorized as exhibiting a place aversion. No other dose levels included rats categorized as exhibiting a place aversion. Although a dose-response relationship was obtained within the dose range of 0.1–0.8 mg/kg, multiple contrasts performed on treatment groups versus their corresponding controls indicated that only three dose levels produced a robust place preference: 0.4 mg/kg, $F(1,150)=5.03$, $p<0.03$; 0.6 mg/kg, $F(1,150)=9.31$, $p<0.003$; 0.8 mg/kg, $F(1,150)=17.55$, $p<0.0002$.

An analysis of both the previous and present data from the control rats from which the two control intervals were estimated indicated that both groups were normally distributed (Kolmogorov-Smirnov $D=0.079$ and 0.053 for the previous and present data, respectively; both $p>0.15$). One rat injected with 2 mg/kg NIC died during its first NIC-conditioning exposure. A two sample t-test revealed no significant difference between the 2 mg/kg NIC-treatment group and its corresponding controls, $p>0.5$, indicating that this dose of NIC did not induce a place preference nor aversion.

Onset of the Nicotine Response Cue

Only rats administered 0.8 mg/kg NIC immediately prior to conditioning sessions demonstrated a significant place preference. The analysis of the groups administered NIC or SAL at $t_{-0'}$, $t_{-60'}$ and $t_{-120'}$ revealed no significance for the factor associated with the treatment prior to conditioning (NIC or SAL). Significance was found for the factor associated with the week of conditioning, $F(4,150)=2.91$, $p<0.025$ and for the treatment × week interaction, $F(4,150)=2.65$, $p<0.04$. The Least Squares Means analysis was used to compare the NIC treatment groups to their corresponding controls in order to interpret the interaction. The only difference found was at zero min prior to conditioning, $t(150)=2.86$, $p<0.005$; indicative of NIC-induced place preference (Fig. 2).

Similarly, rats administered 0.8 mg/kg NIC 20 min prior to conditioning did not evidence a conditioned place preference. The ANOVA showed significance for the factor associated with treatment (NIC or SAL) and time of treatment ($t_{-0'}$ or $t_{-20'}$), $F(3,54)=4.59$, $p<0.01$, and for the factor associated with the two boxes, $F(1,54)=14.7$, $p<0.0004$. The main effect interaction was not significant. A Least Squares Means analysis performed on the combined data from both boxes showed a place preference for NIC $t_{-0'}$ versus controls $t_{-0'}$, $t(54)=3.45$, $p<0.002$, but not for NIC $t_{-20'}$ versus controls $t_{-20'}$ $p>0.4$. Additionally, the $t_{-0'}$ controls were found to be different from the $t_{-20'}$ controls, $t(54)=2.86$,

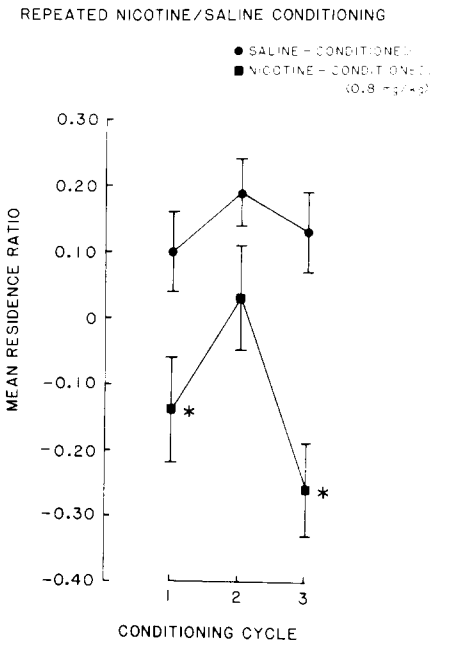

FIG. 4. The effects of three repeated conditioning and testing cycles on nicotine-induced conditioned place preference. The squares and circles represent the mean residence ratios (±S.E.) from groups of rats (N=16) administered nicotine 0.8 mg/kg or saline, respectively, over three conditioning and testing cycles with two days between cycles. *Indicates significant difference from control values, $p<0.05$.

$p<0.01$, suggesting that procedural differences involved in administering the injections at the different times affected the rats' baseline preference.

Effects of Single Daily Nicotine Injections Between Two Cycles of Nicotine Place Conditioning and Testing

Rats conditioned with NIC showed a significant place preference when compared to their corresponding controls regardless of conditioning cycle or treatment between cycles (Fig. 3). For those rats that received daily NIC injections between conditioning and testing cycles, there was a significant difference between NIC and SAL groups, $F(1,30)=13.87$, $p<0.0009$. There were no differences between the first and second cycle measurements and there was no treatment × cycle interaction. Analysis of the simple effects showed that for both the first and second cycles, NIC-conditioned groups were significantly different from the controls, $t(60)=2.77$, $p<0.01$ and $t(60)=2.48$, $p<0.02$, respectively.

For those rats that received no treatment between conditioning and testing cycles, there was also a significant difference between NIC and SAL groups, $F(1,29)=9.93$, $p<0.005$. There were no differences between the first and second cycle measurements and there was no treatment × cycle interaction. Analysis of the simple effects showed that for both the first and second cycles, NIC-conditioned groups were significantly different from the controls, $t(54)=2.71$, $p<0.01$ and $t(54)=2.35$, $p<0.03$, respectively.

Repeated Nicotine Place Preference Conditioning and Testing Over Three Consecutive Cycles

Tolerance to nicotine's place conditioning effects did not develop over three conditioning and testing cycles (Fig. 4). The F tests for the factor associated with treatment (NIC or SAL), $F(1,30)=11.24$, $p<0.003$, and for the factor associated with cycle, $F(2,60)=8.57$, $p<0.0006$, were significant. However, the treatment × cycle interaction term was also significant, $F(2,60)=3.29$, $p<0.05$, indicating that variation from cycle to cycle was different for the two treatments. Consequently, the simple effects of treatment and cycle were examined. NIC induced a place preference on cycle I, $t(57)=2.46$, $p<0.02$, and cycle III, $t(57)=4.14$, $p<0.0002$. There was not, however, a significant place preference observed at the end of cycle II.

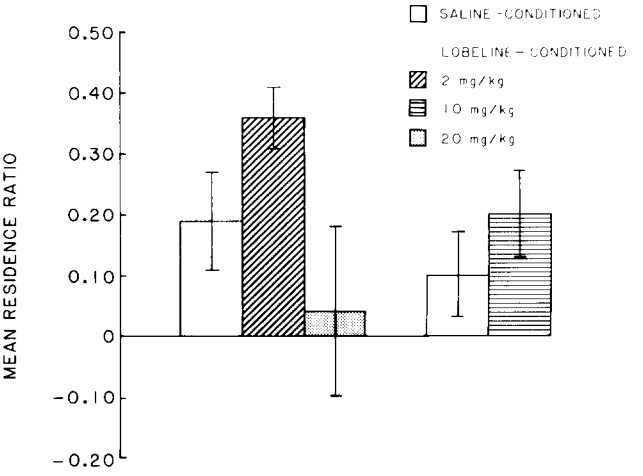

FIG. 5. The effects of lobeline in the conditioned place preference paradigm. The histograms represent the mean residence ratios (±S.E.) from groups of rats (N=10 or 16) conditioned with lobeline (2, 10 or 20 mg/kg) or saline.

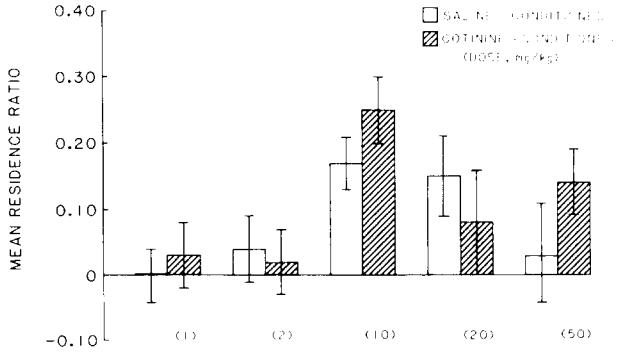

FIG. 6. The effects of cotinine in the conditioned place preference paradigm. The histograms represent the mean residence ratios (±S.E.) from groups of rats (N=14 or 16) conditioned with cotinine (1, 2, 10, 20 or 50 mg/kg) or saline.

Effects of Lobeline and Cotinine in the Place Conditioning Paradigm

Neither lobeline nor cotinine produced significant place preferences or aversions. A one-way ANOVA performed on all of the LOB-conditioned groups and their corresponding controls showed no significant differences between groups, $p>0.14$. Multiple contrasts between each treatment group

TABLE 2

MEAN RESIDENCE RATIOS (± S.E.) FOR GROUPS OF RATS (N=8) THAT RECEIVED NICOTINE (0.1 or 1.2 mg/kg) OR SALINE ON POSTNATAL DAYS 5 THROUGH 8 AND WERE THEN CONDITIONED WITH NICOTINE 0.8 mg/kg IN THE PLACE PREFERENCE PARADIGM OVER TWO SEPARATE CYCLES BEGINNING ON POSTNATAL DAYS 39 OR 40 AND 70 OR 71

		Residence Ratio	
Sex	Treatment	Cycle I	Cycle II
F	NIC 0.1	−0.23 ± 0.05	−0.08 ± 0.08
F	SAL	−0.25 ± 0.07	−0.11 ± 0.07
M	NIC 0.1	−0.08 ± 0.08	−0.15 ± 0.04
M	SAL	−0.21 ± 0.04	−0.14 ± 0.11
F	NIC 1.2	−0.28 ± 0.06	−0.18 ± 0.05
F	SAL	−0.18 ± 0.05	−0.12 ± 0.09
M	NIC 1.2	−0.15 ± 0.04	−0.14 ± 0.07
M	SAL	−0.22 ± 0.06	−0.17 ± 0.06

and its corresponding control showed no significant differences at the 0.05 level of significance (Fig. 5). A one-way ANOVA performed on all of the COT-conditioned groups and their corresponding controls showed a significant difference between groups, $F(9,148)=2.24$, $p<0.03$. Multiple contrasts were examined to determine whether any treatment group was significantly different than its corresponding control group. No differences were found at 0.05 level of significance (Fig. 6).

Effects of Perinatal Nicotine Treatment on Subsequent Measures of Nicotine-Induced Conditioned Place Preference, Spontaneous Motor Activity, Negative Geotaxis and Forelimb Grip Strength

Conditioned place preference. Although there were no SAL-conditioned controls in these place preference experiments, the consistently negative mean group RRs obtained (Table 2) suggested that a conditioned place preference was produced (the rats spent greater amounts of time in the chamber paired with NIC). None of the main effect factors were significant for the groups associated with the perinatal administration of 0.1 mg/kg NIC: Perinatal treatment (NIC or SAL), $p>0.8$; sex, $p>0.6$; cycle of conditioning, $p>0.1$. Similarly, none of the main effect interactions (treatment × cycle, sex × cycle, sex × treatment, and treatment × sex × cycle) were significant. The groups associated with the perinatal administration of 1.2 mg/kg NIC also showed no significant differences with respect to main effect factors (treatment, $p>0.4$; sex, $p>0.6$ and cycle, $p>0.1$) or factor interactions.

Spontaneous motor activity. There were no differences between groups with respect to the main effect factors of sex or perinatal treatment on any of the nine behavioral measurements.

Negative geotaxis. There were few differences noted between groups with respect to reorientation times. There were no differences associated with the main effect factors of perinatal treatment, $p>0.7$ or sex, $p>0.6$. There was, however, a significant effect associated with the factor day of testing, $F(5,140)=14.13$, $p<0.0002$, and the interaction of treatment × day of testing, $F(5,140)=2.87$, $p<0.02$. Pairwise comparisons performed between groups on each testing day yielded three significant comparisons: day 52 or 53, NIC-male versus SAL-male (male rats which received NIC or SAL perinatally), $t(164)=2.183$, $p<0.04$; day 78 or 79, NIC-male versus SAL-male, $t(164)=2.959$, $p<0.004$ and NIC-female versus SAL-male, $t(164)=2.703$, $p<0.008$.

Forelimb grip strength. Overall, female rats displayed a greater grip strength to body weight ratio when compared to the males; the ratio decreased in both groups over time. There was no significant difference for the main effect factor associated with perinatal treatment, $p>0.6$. There was significance for the main effects of sex, $F(1,28)=5.27$, $p<0.03$, and day of testing, $F(3,84)=99.16$, $p<0.0002$. None of the main effect factor interactions were significant at the 0.05 level. Pairwise comparisons performed between groups on each testing day yielded one significant comparison: day 79 or 80, NIC-male versus NIC-female, $t(110)=2.047$, $p<0.05$.

DISCUSSION

The present data, in agreement with our previous findings [4], demonstrate that nicotine, at doses between 0.1 and 1.5 mg, induces a dose-dependent place preference as assessed by the conditioned place preference paradigm. In contrast to our previous findings, no consistent dose-related place aversion was noted. This difference may have arisen through the past use of a too liberal categorization of place preference and aversion. In our previous report, we categorized rats as exhibiting either preference or aversion based on a confidence interval (CI) derived from the mean of the control group: 95% CI = mean RR±t × standard error of the mean. The present data were analyzed using a confidence interval based on the expected range of all of the animals in the control group: 95% CI = mean RR±1.96 × standard deviation from the mean. Since the interval used in the present study encompasses a wider range of values, it would be expected that fewer animals would be classified into the preference and aversion categories and this was found to be the case. Within the 0.1 to 0.8 mg/kg dose range, we presently found percent place preference values ranging from 6 to 31%, as compared to the 44 to 89% values reported previously. In addition, when the treatment group means were compared to the corresponding controls, only three dosage levels (0.4, 0.6 and 0.8 mg/kg) yielded results that were indicative of a nicotine-induced place preference and none suggested a place aversion. Thus, in the present study, we have stipulated that in order to achieve the preference or aversion categorization, an animal must have an RR value approximately two standard deviations from the control mean. We feel that this basis for categorization is sufficiently conservative.

The time spent in the gray chamber (which was novel to all rats on test day) was not significantly altered by conditioning the rats with nicotine [4] and this was verified in the present study (data not shown). The time spent in the gray chamber was unaffected by the animals' preference for either the white or black chambers. In addition, we found that the distribution of RRs obtained from the control animals did not deviate from normality. This is an important finding since a substantially non-normal distribution of control RRs may have confounded some of the statistical methods used.

Nicotine, 0.8 mg/kg SC, conditioned a place preference when administered immediately prior to conditioning sessions, but not when administered at 20, 60 or 120 minutes prior to conditioning. We conclude that the nicotine re-

sponse cue occurs within a time period when brain nicotine levels are rising toward a maximum, even though substantial plasma nicotine levels may be sustained for 30 to 60 minutes [10, 20, 21]. Although nicotine levels were not measured in our animals, our data suggest that there must be a close temporal pairing between the unconditioned stimulus (the injection of nicotine) and the conditioned stimulus (the environmental context).

Our data also indicate that tolerance did not develop to the place conditioning effects of nicotine when nicotine was administered in single daily doses for 14 days between two conditioning and testing cycles or when nicotine conditioning and subsequent testing was carried out over three consecutive cycles. When nicotine was administered between cycles, the magnitudes of the nicotine-induced place preferences in each of the cycles were almost equal (when compared to controls), as they were when no treatment was administered between cycles. When the effects of three consecutive conditioning and testing cycles were evaluated, nicotine induced a significant place preference on cycles I and III but not on cycle II; the effect at cycle II approaching significance. The data indicate a trend for a consistent place preference across cycles with a possible decrease, then increase, in preference magnitude across cycles. In other experiments [12], it was found that various doses of morphine and naloxone that produced a maximal place conditioning effect after four drug/environmental context pairings showed an onset of effect only after three pairings. However, when ethanol was used as the unconditioned stimulus, it was found that the magnitude of the conditioned place preference produced did not increase over three conditioning and testing cycles [17]. The continued conditioning and testing of nicotine-conditioned rats over more than three cycles may elucidate more precisely the nature of the conditioned response over time. These experiments have import upon human smoking, a behavior known to span decades.

Neither lobeline (2, 10 or 20 mg/kg) nor cotinine (1 to 50 mg/kg) produced a conditioned place preference or aversion. It was previously reported that lobeline did not generalize to the nicotine discriminative cue in rats [23]. Cotinine has also been reported not to generalize to the nicotine discriminative cue in the rat [19] although variable effects on schedule-controlled responding have been reported in the monkey and dog [18]. Our results indicate that both agents are inactive as unconditioned stimuli in the CPP paradigm.

The perinatal administration of nicotine, 0.1 and 1.2 mg/kg, had no measurable effect on subsequent nicotine place preference conditioning. Nicotine was administered perinatally on postnatal days 5 through 8; this time interval partially overlaps with the brain "growth spurt" in the rat [2]. Our data indicate that the place conditioning effects of nicotine are not altered by perinatal nicotine exposure. It is possible that the CPP paradigm is not sensitive to whatever change the perinatal nicotine exposure may have induced, such as an alteration in nicotine binding characteristics. Also, a carry-over effect from the drug or conditioning sessions from cycle to cycle cannot be ruled out. Since we used what we determined to be the most effective dose of nicotine (0.8 mg/kg) in our place conditioning sessions, subtle changes in animal response behavior may have been overshadowed. Also, conditioning at times earlier or later than those chosen may have produced an observable effect.

As previously noted, prenatal and perinatal nicotine exposure may produce later appearing effects in the offspring. Our data demonstrated few significant effects of perinatal nicotine exposure on the various neuromotor parameters assessed. With respect to the negative geotaxis measurements, the raw data indicated that it was the control-male rats which did not fit the performance pattern exhibited by the other groups during the last two measurement periods. Although the reason for these differences is not clear, we do not believe that the results indicate an effect of perinatal nicotine treatment. Regarding the grip strength assessments, the difference between the males and females which received nicotine perinatally occurred on the last measurement day when the values obtained from all the groups were approaching an asymptote. It is doubtful that this single difference represents a real effect of perinatal nicotine treatment. The data do not provide conclusive evidence for any particular type of neurotoxicity or motor deficit.

In summary, nicotine induced a dose-dependent place preference, but not aversion, in the conditioned place preference paradigm. The conditioned effect required a close temporal pairing between nicotine and the environmental context of conditioning. Tolerance did not develop to nicotine's place conditioning effects with the dosage regimens used and lobeline and cotinine produced no significant place preference or aversions. Perinatal nicotine exposure did not affect subsequent nicotine conditioned place preferences and produced no observable effects on the various measures of neuromotor functioning examined.

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REFERENCES

1. Clarke, P. B. S. and R. Kumar. The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. *Br J Pharmacol* **78:** 329–337, 1983.
2. Davison, A. N. Biochemical, morphological and functional changes in the developing brain. In: *Biochemical Correlates of Brain Structure and Function*, edited by A. N. Davison. New York: Academic Press Inc., 1977, pp. 1–13.
3. Dougherty, J., D. Miller, G. Todd and H. B. Kostenbauder. Reinforcing and other behavioral effects of nicotine. *Neurosci Biobehav Rev* **5:** 487–495, 1981.
4. Fudala, P. J., K. W. Teoh and E. T. Iwamoto. Pharmacologic characterization of nicotine-induced conditioned place preference. *Pharmacol Biochem Behav* **22:** 237–241, 1985.
5. Gorrod, J. W. and P. Jenner. The metabolism of tobacco alkaloids. In: *Essays in Toxicology*, vol 6, edited by W. J. Hayes. New York: Academic Press, Inc., 1975, pp. 35–78.
6. Hendry, J. S. and J. A. Rosecrans. The development of pharmacological tolerance to the effect of nicotine on schedule-controlled responding in mice. *Psychopharmacology* **77:** 339–343, 1982.

7. Johns, J. M., T. M. Louis, R. F. Becker and L. W. Means. Behavioral effects of prenatal exposure to nicotine in guinea pigs. *Neurobehav Toxicol Teratol* **4:** 365–369, 1982.
8. Jones, R. T., T. R. Farrell, III and R. I. Herning. Tobacco smoking and nicotine tolerance. In: *Self-Administration of Abused Substances: Method for Study. NIDA Research Monograph No. 20*, edited by N. A. Krasnegor. Rockville: U.S. Dept. of Health, Education and Welfare, 1978, pp. 202–208.
9. Jonsson, G. and H. Hallman. Effects of neonatal nicotine administration on the postnatal development of central noradrenaline neurons. *Acta Physiol Scand (Suppl)* **479:** 25–26, 1980.
10. Martin, B. R., H. L. Tripathi, M. D. Aceto and E. L. May. Relationship of the biodisposition of the stereoisomers of nicotine in the central nervous system to their pharmacological actions. *J Pharmacol Exp Ther* **226:** 157–163, 1983.
11. Morrison, C. F. and J. A. Stephenson. The occurrence of tolerance to a central depressant effect of nicotine. *Br J Pharmacol* **46:** 151–156, 1972.
12. Mucha, R. F. and S. D. Iversen. Reinforcing properties of morphine and naloxone revealed by conditioned place preferences: a procedural examination. *Psychopharmacology (Berlin)* **82:** 241–247, 1984.
13. Neter, J. and W. Wasserman. *Applied Linear Statistical Models*. Homewood: Richard D. Irwin, Inc., 1977.
14. Olin, B. R., (ed.). *Facts and Comparisons*. St. Louis: J. B. Lippincott Co., 1986.
15. Peters, D. A. V. Prenatal nicotine exposure increases adrenergic receptor binding in the rat cerebral cortex. *Res Commun Chem Pathol Pharmacol* **46:** 307–317, 1984.
16. Peters, D. A. V. and S. Tang. Sex-dependent biological changes following prenatal nicotine exposure in the rat. *Pharmacol Biochem Behav* **17:** 1077–1082, 1982.
17. Reid, L. D., G. A. Hunter, C. M. Beaman and C. L. Hubbell. Toward understanding ethanol's capacity to be reinforcing: A conditioned place preference following injections of ethanol. *Pharmacol Biochem Behav* **22:** 483–487, 1985.
18. Risner, M. E., S. R. Goldberg, J. A. Prada and E. J. Cone. Effects of nicotine, cocaine and some of their metabolites on schedule-controlled responding by beagle dogs and squirrel monkeys. *J Pharmacol Exp Ther* **234:** 113–119, 1985.
19. Rosecrans, J. A. Nicotine as a discriminative stimulus to behavior: Its characterization and relevance to smoking behavior. In: *Cigarette Smoking as a Dependence Process. NIDA Research Monograph No. 23*, edited by N. A. Krasnegor. Rockville: U.S. Dept. of Health, Education and Welfare, 1979, pp. 58–69.
20. Rosecrans, J. A. Brain area nicotine levels in male and female rats with different levels of spontaneous activity. *Neuropharmacology* **11:** 863–870, 1972.
21. Rosecrans, J. A. and M. D. Schechter. Brain area nicotine levels in male and female rats of two strains. *Arch Int Pharmacodyn* **196:** 46–54, 1972.
22. SAS Institute, Inc. *SAS User's Guide: Statistics, 1982 Edition*. Cary, NC: SAS Institute, Inc., 1982.
23. Schechter, M. D. and J. A. Rosecrans. Nicotine as a discriminative cue in rats: Inability of related drugs to produce a nicotine-like cueing effect. *Psychopharmacolgia* **27:** 379–387, 1972.
24. Sershen, H., M. E. A. Reith, M. Banay-Schwartz and A. Lajtha. Effect of prenatal administration of nicotine on amino acid pools, protein metabolism and nicotine binding in the brain. *Neurochem Res* **7:** 1515–1522, 1982.
25. Steel, R. G. D. and J. H. Torrie. *Principles and Procedures of Statistics. A Biometrical Approach*, 2nd edition. New York: McGraw-Hill Book Co., 1980.