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Pharmacologic Characterization of Nicotine-Induced Conditioned Place Preference[1]

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FUDALA, P. J., K. W. TEOH AND E. T. IWAMOTO. *Pharmacologic characterization of nicotine-induced conditioned place preference.* PHARMACOL BIOCHEM BEHAV 22(2) 237–241, 1985.—Rats received subcutaneous injections of either nicotine (0.1 to 1.2 mg/kg) or saline (1.0 ml/kg) immediately prior to conditioning sessions in a conditioned place preference (CPP) paradigm. The drug was paired for 3 conditioning sessions with the non-preferred environment of a 3 compartment place preference apparatus; saline was paired with the preferred environment. The animals were then tested for place preference by determining the proportion of time spent in the preferred and non-preferred compartments during a 15 min test session. Using a statistical method developed for the CPP paradigm, dose-response curves were obtained for the rewarding and aversive effects of nicotine as measured by its ability to alter previously determined baseline preferences obtained from the control animals. Nicotine's rewarding and aversive effects were linearly correlated with respect to dosage within the range of 0.1–0.8 mg/kg (reward increased and aversion decreased). A decrease in reward and an increase in aversion was measured at the 1.2 mg/kg treatment level. Mecamylamine hydrochloride and hexamethonium bromide (at 1.0 mg/kg of the base or ion, respectively) were also tested using the CPP paradigm. While neither compound produced place preferences when administered alone, mecamylamine did block the rewarding effects of 0.8 mg/kg of nicotine when administered 30 minutes prior to the nicotine conditioning sessions. Hexamethonium did not alter nicotine-induced reinforcement. The data suggest that nicotine and its rewarding effects as measured by CPP are primarily mediated by central rather than peripheral events.

Conditioned place preference Reward Aversion Nicotine Dose response Mecamylamine Hexamethonium Rat

ALTHOUGH human smoking behavior is a complex process with psychological and pharmacologic components, nicotine appears to be the primary substance involved in this behavior. Animal studies using various species including the rat [17,19], baboon [1], rhesus monkey [18], squirrel monkey [4, 5, 6, 7] and beagle dog [6,16] have shown that these animals will self-administer nicotine on various schedules of reinforcement.

Conditioned place preference (CPP) has been used to characterize the reinforcing or aversive properties of various drugs, chemicals and ionizing radiation. Rats conditioned with gamma or X-rays [3], ethanol [21] and lithium chloride [12] showed an aversion to the environment with which these stimuli were paired whereas a place preference was induced by cocaine [12], opioids [10,12] and amphetamine [2,15]. The effects of nicotine in conditioned place preference have not yet been investigated.

The success of CPP at measuring reinforcing or aversive effects depends on the association of the internal feeling-state produced by the unconditioned stimulus (the drug) with an external stimulus to be conditioned (a particular chamber of the CPP apparatus). Since the testing of the animal occurs under drug-free conditions, CPP may be particularly useful for screening agents which induce overt psychomotor disturbances after their administration. In addition, the CPP paradigm offers a time and cost efficient measure of reinforcement and aversion since the time required for drug-conditioning can be short as 20 min, animals may be conditioned in pairs in separate chambers of the same apparatus, and the apparatus is simple to construct.

The present experiments were designed to determine whether (1) nicotine produces a dose-dependent reward as measured by the CPP paradigm and, if so, whether (2) nicotine's reinforcing effects can be antagonized by the administration of central or peripheral nicotinic ganglionic blocking agents.

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METHOD

Animals

Experimentally naive, male Sprague-Dawley rats (Harlan Sprague-Dawley Inc., Indianapolis, IN) weighing 255–430 g on the day of testing were initially quarantined for 10 days before being housed in groups of two for one to two weeks. The animals 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.

Apparatus

The experimental apparatus was constructed of plywood and consisted of three distinctive interconnected chambers. One chamber was cubical (25.4 cm side) with black walls and a grid floor. The middle chamber was 10.2×10.2×25.4 cm high with gray walls and a wood floor. The third chamber had an equilateral triangular mesh floor 25.4 cm on a side with white walls 25.4 cm in height. Sliding removable doors separated the middle chamber from the other two. The doorways measured 10.2 cm by 12.7 cm. A 0.32 cm thick clear Plexiglas hinged door covered the top of the apparatus.

Circular holes (1.6 cm in diameter) were drilled through the sides of the apparatus 1.6 cm above the floor to accommodate the paths of photobeam detectors and transducers (Coulbourn Instruments, Lehigh Valley, PA) 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 6.35 cm from the doorways. The photobeam detectors and transducers did not interfere with the animals' activity.

A cumulative timer (Coulbourn Instruments) was activated when the infrared beam passing through the white chamber was initially interrupted. Subsequent interruptions 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.

Drugs

Nicotine base (>98%) was obtained from Eastman Kodak Co., Rochester, NY. Hexamethonium bromide and mecamylamine hydrochloride were purchased from Sigma Chemical Co., St. Louis, MO. Nicotine solutions were prepared fresh daily. Nicotine dose was expressed in terms of the free base assuming a density of 1 mg/μl. Mecamylamine hydrochloride and hexamethonium bromide solutions were prepared weekly and stored at 6°C; the doses were expressed in terms of the base or ion. All drug solutions were prepared with sterile normal saline to give an injection volume of the desired dose equal to 1 ml/kg body weight. All drug injections were made subcutaneously.

Procedure

All experiments were performed in a small room (4 sq. m in floor area) illuminated by a partially shaded 60 watt lightbulb. On conditioning and testing days, the removable floor of the place preference apparatus was washed with tap water and dried after each animal. The mesh floor portion of the white chamber was swabbed with a 0.06% acetic acid solution immediately before each conditioning or testing session to provide another distinguishing feature between the white and black chambers.

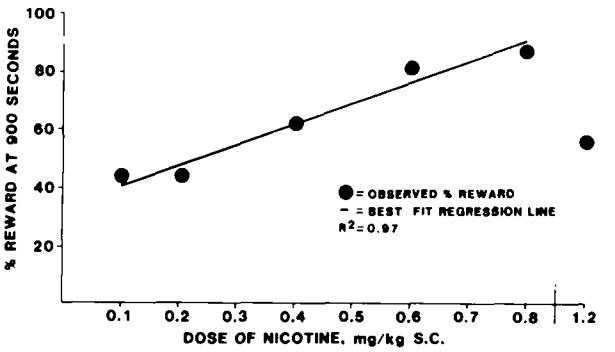

FIG. 1. Percent Reward induced by nicotine in rats. Each point depicts the proportion of groups of 16 to 26 rats exhibiting reward on test preference day after conditioning with nicotine using the place preference paradigm.

The body weights of two rats were recorded. One was injected subcutaneously (SC) with saline (1 ml/kg) and placed in the closed (doors in place) white chamber. The other was injected SC with drug solution and placed in the closed black chamber. Conditioning time was 20 minutes per day. The conditioning chambers and treatments were reversed on the following day. 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 I: Nicotine-induced CPP

Vehicle control group. Over a period of 10 weeks, a vehicle control group, comprised of four groups of 16 animals, was conditioned with saline in both the white and black chambers and then tested for place preference. In Experiment I, all conditioning took place in the presence of background white noise (Coulbourn Instruments) in addition to the conditions described in Procedures. The amounts of time spent in the white and black chambers for each rat during the 15 min test sessions were used in the calculation of a 95% confidence interval (see Statistical Treatment of the Data). The conditioning of this vehicle control group took place either concurrently or during alternating weeks with the treatment groups (below).

Treatment groups. Over a period of 12 weeks, six groups of 16 and one group of 10 rats were conditioned with various

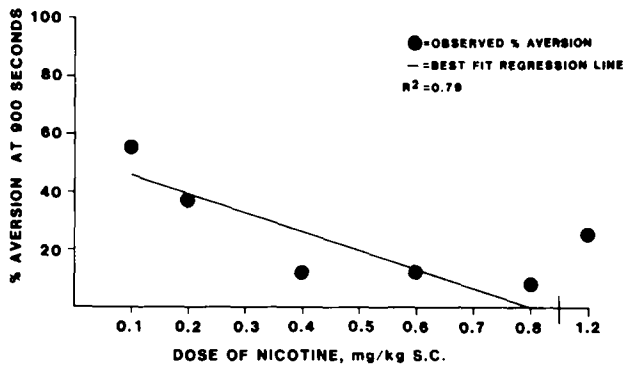

FIG. 2. Percent Aversion induced by nicotine in rats. Each point represents the proportion of animals that exhibited aversion in the same experiment depicted in Fig. 1.

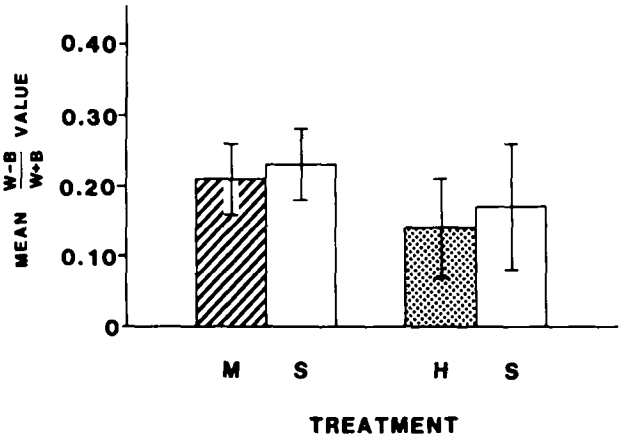

FIG. 3. The effects of mecamylamine (M) and hexamethonium (H) in the conditioned place preference paradigm. The histograms represent the mean residence ratios (±S.E.) from groups of rats conditioned with 1 mg/kg of M or H, or saline (S). N=10 for each group. as a point of information, a mean RR of +0.33 indicates that animals spend twice as much time in the saline-paired, white chamber than in the drug-paired, black chamber of the place preference apparatus.

doses of nicotine in the black chamber and saline in the white chamber and then tested for place preference. The doses of nicotine used were 0.1, 0.2, 0.4, 0.6, 0.8 and 1.2 mg/kg; N=16 for all groups except 0.8 mg/kg where N=26. The amounts of time spent in the white and black chambers for each rat during the 15 min test sessions were used to calculate % Reward or Aversion (see Statistical Treatment of the Data).

Experiment II: Mecamylamine and Hexamethonium Effects on Nicotine-Induced CPP

In Experiment II, all conditioning took place in the absence of the background white noise since preliminary data from an unrelated experiment suggested that the white noise might interfere with nicotine-induced and morphine-induced reward in our CPP paradigm (Fudala, Williamson and Iwamoto, unpublished observations). Subsequent investigation showed no significant differences in the results obtained from experimentation in the presence or absence of the white noise.

In the test for independent effects of mecamylamine (MEC) and hexamethonium (HEX), rats in the drug-treatment group were injected with either 1 mg/kg of MEC or HEX 30 min before conditioning in the black chamber, or were injected with saline and conditioned in the white chamber (N=10 each). The two saline vehicle-control groups (N=10 each) were conditioned in both the white and black chambers.

In testing for the potential antagonism of nicotine-induced reward by the nicotinic blockers, separate groups of rats (N=14 each) received injections of either 1 mg/kg of MEC or HEX 30 min before the alternating saline/nicotine conditioning sessions. The two vehicle control groups (N=14 each) received saline 30 min before conditioning with saline/nicotine. On nicotine conditioning days, 0.8 mg/kg of nicotine was paired with the black chamber.

Statistical Treatment of the Data

Calculation of percent reward or aversion. Using the data from the saline vehicle-control groups in Experiment I, a quantity called the Residence Ratio, (W−B)/(W+B), was calculated. 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 (95% CI) was obtained for the mean Residence Ratio (RR) for the vehicle control groups according to the following formula [13]: mean RR±t × (standard error of the mean) = 95% CI; where t was obtained from a standard Student's t distribution at the 0.025 level of significance. Using the RRs calculated for each rat in the drug-treatment group of Experiment I, the animals at each dose level were categorized as showing reward, aversion or no difference from vehicle control according to the following assignments: Reward, if the observed RR<lower limit of the CI; aversion, if the observed RR>upper limit of the CI; no response, if lower limit of CI≤observed RR≤upper limit of CI. The lower limit for the CI was 0.14 and the upper limit was 0.25 in Experiment I (N=64). The data are plotted as % Reward or % Aversion (100 × the fraction of the rats per group at a given dose exhibiting reward or aversion) versus dose of nicotine. The % Reward and % Aversion for nicotine doses between 0.1 and 0.8 mg/kg were analyzed by linear regression. The regression equations and the coefficients of determination (R^2) were obtained using statistical methods [13] with the aid of the Statistical Analysis System at the University of Kentucky.

Calculation of the Effects of Mecamylamine and Hexamethonium Conditioning and Their Effects on Nicotine Conditioning

The RR was calculated for each rat in the four drug-treatment groups and their corresponding controls in Experiment II. A mean RR was obtained for each treatment and control group and the means analyzed using a two-sample t-test for significance [13].

RESULTS

Nicotine Dose-Response

Nicotine produced both dose-dependent reward and aversion in the same experiment (Figs. 1 and 2). In rats which exhibited reward in Experiment I, the conditioned response was a direct function of dose between 0.1 and 0.8

mg/kg (Fig. 1). The percent reward values ranged from 43.8 to 88.5% and the coefficient of determination (R^2) for the linear regression was 0.97. The 1.2 mg/kg treatment level was not used to estimate the regression since the abrupt decrease in percent reward suggested a biphasic response. The regression equation for predicted percent response is given by: % Reward=0.34 + 0.71 (DOSE). The F test for significant regression yielded $F(1,3)=92.7$, $p<0.0025$.

In those rats that exhibited aversion in Experiment I, the percent aversion responses varied inversely with the dose of nicotine and ranged from 56.3 to 7.7% (Fig. 2). Although a linear model of regression provided an adequate description of the dose-% Aversion relationship: $R^2=0.79$, $F(1,3)=11.6$, $p<0.05$, a quadratic model yielded a substantially better fit of the observed data ($R^2=0.97$) with the regression equation: % Aversion=$0.73-2.05$ (DOSE) + 1.56 $(DOSE)^2$.

The amount of time spent in the gray chamber was not significantly altered by conditioning the rats with nicotine. After estimating the time spent in the gray chamber by subtracting W and B from 900 sec for each rat used in Figs. 1 and 2, mean gray chamber time for the 64 saline-saline vehicle control rats was 220 sec±11 (S.E.M.), and for the 90 saline-nicotine conditioned rats, the gray chamber time was 203 sec±11. Thus, the time spent in the gray chamber was unaffected by the animals' preference for either the white or black chambers.

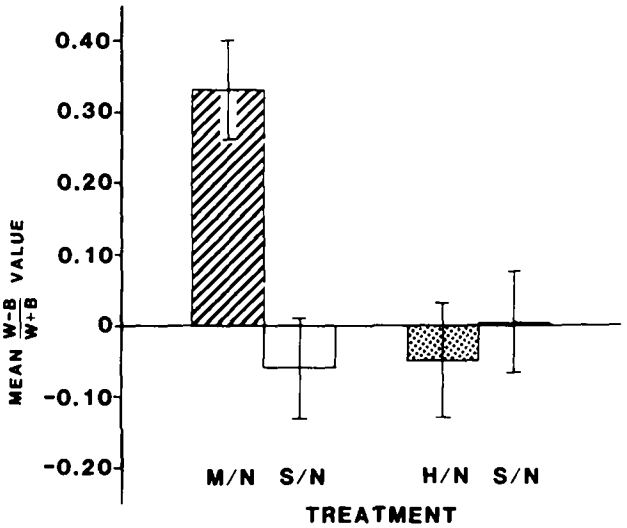

FIG. 4. The effects of mecamylamine (M) and hexamethonium (H) on nicotine-induced reward in the conditioned place preference paradigm. The histograms represent the mean residence ratios (±S.E.) from groups of rats pretreated with 1 mg/kg of M or H, or saline (S), 30 min prior to conditioning with 0.8 mg/kg of nicotine. N=14 for each group. M/N was significantly different from S/N. $p=0.0005$.

Effects of Nicotinic Antagonists

Mecamylamine and hexamethonium had no intrinsic rewarding or aversive effects as assessed by the place preference model when compared with saline controls (Fig. 3). As a point of information, a mean RR of +0.33 indicates that animals spend twice as much time in the saline-paired, white chamber than in the drug-paired, black chamber of the place preference apparatus. Thus, the control rats used in this experiment, as well as in Experiment I, had a preference for the white chamber. Neither mecamylamine nor hexamethonium, at a dose of 1 mg/kg, altered this preference.

Mecamylamine, but not hexamethonium, antagonized the rewarding effects of 0.8 mg/kg of nicotine (Fig. 4). Rats administered saline 30 min before the conditioning dose of nicotine (S/N) exhibited reward as evidenced by a decreased mean RR value (−0.06±0.07) relative to those that received mecamylamine prior to nicotine conditioning (M/N: 0.33±0.07). Decreased RR values indicate that rats spend more time in the drug-paired, black chamber. As can be seen in Fig. 4, hexamethonium did not alter nicotine-induced reward (H/N not significantly different from S/N; $p>0.1$). Thus, 1 mg/kg of mecamylamine antagonized nicotine-induced reward in the place preference paradigm.

DISCUSSION

These data clearly demonstrate that nicotine, at doses from 0.1 to 1.2 mg/kg, induces both rewarding and aversive effects in rats as assessed by the conditioned place preference paradigm. Neither the peripheral nicotine receptor antagonist hexamethonium [14] nor the central/peripheral antagonist mecamylamine [11,20] induced place preference when administered alone. However, pretreatment with 1 mg/kg mecamylamine, but not hexamethonium, blocked the reinforcing effects of a 0.8 mg/kg dose of nicotine in the CPP paradigm.

Although previous workers using the conditioned place preference paradigm usually present their data in terms of "mean time in seconds" spent in the saline-paired and drug-paired sides [2, 10, 12], we chose to present our data using the Residence Ratio for the following reasons. The numerator, W−B, reflects the dynamics of the place preference paradigm. When rats spend more time in the white chamber than in the black chamber, the term will be positive. Such was the case for our saline-saline conditioned vehicle control rats which had a natural bias for the white chamber. In the present experiments, we hypothesized that if nicotine-pairing produced approach to environmental cues, then pairing the black chamber with nicotine should result in W−B values less than the control values (B gets larger). Conversely, if nicotine pairing produced avoidance of environmental cues, then pairing the black chamber with nicotine should result in W−B values larger than control values. The denominator, W+B, is the total time spent by an animal in the white and black chambers. This term was used because of the slight individual differences between animals with respect to the time spent in the gray chamber. Although nicotine conditioning did not significantly alter the mean gray chamber time (see Results), it was considered appropriate to correct for these individual differences between rats by using the denominator, W+B. Thus, the Residence Ratio is a single term that expresses the proportion of the total time spent by an animal in the white and the black chambers. Ratios which approach −1 are indicative of sample populations that spend increasing amounts of time in the drug-paired, black chamber.

It is important to run appropriate saline-conditioned groups along with the drug-conditioning groups in the place preference paradigm in order to control for natural biases rats may have for a particular environment of the apparatus. From our saline-saline conditioned groups (saline injections on "white chamber" days and saline injections on "black chamber" days), we derived the 95% confidence interval. The significance of the 95% confidence interval is that if 20

saline-saline groups of rats were run, 19 of the groups' mean RR values should fall within this interval. On the other hand, if separate groups of rats were conditioned with a drug that significantly alters place preferences, the groups' mean RR values should lie outside the vehicle control confidence interval. This highlights the efficiency of the method. Depending on the relation of an animal's RR value to the 95% confidence interval, we can classify three different categories of responses in the place preference paradigm, namely Reward, Aversion, and No Difference from Control.

Our investigation has shown nicotine to be both rewarding and aversive in the same experiment. At the lowest dose tested (0.1 mg/kg SC), nicotine was rewarding and aversive in an almost equal proportion of animals. These data suggest the possibility that there are distinct populations for which a given dose of nicotine is initially either rewarding or aversive. As the dose of nicotine was increased to 0.8 mg/kg, the % Reward increased linearly and the level of aversion diminished. A dose of 1.2 mg/kg of nicotine appeared to induce more aversion and less reward than did the 0.8 mg/kg dose. Thus, the multiple effects of nicotine in the place preference paradigm depend on the dose of nicotine and are biphasic with respect to dose. Mecamylamine, but not hexamethonium, antagonized nicotine induced reward. Whether nicotinic antagonists block nicotine induced aversion in the CPP paradigm is currently being investigated.

In addition to the positive reinforcing effects of nicotine, nicotine can also suppress its own self-administration in animals [1,4] and in human subjects [8,9]. Both nicotine's dose and schedule of administration appeared as determinants of its reinforcing or punishing effects. Our data extend these findings by showing that both aversion and reward can be induced by the same dose of nicotine and that this duality of response is also dose-related.

In summary, nicotine induces both reward and aversion in rats using the place preference paradigm. Nicotine-induced reward is probably central in origin since mecamylamine, and not hexamethonium antagonized nicotine-induced place preference.

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