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BRIEF COMMUNICATION

Dissociation of Buprenorphine-Induced Locomotor Sensitization and Conditioned Place Preference in Rats

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ROWLETT, J. K., T. R. GIBSON AND M. T. BARDO. *Dissociation of buprenorphine-induced locomotor sensitization and conditioned place preference in rats.* PHARMACOL BIOCHEM BEHAV **49**(1) 241-245, 1994.—The locomotor and rewarding effects of the opioid mixed agonist-antagonist buprenorphine were assessed in a conditioned place preference (CPP) experiment. Separate groups of rats were given one of three doses of buprenorphine (0.3, 1.0, or 3.0 mg/kg) or saline paired with the white compartment of a CPP apparatus. The following day, all rats received saline paired with the black compartment. After six conditioning trials, rats were given free access to all compartments of the CPP apparatus. Horizontal activity data obtained during conditioning revealed increased activity (i.e., behavioral sensitization) for the three doses on trial 6. Vertical activity data revealed significant increases on trial 6 for the 1.0 and 3.0 mg/kg doses only. Significant CPP was obtained with the 0.3 mg/kg and 1.0 mg/kg doses of buprenorphine, but not with the 3.0 mg/kg dose. These data indicate that buprenorphine elicits locomotor sensitization after repeated exposures that follows a linear dose-response relationship. In contrast, these data suggest that the rewarding effects of buprenorphine follow an inverted U-shaped dose-response curve.

Buprenorphine Conditioned place preference Locomotor activity Behavioral sensitization
Opioid mixed agonist-antagonist Drug reward

BUPRENORPHINE is an opioid mixed agonist-antagonist that has been proposed as a pharmacological treatment of opioid dependence (10,11). Buprenorphine has been shown to act as a full morphine-like agonist in tests using self-administration (15,16,20,21) and intracranial self-stimulation (9) in animals. In humans, buprenorphine elicits opioid agonist-like subjective effects (10) and abuse of buprenorphine has been reported (7,8,17).

In some behavioral tests, buprenorphine exhibits an unusual dose-response function. Specifically, buprenorphine produces a biphasic inverted U-shaped dose-response curve in tests of analgesia (5,6), catalepsy (5), respiratory depression (4), and gastrointestinal motility [(4), see (14) for review]. Recently, this drug was shown to produce an inverted U-shaped dose-response curve in the conditioned place preference (CPP) paradigm (2). This finding suggests that CPP may be a potentially useful procedure for elucidating the neuropharmacological mechanism of the inverted U-shaped dose-response curve. However, experiments in our laboratory (18) have demonstrated a linear dose-response function for buprenorphine-induced CPP over a dose range similar (i.e., 0.001-1.0 mg/kg) to that studied by Brown et al. (2). The present experiment, therefore, sought to determine the dose-response curve for buprenorphine-induced CPP at a slightly higher dose range (0.3 to 3 mg/kg). This dose range was chosen because a similar dose range has been shown to produce an inverted U-shaped dose-response curve with tests such as analgesia (4-6).

Another objective of the present study was to examine whether the locomotor effects of repeated buprenorphine

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treatment also follows a biphasic, inverted U-shaped dose-response function. Morphine produces an augmented locomotor response with repeated injections [i.e., behavioral sensitization, see (13) for review]. The present experiment examined the acute and repeated locomotor-activating effects of buprenorphine at 0.3, 1, and 3 mg/kg during the conditioning trials of the CPP procedure.

METHOD

Animals

Thirty-six male Sprague–Dawley rats (Harlan Industries, Indianapolis, IN), that weighed between 200 and 250 g at the beginning of the study, were used. Rats were housed individually in mounted rack cages (lights on from 0700 h to 1900 h, food and water available continuously).

Apparatus

The apparatus consisted of a rectangular wooden chamber with three compartments separated by removable panels. The two end compartments measured 22 × 26 × 30 cm high, while the middle compartment measured 22 × 14 × 30 cm high. One end compartment had white walls, a wire mesh floor, and pine bedding beneath the floor. The other end compartment had black walls, a metal grid floor, and cedar chips beneath the floor. The middle compartment had grey walls and a solid wood floor. For conditioning trials, the removable panels had no openings and were identical to the walls of the chamber (i.e., the rat was confined to a particular end compartment). For testing, a second set of removable panels was used that had a 10 × 10 cm opening at the bottom of the panels (i.e., the rat had free access to all three compartments). Two identical CPP chambers were used. Both chambers were placed in a room separate from the colony room which contained a white noise generator (ambient background of 70 dB). A video camera was hung directly over the CPP chambers to videotape behavior on a videocassette recorder located in an adjacent room.

Drug

Buprenorphine hydrochloride was synthesized by Reckitt & Coleman (Dansom Lane, Kingston-upon-Hull, UK) and supplied by the National Institute on Drug Abuse (Rockville, MD). The drug was dissolved in saline at 1.5 mg/ml with a drop of glacial acetic acid per 10 ml of solution added. This solution was sonicated for approximately 10 min, and lower doses were then diluted in the same drug vehicle. The injections were given via the intraperitoneal (IP) route at a volume of 2.0 ml/kg for all doses. The doses were based on the salt form of the drug.

Procedure

The rats were randomly assigned to one of four dose groups (n = 9 per group). Animals were administered either 0 (saline only), 0.3, 1.0, or 3.0 mg/kg buprenorphine paired with the white end compartment. On each conditioning day, the rat was transported in its home cage to the room with the CPP chambers, injected with saline or drug, and then immediately placed into the white compartment. After 30 min, the rat was removed from the compartment and returned to the home cage. On the following day, rats from all treatment groups were injected with saline and placed immediately into the black compartment for 30 min. The order of exposure to the white and black compartments was counterbalanced within each treatment group. This conditioning procedure was repeated 12 consecutive days such that each animal received six drug-white compartment pairings and six saline-black compartment pairings.

During conditioning, rats were videotaped for horizontal and vertical activity on the first and sixth trial on which the drug was paired with the white compartment. Activity was scored by an observer unaware of the treatment conditions for each individual rat. Horizontal activity consisted of both forepaws crossing a line drawn on a video monitor screen that bisected the white compartment. Line crosses were counted when the animal moved forward or backward over the line. Vertical activity consisted of both forepaws leaving the compartment floor and included rearing with forepaws against the compartment wall or nonsupported rearing and jumping, but excluded grooming behavior. Both activity measures were sampled over three 5-min blocks that occurred 5–10, 15–20, and 25–30 min after the injection. The data obtained across the three blocks were cumulated for statistical analyses. Activity data were not recorded when rats were given saline-black pairings.

On the day after the last conditioning trial (day 13), the solid removable panels in each CPP chamber were replaced with the panels with openings. Each rat was placed into the middle compartment and given free access to all three compartments for 15 min. An observer, unaware of the individual treatments, recorded the time spent in the white and black compartments, as well as the number of entries into each compartment.

Data Analysis

Horizontal and vertical activity data during conditioning trials were analyzed separately using mixed factorial analyses of variance (ANOVA), with drug group as the between-subjects factor and conditioning day as the repeated measure. On the CPP test day, the durations and entries spent in the white and black end compartments were analyzed with separate mixed factorial ANOVA with drug group as the between-subjects factor and end compartment as the repeated measure. Multiple comparisons were made using Dunnett's tests comparing each drug group to the saline control and using the Bonferroni t procedure for repeated measures. All statistical tests were two tailed, with an alpha level of $p \leq 0.05$.

RESULTS

The horizontal activity results are shown in the top panel of Fig. 1. A mixed factorial ANOVA revealed significant main effects of dose and trial, $F(3, 32) = 4.77$, $p < 0.01$, and $F(1, 32) = 25.86$, $p < 0.0001$, respectively. The interaction of trial and dose was significant, $F(3, 32) = 3.71$, $p < 0.05$. No effect of buprenorphine on trial 1 horizontal activity was obtained. However, multiple comparisons revealed that horizontal activity produced by 0.3, 1.0, and 3.0 mg/kg buprenorphine on trial 6 was significantly higher than the saline controls (Dunnett's tests, $p < 0.05$). Horizontal activity increased significantly from trial 1 to trial 6 for the 1.0 and 3.0 mg/kg buprenorphine groups only (Bonferroni t-test, $p < 0.05$).

The vertical activity results are shown in the bottom panel of Fig. 1. A mixed factorial ANOVA revealed a significant main effect of trial only, $F(1, 32) = 18.84$, $p < 0.0001$. The interaction of trial and dose was significant, $F(3, 32) = 3.39$, $p < 0.05$. No effect of buprenorphine on trial 1 vertical activ-

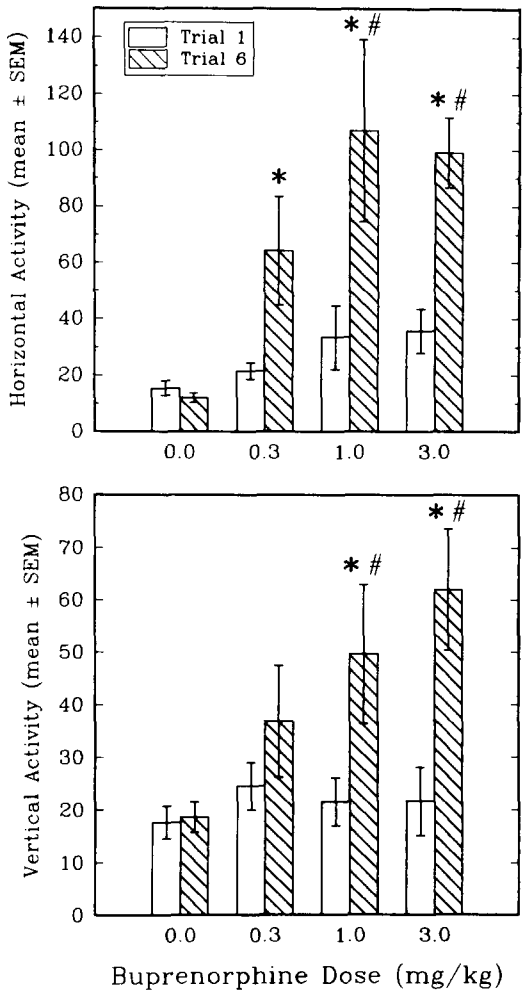

FIG. 1. Horizontal (top panel) and vertical (bottom panel) locomotor activity data for saline- and buprenorphine-treated rats during conditioning in the white compartment (n = 9/group). Note that * represents a significant between-group difference compared to saline control within a trial (Dunnett's test, $p < 0.05$) and # represents a significant difference from trial 1 to trial 6 (Bonferroni t-test, $p < 0.05$).

ity was obtained. Multiple comparisons revealed that vertical activity produced by 1.0 and 3.0 mg/kg buprenorphine on trial 6 was significantly higher than the saline controls (Dunnett's tests, $p < 0.05$). In addition, vertical activity produced by 1.0 and 3.0 mg/kg buprenorphine increased significantly from trial 1 to trial 6 (Bonferroni t-test, $p < 0.05$).

The durations spent in the white and black end compartments on the CPP test day are shown in Fig. 2 (top panel). A mixed factorial ANOVA revealed a significant main effect of end compartment, $F(1, 32) = 4.61$, $p < 0.05$, and a significant dose by compartment interaction, $F(3, 32) = 7.64$, $p < 0.001$. Multiple comparisons revealed significantly higher durations in white for the 0.3 and 1.0 mg/kg doses of buprenorphine compared to the saline control group (Dunnett's tests, $p < 0.05$). In addition, the duration in black was significantly lower at the 0.3 mg/kg dose compared to the saline control group (Dunnett's test, $p < 0.05$). However, the 3.0 mg/kg dose of buprenorphine did not significantly increase the duration in the white end compartment relative to control. Finally, Bonferroni t-tests revealed significant differences in duration spent in white compared to black for both the 0.3 and 1.0 mg/kg doses ($p < 0.05$), but not for the 3 mg/kg dose. For the saline group, the difference in white compared to black approached, but did not achieve, significance (Bonferroni t-test, $p < 0.10$).

The entries into the white and black end compartments on the CPP test day are shown in Fig. 2 (bottom panel). A mixed factorial ANOVA revealed a significant end compartment by dose interaction, $F(3, 32) = 9.56$, $p < 0.05$. Multiple comparisons revealed that entries into the black compartment were decreased by 0.3 mg/kg buprenorphine, while entries into the white compartment were increased by 1.0 mg/kg buprenorphine compared to the saline control (Dunnett's test, $p < 0.05$). In addition, rats in the saline group made more entries into black than into the white end compartment (Bonferroni t-test, $p < 0.05$).

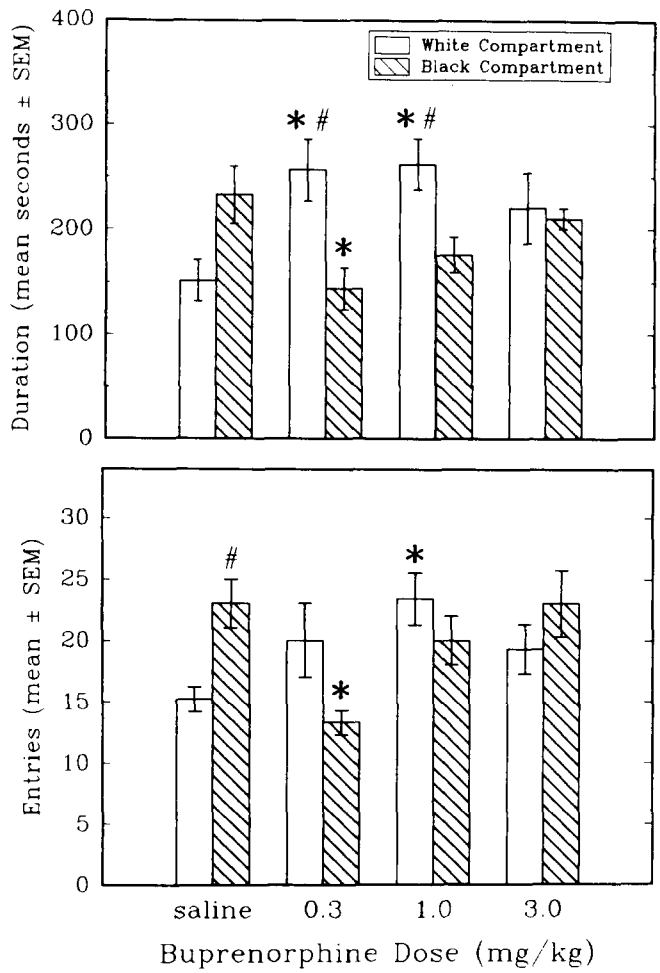

FIG. 2. Duration (top) and entries (bottom) in the white and black end compartments of the CPP apparatus for saline- and buprenorphine-treated rats on the test day (n = 9/group). Rats received six buprenorphine conditioning trials prior to the test day. Note that * represents a significant between-group difference compared to saline control within a trial (Dunnett's test, $p < 0.05$) and # represents a significant difference between white and black end compartments (Bonferroni t-test, $p < 0.05$).

DISCUSSION

The locomotor activity results from the conditioning trials demonstrated that buprenorphine produced enhanced horizontal and vertical activity on trial 6 compared to trial 1, an effect indicative of behavioral sensitization (13). Moreover, sensitization appeared to be developing at 0.3 mg/kg for horizontal activity, measured as an increase in activity compared to the saline controls but not as an increase from trial 1 to trial 6. For vertical activity (i.e., rearing, jumping), sensitization was observed only at 1.0 mg/kg and above. This difference in dose-response functions for horizontal and vertical activity may represent the manifestation of a different behavioral syndrome after repeated high doses of buprenorphine. For both horizontal and vertical activity, however, the sensitization effect appeared to be linear, i.e., no evidence of a biphasic dose-response function was obtained.

The behavioral sensitization effect observed with repeated buprenorphine injections is similar to that observed with repeated morphine treatment (1,12,13). For example, rats treated with 10 mg/kg morphine show a slight increase in acute locomotor activity relative to saline-treated rats, an effect that sensitizes after daily injections (1,12). This sensitization effect of morphine is characterized by increases in a variety of specific behaviors (e.g., rearing, sniffing, grooming, and activity bursts), as well as increases in dopamine neurotransmission (12,13). In some conditions, the sensitization effect produced by morphine may reflect the development of conditioned hyperactivity [(19), but see (12)]. Because buprenorphine has other pharmacological sites of action in addition to morphine-like μ agonist receptor interactions (14), it will be important to determine whether a pattern of behavioral (i.e., stereotypy, conditioned hyperactivity) and neurochemical (i.e., enhanced dopamine neurotransmission) results similar to that seen with morphine also occur following repeated buprenorphine treatment.

The present experiment replicates and extends a previous study that demonstrated significant CPP with buprenorphine (2), indicating that this drug has rewarding properties similar to that obtained with morphine [see (3) for review]. Evidence for an inverted U-shaped dose-response function for buprenorphine-induced CPP was obtained, because the 3.0 mg/kg dose did not produce significant CPP. This finding should be interpreted with some caution, however, because a trend for CPP was obtained with the 3.0 mg/kg dose of buprenorphine. Nevertheless, this pattern of results is consistent with the findings of Brown et al. (2), in that a relatively high dose of buprenorphine did not produce significant CPP. Taken together, the dose-response functions reported here suggest that the neural mechanisms involved in locomotor sensitization produced by buprenorphine may be different from the the neural mechanisms involved in the rewarding effects of buprenorphine.

It is possible that the occurence of an inverted U-shaped dose response curve for buprenorphine-induced CPP reflects the development of conditioned hyperactivity. Drugs often elicit conditioned increases in activity when animals are tested in a drug-free state in an environment previously paired with the drug [e.g., (19)]. Perhaps 3.0 mg/kg buprenorphine produced greater conditioned hyperactivity than 0.3 or 1.0 mg/kg buprenorphine and this expression of hyperactivity interfered with the expression of conditioned place preference. However, shuttling behavior in the 3.0 mg/kg group, as measured by entries into the white or black end compartments, was not increased relative to the saline control. Indeed, entries into the white compartment were significantly enhanced in the 1.0 mg/kg buprenorphine group, suggesting that increased activity may have occurred in this group, even though significant CPP was observed as well. Thus, these results suggest a dissociation of locomotor activity and the expression of reward on the test day.

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