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Effects of Apomorphine on Novelty-Induced Place Preference Behavior in Rats

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BARDO, M. T., M. LACY AND B. A. MATTINGLY. *Effects of apomorphine on novelty-induced place preference behavior in rats.* PHARMACOL BIOCHEM BEHAV **37**(1) 89–93, 1990.—Adult male rats were exposed to one of two different stimulus compartments by being placed into the compartment for 30 min on each of eight consecutive days. Following this exposure, each rat was administered 0, 0.05, 0.1, 0.5 or 5.0 mg/kg apomorphine. Thirty min after injection, each animal was given free-choice access to the familiar (exposed) compartment and to the novel (nonexposed) compartment. As expected, saline-injected control animals displayed a preference for the novel compartment. This novelty preference was disrupted in animals given either 0.05 or 0.1 mg/kg apomorphine, but not in animals given either 0.5 or 5.0 mg/kg apomorphine. The disruption in novelty preference by the low doses of apomorphine did not reflect a disruption of locomotor activity, as there was no direct relationship between the preference for novelty and the rate of horizontal or vertical activity among the different treatment groups. Instead, the low doses of apomorphine may have inhibited dopamine function by blocking presynaptic autoreceptors selectively, and thus the reinforcing effect of the novel stimulation may have been attenuated.

Place preference Apomorphine Novelty Locomotor activity Dopamine

EXPOSURE to novel environmental stimuli may activate the mesolimbic dopamine (DA) pathway in a manner analogous to the administration of stimulant drugs. Similar to the effects of amphetamine, rats exposed to novel environmental stimuli display an increase in locomotor activity (2, 3, 28) and find novel stimuli to be reinforcing as assessed in the place preference paradigm (6, 11, 23). Lesions of the mesolimbic DA system disrupt the increase in locomotion, rearing and approach behaviors normally elicited by novel stimuli (7, 8, 19). Further, novelty-induced place preference is blocked when rats or mice are tested under the influence of DA antagonist drugs such as haloperidol and thioridazine (1,18).

While DA antagonists disrupt novelty-induced place preference, the effect of DA agonists on novelty preference behavior is less clear. Methamphetamine has been reported to decrease novelty preference behavior as either a direct function of dose (17) or as a U-shaped function of dose (12). More recently, our laboratory failed to detect any effect of d-amphetamine (0.1–1.0 mg/kg) on novelty-induced place preference (1). The lack of effect obtained with d-amphetamine may reflect the relatively weaker action of this drug compared to methamphetamine within the central nervous system (27). However, other methodological differences between experiments cannot be ruled out.

The purpose of the present experiment was to examine the effect of the direct DA agonist apomorphine on novelty-induced place preference behavior in rats. Unlike the indirect DA agonist amphetamine, which evokes the release of DA from presynaptic terminals, apomorphine has a a direct agonist action at both presynaptic and postsynaptic receptors (24). We chose a range of apomorphine doses (0, 0.05, 0.1, 0.5 and 5.0 mg/kg) which would presumably assess both the low- and high-dose receptor actions of apomorphine.

METHOD

Animals

The animals were male Sprague-Dawley rats obtained from Harlan Industries (Indianapolis, IN) at 225–250 g body weight. Animals were caged individually with food and water available continuously in the home cage. Prior to the start of the experiment, animals were acclimated to the colony room (22 ± 1°C, humidity 45 ± 5%) for at least one week and were handled for 2 days.

Apparatus

The apparatus consisted of a rectangular wooden chamber that had three different compartments separated by removable partitions. The two end compartments measured $24 \times 30 \times 45$ cm high, while the middle compartment was smaller and measured $24 \times 10 \times 45$ 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 bedding beneath the floor. The middle compartment had gray walls and a solid wood floor. The solid partitions could be

replaced with similar partitions containing a 10 × 10 cm opening, which allowed the animals access to all compartments. The apparatus was located in a laboratory room that was separate from the colony room and was equipped with a white noise generator and audio speaker (ambient background of 70 dB). Suspended from the ceiling above the apparatus was a video camera which was used to record the animals' behavior on test days.

Procedure

Two separate experiments were performed. In both experiments, one-half of the animals were exposed to the white compartment and the other half were exposed to the black compartment. Exposure consisted of placing the animal into the compartment for 30 min daily on each of eight consecutive days with the solid partitions in place.

On the day after the last exposure session, each animal in Experiment 1 was assigned to one of the following drug treatment groups (n = 10 per group) which were counterbalanced for prior placements into either the white or black compartments: 0, 0.05, 0.5 or 5.0 mg/kg apomorphine HCl. In Experiment 2, animals were administered either 0 or 0.1 mg/kg apomorphine (n = 8 per group). Apomorphine was mixed in 0.001 N HCl and injected SC in a volume of 0.5 ml/kg body weight. The dosage was expressed as the salt form of the drug.

Thirty min after injection, each animal was placed into the center gray compartment with the solid partitions removed and replaced by the partitions having an opening. Using a video monitor, an observer who was unaware of each animal's individual treatment recorded behavior for 15 min in both the novel and familiar end compartments. The following measures were taken: 1) Duration spent in each compartment (defined as both front paws in the compartment); 2) number of entries into each compartment (defined as both front paws breaking the plane of partition between compartments); 3) horizontal activity (defined as both front paws crossing a line drawn on the video monitor screen that bisected each compartment parallel to the partition); and 4) vertical activity (defined as both front paws off the floor, excluding grooming behaviors). The injection and test procedure was repeated again on the next day.

Statistics

Horizontal and vertical activity data were converted to rate measures by taking the number of line crosses and rears observed within each compartment (novel vs. familiar) and dividing it by the total duration spent within each compartment. For each dependent variable, a separate split-plot analysis of variance was then performed on the data obtained from each of the two test days. In cases where significant interactions occurred, Bonferroni *t*-tests were performed to compare the within-subject differences (novel vs. familiar compartment) for each drug treatment group. In addition, further analyses of variance were used to compare the between-subject differences (0, 0.05, 0.5 vs. 5.0 mg/kg) within each compartment. Subsequent pairwise comparisons for between-subject differences between each drug group relative to the saline control group were performed using Dunnett's test. In all cases, the level of statistical significance was declared at either $p<0.05$ or $p<0.01$.

RESULTS

Novelty Preference

As shown in the left panel of Fig. 1, animals displayed a novelty-induced place preference which was disrupted by 0.05 mg/kg apomorphine. Analysis of the data from test day 1 revealed a significant interaction between the test drug and compartment factors, $F(3,36) = 4.55$, $p<0.01$. Subsequent within-subject analyses indicated that animals spent significantly more time in the novel compartment than in the familiar compartment when tested under the influence of 0, 0.5 and 5.0 mg/kg apomorphine, Bonferroni $t(9)$'s >2.68, $p<0.05$ in each case, but not when tested under the influence of 0.05 mg/kg apomorphine, Bonferroni $t(9) = 0.14$, $p>0.05$. Between-group analyses also revealed that, while there was no significant change across apomorphine doses in the duration spent in the novel compartment, $F(3,36) = 2.42$, $p>0.05$, there was a significant decrease across apomorphine doses in the duration spent in the familiar compartment, $F(3,36) = 6.00$, $p<0.01$. Subsequent comparisons revealed that animals tested under the influence of 0.5 mg/kg apomorphine displayed significantly less duration in familiar relative to saline controls, Dunnett's test, $p<0.05$, while there was no significant difference produced by either 0.05 or 5.0 mg/kg apomorphine relative to saline controls, Dunnett's tests, $p>0.05$. On test day 2, there was no significant main effect or interaction evident in the overall analysis of variance of the duration data (see Fig. 1, right panel).

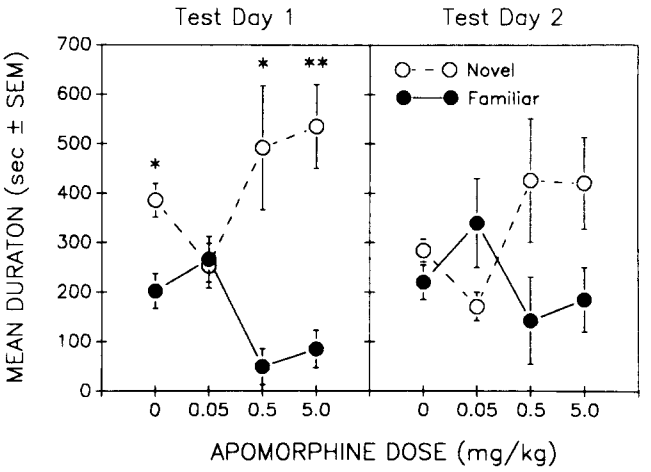

FIG. 1. Mean (± SEM) duration spent in the novel and familiar compartments in animals administered 0, 0.05, 0.5 or 5.0 mg/kg apomorphine on test days 1 and 2 in Experiment 1. The asterisks represent a significant within-subject difference from the duration in the familiar compartment, $*p<0.05$, $**p<0.01$.

The results from the second experiment showed that another low dose of apomorphine (0.1 mg/kg) disrupted novelty-induced place preference on test day 1 (data not shown). As expected, when tested under the influence of saline, animals spent significantly more time in the novel compartment than in the familiar compartment; the means (± SEM) were 321 ± 14 sec in novel and 224 ± 11 sec in familiar, Bonferroni $t(7) = 3.31$, $p<0.05$. However, when tested under the influence of 0.1 mg/kg apomorphine, there was no significant difference in time spent in the novel and familiar compartments; the means (± SEM) were 196 ± 53 sec in novel and 391 ± 97 sec in familiar, Bonferroni $t(7) = 1.40$, $p>0.05$.

Activity

As shown in Fig. 2, apomorphine produced a dose-dependent

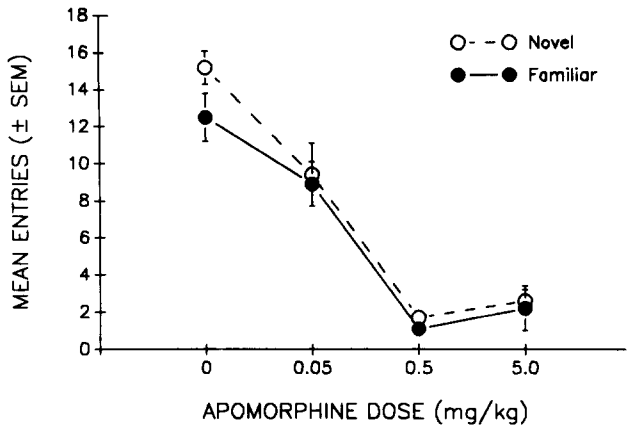

FIG. 2. Mean (±SEM) number of entries into the novel and familiar compartments in animals administered 0, 0.05, 0.5 or 5.0 mg/kg apomorphine on test day 1 in Experiment 1.

decrease in the number of entries into both the novel and familiar compartments on test day 1, $F(3,36)=38.13$, $p<0.01$. Regardless of whether the compartment was novel or familiar, animals tested under the influence of either 0.5 or 5.0 mg/kg apomorphine made significantly fewer compartment entries than animals tested under the influence of saline, Dunnett's tests, $p<0.05$ in each case, while there was no significant difference between animals given saline or 0.05 mg/kg apomorphine, Dunnett's test, $p>0.05$. On test day 2, there was also a significant dose-dependent decrease in the number of compartment entries, $F(3,35)=18.79$, $p<0.01$ (data not shown). However, there was no significant within-subject difference in the number of entries into the novel and familiar compartments at any drug dose on test days 1 or 2.

Apomorphine also produced a dose-dependent decrease in the rate of horizontal and vertical activity measured in each compartment on test day 1 (see Fig. 3). For horizontal activity, the overall analysis of test day 1 data revealed a significant interaction between the test drug and compartment factors, $F(3,25)=3.89$, $p<0.05$. Subsequent within-subject analyses indicated that animals displayed a significantly lower rate of horizontal activity in the novel compartment than in the familiar compartment when tested under the influence of saline, Bonferroni $t(9)=5.38$, $p<0.01$, but not when tested under the influence of 0.05, 0.5 or 5.0 mg/kg apomorphine, Bonferroni t's<0.69, $p>0.05$ in each case. Between-subject analyses indicated that, relative to saline controls, horizontal activity was decreased significantly in both the novel and familiar compartments across each dose of apomorphine tested, Dunnett's tests, $p<0.05$. On test day 2, apomorphine also produced a dose-dependent decrease in horizontal activity rate, $F(3,25)=11.52$, $p<0.01$. However, there were no significant within-subject differences in the rate of horizontal activity within the novel and familiar compartments at any drug dose on test day 2 (data not shown).

For vertical activity, there was also a dose-dependent decrease in activity rate on test day 1, $F(3,25)=13.70$, $p<0.01$ (see Fig. 3, bottom) and on test day 2, $F(3,25)=8.75$, $p<0.01$ (data not shown). Regardless of whether the compartment was novel or familiar, between-subject analyses from test days 1 and 2 revealed that vertical activity was decreased significantly across each apomorphine dose tested relative to saline controls, Dunnett's tests, $p<0.05$. However, there were no significant within-subject differences in the rate of vertical activity between the novel and familiar compartments at any drug dose on test days 1 or 2.

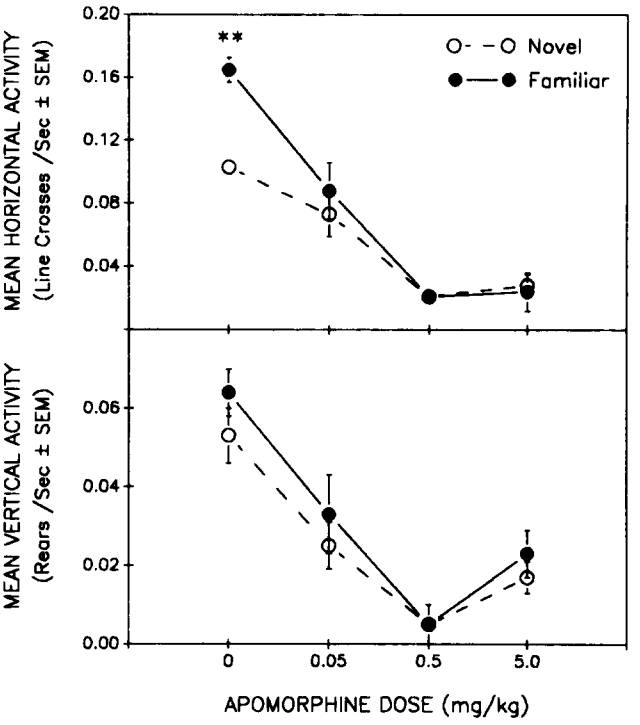

FIG. 3. Mean (±SEM) rate of horizontal and vertical activity in the novel and familiar compartments in animals administered 0, 0.05, 0.5 or 5.0 mg/kg apomorphine on test day 1 in Experiment 1. The asterisks represent a significant within-subject difference from the rate of activity in the novel compartment, $^{**}p<0.01$.

DISCUSSION

As expected from previous work (6, 11, 23), rats given free-choice access to a novel and familiar stimulus environment showed a preference for the novel environment. This preference behavior was evident on the first test day, but not on the second test day. Further, the preference behavior observed on the first test day was accompanied by a decrease in the rate of horizontal activity in the novel environment relative to the familiar environment, a finding which replicates previous work (1,17).

More importantly, on the first test day, apomorphine produced a dose-dependent alteration in novelty-induced place preference. With low doses of apomorphine (0.05 and 0.1 mg/kg), there was a disruption in novelty-induced place preference. With higher doses of apomorphine (0.5 and 5.0 mg/kg), however, there was actually a tendency for the novelty-induced place preference to be enhanced. These results contrast with a previous study by Misslin and colleagues (18) which reported that apomorphine in low doses (0.06–0.25 mg/kg) had no effect on novelty-induced place preference, whereas apomorphine in higher doses (0.5–8.0 mg/kg) disrupted the preference. However, our report is not directly comparable to this previous report, since we tested rats that had been given eight 30-min exposures to one environment, whereas Misslin and colleagues (18) tested mice that had been given a single 24-hr exposure to one environment. Indeed, species differences may represent a critical variable to consider because, unlike the apomorphine-induced stereotypic response characterized in

rats, apomorphine is known to produce a different species-typical climbing response in mice (5).

In the rat, low doses of apomorphine (0.05–0.1 mg/kg) are thought to have a relatively selective action at presynaptic autoreceptors on DA neurons. This notion comes from electrophysiological and neurochemical evidence indicating that low doses of apomorphine inhibit completely the activity of midbrain DA neurons (24) and attenuate the release of DA in the striatum (26). In addition, a low dose of apomorphine depresses locomotor activity in an open-field and reduces exploratory nose-poking behavior (13, 14, 25). This depression in locomotion and exploration is similar to the effect seen following administration of haloperidol (1). Taken together, these findings suggest that the disruption of novelty-induced place preference by low doses of apomorphine in the present study may be related to an inhibition of DA function.

Higher doses of apomorphine (0.5–5.0 mg/kg) are thought to activate postsynaptic DA receptors in addition to the autoreceptors. This notion comes from electrophysiological evidence indicating that the neurons within the striatum are inhibited by apomorphine, but at doses 6-fold higher than those required to inhibit the presynaptic input from midbrain DA neurons (24). This suggests that the higher doses of apomorphine used in the present study (0.5 and 5.0 mg/kg) may have activated postsynaptic terminal neurons directly and thus, the blockade of novelty-induced place preference observed with low "autoreceptor" doses of apomorphine may have been reversed.

While apomorphine produced a biphasic effect on novelty-induced place preference as a function of dose, apomorphine produced a monophasic depression in locomotor activity within the same dose range. Previous reports have been inconsistent about the dose-response relationship between apomorphine dose and level of locomotor activity in rats. That is, although it is widely agreed that low doses decrease activity, higher doses of apomorphine (1–5 mg/kg) have been reported to increase activity (9, 14, 20, 21), decrease activity (10, 13, 22, 25) or have no effect on activity following acute administration (15,16). Numerous procedural differences exist among these reports, including differences in the rat strain and apparatus used, the method of quantifying activity, the injection route, and whether or not animals were habituated to the apparatus. These vast procedural differences preclude any firm conclusion about what may account for the depression in activity observed in the present study following high doses of apomorphine. Nonetheless, the fact that apomorphine produced a biphasic effect on novelty-induced place preference and a monophasic effect on locomotor activity suggests that these two behaviors are dissociable phenomena.

In conclusion, these results are consistent with the hypothesis that preference for novelty depends upon the activation of a DA system within the brain. Strong evidence now implicates the mesolimbic DA system in the reinforcing effect of various stimuli, including food, water, electrical brain stimulation, and drugs of abuse (4,29). Perhaps novel stimuli are reinforcing because they also activate the mesolimbic DA system.

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