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Postconditioning Effects of Magnesium on Cocaine Conditioned Place Preference in Mice[1]

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LAWLEY, S. I. AND K. M. KANTAK. *Postconditioning effects of magnesium on cocaine conditioned place preference in mice.* PHARMACOL BIOCHEM BEHAV **36**(3) 531–538, 1990.—Magnesium chloride ($MgCl_2$) has recently been shown to have stimulant-like properties. Because stimulants are known to induce conditioned place preference (CPP), the CPP procedure was used to test the hypothesis that cocaine and $MgCl_2$ share similar stimulus properties. This would be shown if cocaine-induced CPP could be enhanced in a postconditioning preference test by $MgCl_2$ and other stimulants. Mice were conditioned with 5.0 mg/kg cocaine to the nonpreferred end of a three-compartment straight shuttle box. All groups showed significant shifts in preference from the preconditioning test to the postconditioning test. There were no changes in place preference over test days in mice that were injected only with saline and therefore not conditioned. When animals were given acute injections of either saline, 5.0 mg/kg cocaine, 1.0 mg/kg amphetamine, 30 mg/kg $MgCl_2$, 10 mg/kg pentobarbital, or 0.25 mg/kg haloperidol following conditioning with cocaine, amphetamine and $MgCl_2$ elevated the conditioned cocaine effect, and pentobarbital and haloperidol decreased the conditioned cocaine effect compared to saline. In addition, there was a dose-dependent influence of $MgCl_2$, with 30 mg/kg producing the maximum effect on the conditioned cocaine effect.

Amphetamine Cocaine Conditioned place preference Haloperiodol Magnesium chloride Pentobarbital Reinforcement Stimulants

CONDITIONED place preference (CPP) is a procedure used to investigate potential reinforcing stimulus properties of drugs. A wide variety of methods and drugs have been utilized in this procedure since Kumar (24) and Rossi and Reid (35) investigated the consequences of rats given intraperitoneal and subcutaneous injections of morphine in distinctive environments. Different routes of administration, i.e., intraperitoneal (31, 37, 38), intraventricular (29,32), and intracranial to the lateral hypothalamus, nucleus accumbens, and the periaqueductal grey (42), can support CPP induced by various compounds. Substances which have induced CPP in rats include stimulants (see below), opiates (4, 22, 25, 31), clonidine (1), apomorphine (41), and food (39).

Both d-amphetamine (11, 25, 28, 41) and cocaine (25, 30, 32, 40) induce CPP. The shift in place preference is thought to be due to the interactions of these drugs with the neurotransmitter dopamine (DA) which is thought to mediate the reinforcing stimulus properties of stimulants (44). Although there are many similarities between amphetamine and cocaine CPP, there are some differences. Amphetamine CPP is blocked by DA antagonists haloperidol (28,41) and alpha flupenthixol (2,25), whereas cocaine CPP is not blocked by the administration of DA antagonists (25,40), unless the cocaine is administered intracerebrally (2,30). Other stimulants such as methylphenidate, apomorphine and methamphetamine (12, 28, 41) produce CPP.

Magnesium chloride ($MgCl_2$) has recently been shown to have stimulant-like behavioral properties (17). Low doses of the stimulants cocaine and d-amphetamine can increase aggression, whereas higher doses attenuate it (21, 26, 27). These results are paralleled by $MgCl_2$. Low doses of $MgCl_2$ (15 and 30 mg/kg) increase aggression and higher doses (125 and 250 mg/kg) reduce it (17). In addition, tolerance to some of the behavioral activating effects but not to the behavioral inhibiting effects of chronically administered cocaine and amphetamine is produced (13, 33, 36). At doses of $MgCl_2$ which enhance aggression tolerance develops, and at doses where it attenuates aggression tolerance does not develop (17). $MgCl_2$ also potentiates the dose-dependent effects of cocaine and attenuates the dose-dependent effects of haloperidol on mouse aggression (19). Further studies demonstrate that $MgCl_2$ enhances stereotyped sniffing behavior produced by apomorphine and locomotor behavior produced by l-amphetamine (20). Shifts to the left in the dose-response functions were observed which indicate that $MgCl_2$ enhances the potency of these drugs.

A CPP procedure was developed in mice for the present series of experiments using cocaine as the conditioning agent in order to

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determine if $MgCl_2$ shares stimulus properties with cocaine as the above studies would predict. One way to demonstrate this using a CPP procedure would be to test the mice with $MgCl_2$ and other drugs following conditioning with cocaine. Similar postconditioning effects of $MgCl_2$ to other stimulants, which differ from a saline control, would indicate that $MgCl_2$ shares stimulus properties with cocaine. Three experiments were conducted. In the first experiment the stability of side preference over time was determined in the absence of any drug cues. Also, the postconditioning effect of cocaine on cocaine-induced CPP was examined. The effect of the acute administration of $MgCl_2$ after the induction of cocaine place preference was examined in the second experiment and compared with drugs known to share stimulus properties or not to share stimulus properties with cocaine. In the third experiment, the dose response of $MgCl_2$ administration after the induction of cocaine place preference was determined.

METHOD

Animals

Adult male CFW strain mice (42 days old) from Charles River Breeding Labs, Portage, MI, were housed in $11.5 \times 7 \times 5$ inch clear plastic boxes in groups of 2 or 3 and were ear punched for individual identification. All animals were housed 2 weeks prior to conditioning and testing. A continuous 12-hour light/dark cycle beginning at 8 a.m. and constant temperature were maintained ($72 \pm 4°F$). The number of animals per drug group ranged from 4 to 9. All subjects had ad lib access to tap water and standard Purina Lab Chow.

Apparatus

Conditioning and testing of animals took place in painted wooden boxes measuring $38 \times 4 \times 5$ inches. Each box consisted of 3 dissimilar sections separated by 2 guillotine doors. The top of the boxes were covered with wire mesh. The central area measured 21 inches long and was painted grey. Most conditioning apparatus used in CPP experiments have only 2 compartments (6), however, there are several reasons for a central third compartment. First, by using the central grey area as a start box during testing, the animal was free to choose to go to the black or white side without being initially biased by starting in one or the other end (39). Second, a relatively large neutral area can be used to detect place aversion. If one end were aversive to the animal, it could go to the neutral grey area without spending more time in the opposite end. If the animal were to spend a greater percent of the time in one end chamber relative to the time spent in both ends, this would reflect an actual preference for that end and not merely an aversion of the other. Third, the central area would allow for the number of visits to a side to be unbiased. An animal could leave and reenter one side without entering the other side.

Three distinctive cues, a visual, an olfactory and a tactile cue, were associated with the end chambers. The two end chambers were alternately white and black, and measured 8.5 inches long. The black end during testing and conditioning was swabbed with a 2–3% apple cider vinegar solution to provide a weak odor cue, and the floor was covered with a wire-mesh screen to provide a tactile cue (29). The white end had no odor cue and the floor had a smooth painted surface. Ten boxes were used for testing and conditioning, and each animal was tested in the same box throughout the experiment. An Epson portable computer and Stoelting Behavioral Event Software were used to collect the time of occurrence of entry into each compartment.

Drugs

Cocaine hydrochloride (5.0 mg/kg, Mallinckrodt, St. Louis, MO), d-amphetamine sulfate (1.0 mg/kg, Sigma, St. Louis, MO), sodium pentobarbital (10.0 mg/kg, Lemmon Company, Sellersville, PA) and haloperidol (0.25 mg/kg, Sigma, St. Louis, MO), were injected intraperitoneally (IP) in a 0.9% saline vehicle. Magnesium chloride ($MgCl_2 \cdot 6H_2O$, Fisher Scientific) doses were expressed as the anhydrous salt. Three doses of $MgCl_2$ were administered, 15, 30, and 125 mg/kg, and were injected subcutaneously (SC) with distilled water as vehicle. All injection volumes were 1 ml/100 g body weight. Drugs were administered immediately prior to testing except for amphetamine and haloperidol which were injected 30 and 60 minutes prior to testing, respectively. These doses and times were chosen based upon our experience and previous literature (15, 23, 34, 40).

Procedure

Experiment 1: Stability of side preference and postconditioning effects of cocaine. The first part of this experiment tested the stability of preference with no cocaine conditioning. For 3 days each animal was allowed to explore all 3 sections of the conditioning chamber for 15 minutes per day. On the third, twelfth and thirteenth days, the amount of time spent in each of the end sections and the number of visits to each end were recorded. From these data, four measures were calculated: 1) percent of time on the cocaine conditioned side (day 3 nonpreferred side) as a primary index of place preference, 2) total number of visits to the sides as an index of activation or sedation, 3) number of visits to the preferred vs. nonpreferred sides as a secondary index of place preference, and 4) total time in ends as an index of place aversion. Place preference was determined by establishing that side in which the animal spent greater than 50% of the total time spent in the ends. Experimenter assignment to side was not incorporated in the design in order to observe the shifts in natural preferences with conditioning. In these experiments with mice, initial preferences were distributed equally between the black and white sides, indicating that this is a balanced procedure (56). In addition, this is a biased procedure because the side paired with the conditioning drug was not randomly assigned (6).

Saline injections started on the fourth day. For the next eight days animals were given daily 30-minute sessions of exposure where they were alternately confined to either the black side or the white side. On the first day of saline injections the animals were confined to the side demonstrated to be less preferred on day 3 followed by the more preferred side on the second day of saline injections and so on.

On the first day following the last saline injection (day 12), each animal was again tested to determine if side preference had switched. No drugs were administered on this day, though a sham IP injection was given. On day 13 the animals were given single IP injections of saline and tested for side preference. A second group of animals was tested to determine the stability of side preference when animals were conditioned with cocaine and then tested under both nondrug conditions and drug conditions with cocaine. The habituation or preconditioning phase of this group was identical to the previous group. The second phase differed, however, in that the subjects were conditioned in 30-minute sessions on alternate days with either saline or cocaine. During the conditioning sessions the animal was confined to either the black or white end. The nonpreferred side was paired with cocaine on days 4, 6, 8, and 10, while the preferred side was paired with saline on days 5, 7, 9, and 11. Thus, each animal had 4 pairings with cocaine and 4 pairings with saline for an 8-day total conditioning period.

During the day 12 test phase in this group, the animals were treated the same way as the saline group, and on day 13 the

TABLE 1

EFFECTS OF SALINE AND COCAINE DURING AND AFTER CONDITIONING

	(n)	Day 3	Day 12	Day 13
% Time on Less Preferred Side				
Saline	(4)	19.25 ± 10.64	20.25 ± 5.65	20.75 ± 2.84
Cocaine	(5)	26.60 ± 7.64	75.80 ± 12.77	84.20 ± 10.22
Total Time (sec)				
Saline		554.50 ± 55.85	723.75 ± 28.85*	731.25 ± 21.90*
Cocaine		520.80 ± 101.53	556.20 ± 96.02	653.40 ± 94.34
Visit Frequency Saline				
Preferred		18.00 ± 1.87	13.75 ± 1.25	13.25 ± 2.02
Nonpreferred		11.75 ± 3.97	11.25 ± 2.29	13.50 ± 2.72
Cocaine				
Preferred		18.20 ± 5.48	26.80 ± 6.07	46.20 ± 6.67*
Nonpreferred		13.60 ± 4.42	15.00 ± 4.65	17.00 ± 17.17

Values are the mean ± S.E.M. Measures were taken on days 3, 12 and 13. The saline group was injected with saline on all conditioning days and the cocaine group was administered 5.0 mg/kg cocaine on alternate conditioning days and prior to the preference test on day 13. Saline conditioning group was administered saline on day 13. *$p<0.05$ compared to total time on day 3 and number of visits on nonpreferred side.

animals were given IP injections of cocaine (5.0 mg/kg) and tested for side preference.

Experiment 2: Postconditioning drug effects on cocaine CPP. The preconditioning phase of this experiment was identical to the previous experiment. During second phase the subjects were conditioned in 30-minute sessions on alternate days with either saline or cocaine as in the second group of the previous experiment.

On the first day following conditioning (day 12), each animal was again tested to determine if side preference had switched due to conditioning. No drugs were administered on this day, though a sham injection was given as in Experiment 1. On day 13 the animals were given injections of either saline, amphetamine (1.0 mg/kg), pentobarbital (10.0 mg/kg), haloperidol (0.25 mg/kg), or $MgCl_2$ (30.0 mg/kg) prior to testing. These drugs were used to calibrate this postconditioning drug injection procedure based upon their effects in a self-administration procedure. Amphetamine substitutes for cocaine in a self-administration paradigm (3,16), and therefore shares stimulus properties. It has been shown, however, that pentobarbital, which is a self-administered substance, will not substitute for psychomotor stimulants (45), and therefore does not share stimulus properties with stimulants. Dopamine antagonists are known to block the self-administration of cocaine (8), and therefore do not share stimulus properties with cocaine. Thus the effects of $MgCl_2$ can be directly compared to the effects of drugs known to share stimulus properties and not to share stimulus properties with cocaine.

Experiment 3: Postconditioning $MgCl_2$ dose effects on cocaine CPP. In order to test the dose effects of $MgCl_2$when acutely administered after cocaine-induced CPP, the basic procedure of Experiment 2 was repeated, except that different doses of $MgCl_2$ were administered. The data from the saline and 30 mg/kg $MgCl_2$-treated animals from Experiment 2 were analyzed with data from two groups given 15 and 125 mg/kg $MgCl_2$ on day 13. The preconditioning and conditioning phases of this experiment were the same as the previous experiment.

Statistics

Data were analyzed by the appropriate one-way or two-way analysis of variance with repeated measures. For the visit data, the DAY main effect was used to measure any differences in the total number of visits, and the SIDE × DAY interaction was used to measure any differences in visits to the preferred vs. nonpreferred sides. Multiple comparisons among group means were made with the Duncan Multiple Range test.

RESULTS

Experiment 1: Stability of Side Preference and Postconditioning Effects of Cocaine

Analysis of percent of time spent on the conditioned side revealed that there were significant effects due to conditioning drug, $F(1,7)=33.11$, $p<0.001$, and due to test day, $F(2,14)=6.94$, $p<0.01$. The interaction of day with drug was significant, $F(2,14)=6.30$, $p<0.05$. Post hoc analysis of the saline-conditioned animals revealed that there were no shifts in preference on days 3, 12 and 13 (Table 1). This indicates that without drug conditioning cues, preference for a location remains stable. In the cocaine-conditioned group, there was a significant shift in preference from day 3 to days 12 and 13, $p<0.01$ (Table 1). There were no differences between day 12 and day 13. Furthermore, the saline group and cocaine group had similar preferences on day 3, but differences in preference on days 12 and 13, $p<0.01$. These data from days 12 and 13 show that cocaine had effects upon the preference of the animals in the drugged state as well as in the nondrugged state.

The two groups were analyzed for differences in total time spent in the ends of the test apparatus. An analysis of variance for the saline group showed a significant difference between days, $F(2,6)=7.97$, $p<0.05$. Post hoc analysis revealed an increase in the total time in the ends from day 3 to days 12 and 13, $p<0.05$ (Table 1). For the cocaine group, animals tended to spend more time in the ends over days but this effect was not significant.

For the total number of visits to the ends and for visits to the preferred vs. nonpreferred end, analyses of variance revealed that there were no significant main effects or interaction effects. However, in the cocaine group there was a greater number of visits in the drugged state and post hoc analysis showed that there were

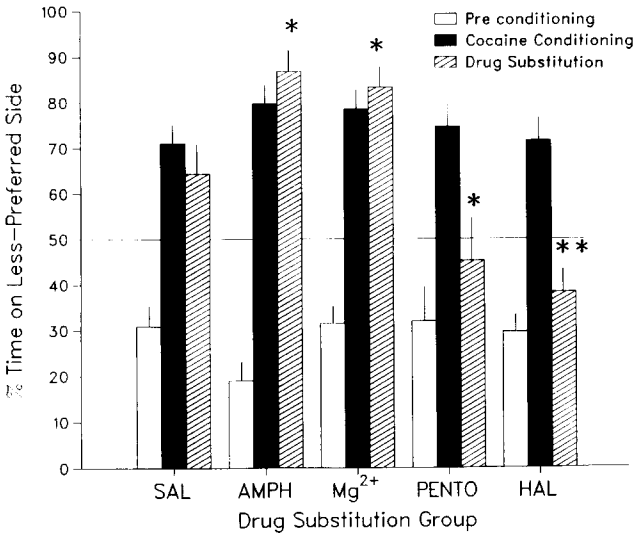

FIG. 1. Mean ± S.E.M. percentage of time spent on the day 3 less-preferred side on day 3 (preconditioning), day 12 (postcocaine conditioning) and day 13 (drug treatment). The drug treatment groups on day 13 were saline (sal), 1 mg/kg amphetamine (amph), 30 mg/kg magnesium chloride (Mg^{2+}), 10 mg/kg pentobarbital (pento) and 0.25 mg/kg haloperidol (hal). Significantly different from the saline treatment, $*p<0.05$, $**p<0.01$.

significantly more visits on day 13 in the cocaine group when compared to those animals that received saline injections on this day and compared to its day 3 preconditioning baseline, $p<0.05$. In addition, the number of visits to the preferred end on day 13 was significantly greater than the visits to the nonpreferred end following cocaine treatment (Table 1).

Experiment 2: Postconditioning Drug Effects on Cocaine CPP

Analysis of the percent of time showed significant differences between groups as a main effect, $F(4,27)=5.29$, $p<0.01$. There was also a significant effect of test day on percent time, $F(2,54)=90.55$, $p<0.0001$. Analysis of the interaction effect revealed significant differences between drug treatment and specific test days, $F(8,54)=4.89$, $p<0.001$ (Fig. 1). Post hoc analysis showed that there were no differences among groups during day 3 preconditioning testing nor during day 12 post cocaine-conditioning testing, and that all groups showed significant differences between percentage of time on day 3 and day 12, $p<0.01$. This indicates a significant effect of cocaine conditioning in reversing placed preference in all groups. On day 13 the different drugs had different effects upon the conditioned cocaine effect. Following saline, the percent of time on the cocaine-conditioned side was maintained at a slightly lower amount than on day 12. For both amphetamine and $MgCl_2$, the percent of time on the cocaine-conditioned side was maintained at a slightly higher amount than on day 12. Consequently, on day 13, the percentage of time spent on the conditioned side was significantly greater following amphetamine and $MgCl_2$, compared to saline ($p<0.05$). On day 13, both pentobarbital ($p<0.05$) and haloperidol ($p<0.01$) reduced the percent of time on the cocaine-conditioned side compared to

TABLE 2

POSTCONDITIONING DRUG EFFECTS ON COCAINE CPP

(n)	Sal (9)	Amph (4)	$MgCl_2$ (9)	Pento (5)	Hal (5)
Day 3 Preconditioning					
Total Time (sec)	510.9 ± 34.8	544.8 ± 63.7	526.9 ± 33.1	488.6 ± 102.1	382.6 ± 57.5
Visits Preferred	20.1 ± 1.6	16.5 ± 3.7	18.0 ± 1.4	17.2 ± 2.6	16.2 ± 3.4
Nonpreferred	16.8 ± 2.0	12.0 ± 2.3	18.2 ± 1.8	14.2 ± 3.8	11.2 ± 2.1
Day 12 Cocaine conditioning					
Total Time (sec)	642.3 ± 33.2†	612.0 ± 57.3	606.6 ± 59.2	626.8 ± 73.0	598.6 ± 38.1†
Visits Preferred	21.8 ± 2.0†	25.0 ± 0.7*	17.6 ± 2.2*	19.8 ± 2.2	23.4 ± 4.3
Nonpreferred	14.8 ± 1.4	11.3 ± 2.7	12.7 ± 2.3	13.2 ± 1.5	21.2 ± 3.1
Day 13 Drug treatment					
Total Time (sec)	709.2 ± 31.6†	690.5 ± 82.2	671.3 ± 59.9	658.0 ± 43.9	615.2 ± 61.4†
Visits Preferred	21.4 ± 3.2†	34.5 ± 6.7*	12.2 ± 1.7*	31.4 ± 4.0	10.4 ± 1.3
Nonpreferred	15.0 ± 2.2	18.8 ± 3.9	7.2 ± 1.5	29.6 ± 7.2	10.2 ± 2.2

Values are the mean ± S.E.M. Doses of drugs used on day 13 (drug treatment) are as follows: saline (Sal), 1 mg/kg amphetamine (Amph), 30 mg/kg magnesium chloride ($MgCl_2$), 10 mg/kg pentobarbital (Pento) and 0.25 mg/kg haloperidol (Hal). Significant differences when compared with nonpreferred visits and total time on day 3 are $*p<0.05$ and $†p<0.01$.

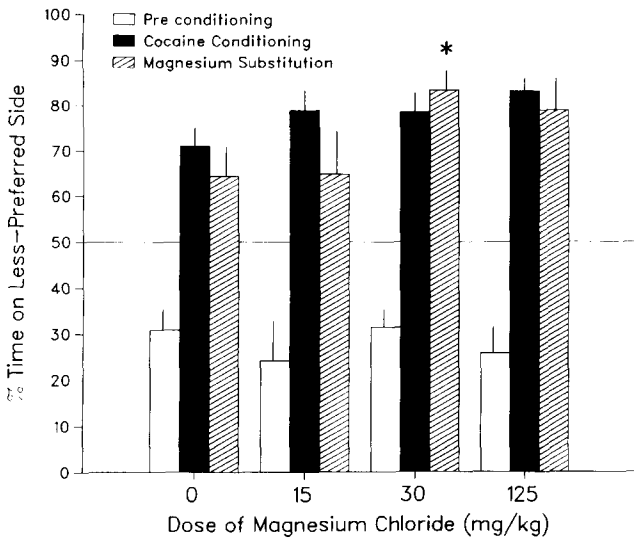

FIG. 2. Mean ± S.E.M. percentage of time spent on the day 3 less-preferred side on day 3 (preconditioning), day 12 (postcocaine conditioning) and day 13 (magnesium chloride treatment). Doses of magnesium chloride on day 13 were 0 (saline), 15, 30, and 125 mg/kg. Significantly different from the saline treatment, $*p<0.05$.

saline. The percent of time on the cocaine-conditioned side following pentobarbital and haloperidol on day 13 was not significantly different from the day 3 preconditioning baseline.

Each of the five drug treatment groups were analyzed separately for differences in total time spent on either end of the test apparatus as a function of test day. As in Experiment 1, the total time spent in the end compartments generally increased from the preconditioning to the postconditioning phases, though only two of the drug groups showed significant differences (Table 2). These were the saline, $F(2,16)=9.18$, $p<0.01$, and haloperidol, $F(2,8)=10.71$, $p<0.01$, treatments. Post hoc analysis for both of these groups revealed that there were significant increases from day 3 to days 12 and 13 ($p<0.01$) for the haloperidol treatment group, and significant differences from day 3 to day 12 ($p<0.05$) and day 13 ($p<0.01$) for the saline treatment group. There were no differences between day 12 and day 13 in either group.

Analysis of the main effects for day of testing showed that all of the treatment groups except the saline treatment group had differential number of visits on different days: pentobarbital, $F(2,8)=12.75$, $p<0.01$; amphetamine, $F(2,6)=7.99$, $p<0.05$; haloperidol, $F(2,8)=10.23$, $p<0.01$; and $MgCl_2$, $F(2,16)=12.82$, $p<0.001$. For both the pentobarbital and the amphetamine treatment groups, post hoc analysis showed significant differences between the total number of visits to the two sides on day 3 and day 13, $p<0.01$, and on days 12 and 13, $p<0.05$ for amphetamine and $p<0.01$ for pentobarbital. Both the haloperidol and $MgCl_2$ treatment groups showed significant decreases on day 13 with respect to day 12, $p<0.01$, but no decreases below the day 3 baseline occurred. Although there was no significant interaction of test day with side preference for the visit data, there was an overall tendency for a greater number of visits to the preferred side following cocaine conditioning on day 12. Post hoc analysis on day 13 revealed that the preferred side was visited significantly more times in the saline ($p<0.01$), amphetamine ($p<0.05$), and $MgCl_2$ treatment groups $p<0.05$) (Table 2). Following pentobarbital and haloperidol, there were equal number of visits to both preferred and nonpreferred sides.

Experiment 3: Postconditioning $MgCl_2$ Dose Effects on Cocaine CPP

There were no significant differences in percent of time spent on the cocaine-conditioned side due to $MgCl_2$ dose, but there was a significant effect due to test day, $F(2,46)=98.83$, $p<0.0001$. Cocaine produced significant place conditioning in all groups, $p<0.01$, and this conditioned effect was maintained by all doses of $MgCl_2$ on day 13 (Fig. 2). Post hoc analysis of day 13 showed that the 30 mg/kg treatment significantly maintained the cocaine-conditioned place preference above saline, $p<0.05$. The 15 mg/kg and 125 mg/kg treatments were not different from saline.

An analysis of the total time spent in the ends showed that there were no significant differences over test days for the $MgCl_2$ treatment groups, though the saline treatment group did show a significant increase over test days on this measure, $F(2,16)=9.18$, $p<0.01$ (Table 3).

The two higher doses of $MgCl_2$, 30 and 125 mg/kg, showed significant differences in number of visits over test days, $F(2,16)=12.82$, $p<0.001$, and $F(2,8)=4.54$, $p<0.05$, respectively. In the 30 mg/kg group, the total number of visits to the two ends decreased on day 13 compared to day 12, $p<0.01$, but was no different than the day 3 baseline. There was a significant increase in the total number of visits to the two ends from day 3 to day 12 in the 125 mg/kg group, $p<0.05$.

As in Experiment 2, animals generally visited the preferred side more often than the nonpreferred side (Table 3). These differences were significant in the saline treatment group, $F(1,8)=33.80$, $p<0.001$, and the 30 mg/kg $MgCl_2$ treatment group, $F(1,8)=8.33$, $p<0.05$. Post hoc analysis of the interaction revealed that there were significant differences between preferred and nonpreferred sides on days 12 and 13 for the saline group ($p<0.01$), and on days 12 and 13 for the 30 mg/kg group ($p<0.05$).

DISCUSSION

These data indicate that mice do show robust cocaine conditioning effects in the CPP paradigm. It has been argued that mice make good subjects in the study of neural mechanisms of reward because they are compact, inexpensive, and vary in number of dopamine and opiate receptors (7). These data appear to support their use in this paradigm because of the strength of the cocaine conditioning and the robustness of acute effects of pharmacological agents upon cocaine-induced CPP when they are administered after conditioning.

The results from Experiment 1 demonstrate that unconditioned preferences remain stable over time. The stability of the side preference of the subjects over time is crucial to the interpretation of the preference changes with cocaine administration. Because of the stability of side preference in the unconditioned state, any significant changes in side preference following drug administration could be attributed to conditioning. Also, mice that were not conditioned spent more time in the end chambers during the latter test days without any changes in the number of visits to each side on all test days. This effect on total time was also seen in many conditioning groups. Therefore, the increase in total time spent in the two end compartments is not what contributed to the drug effects, but could reflect an increased familiarity of the apparatus over the testing periods.

Cocaine CPP in mice was easily obtained with a dose of 5.0 mg/kg in the second part of Experiment 1, as well as in Experiments 2 and 3, Spyraki (40) using rats, was not able to demonstrate any significant changes in side-preference with 0.625 mg/kg cocaine, though was able to with higher doses, ranging

TABLE 3

DOSE RESPONSE OF MAGNESIUM TREATMENT ON COCAINE CPP

(n)	Saline (9)	15 (4)	30 (9)	125 (5)
Day 3 Preconditioning				
Total Time sec	510.9 ± 34.8	553.0 ± 91.4	526.9 ± 33.1	465.4 ± 75.5
Visits Preferred	20.1 ± 1.6	44.3 ± 31.5	18.0 ± 1.4	20.4 ± 3.4
Nonpreferred	16.8 ± 2.0	25.5 ± 15.7	18.2 ± 1.8	15.4 ± 4.4
Day 12 Cocaine conditioning				
Total Time (sec)	642.3 ± 33.2†	584.0 ± 92.9	606.6 ± 59.2	562.0 ± 29.0
Visits Preferred	21.8 ± 2.0†	23.8 ± 5.1	17.6 ± 2.2*	31.0 ± 5.6
Nonpreferred	14.8 ± 1.4	17.3 ± 5.3	12.7 ± 2.3	23.4 ± 4.0
Day 13 Drug treatment				
Total Time (sec)	709.2 ± 31.6†	512.0 ± 38.2	671.3 ± 59.9	579.8 ± 66.7
Visits Preferred	21.4 ± 3.2†	21.8 ± 3.6	12.2 ± 1.7*	24.8 ± 4.0
Nonpreferred	15.0 ± 2.2	26.8 ± 6.9	7.2 ± 1.5	21.8 ± 5.7

Values are the mean ± S.E.M. Doses of $MgCl_2$ used on day 13 (drug treatment) are saline, 15, 30, and 125 mg/kg. Significant differences when compared with nonpreferred visits and total time on day 3 are *$p<0.05$ and †$p<0.01$.

from 1.25 to 20 mg/kg. The greatest effect was produced at 5.0 mg/kg. Since the primary objective of this study was to investigate the postconditioning effects of drugs on cocaine CPP, no dose-responsive curve to cocaine conditioning was measured in mice. The present data show that mice are robustly showing cocaine CPP at this fixed dose. The dose response in mice may be similar to that of rats, though this remains to be determined.

Drugs may have two independent properties when administered during place conditioning: 1) a reinforcing stimulus property, and 2) a discriminative stimulus property (9,10). These discriminative properties of the drug are a part of a stimulus compound that is partially absent in the nondrugged test situation, and then is wholly present during the drugged situation. If it is the internal cues that animals respond to and cause the increase in conditioned place preference during testing in the drugged state, it is arguable that different drugs of a specific class could enhance or maintain CPP induced by drugs within the same class. The slight lowering of the cocaine-conditioned effect on day 13 by saline administration indicates a resistance of the conditioned effect to extinction. Administration of $MgCl_2$ immediately prior to testing enhanced the conditioned preference above the level seen with saline administration, indicating a delay in the extinction process. It was also shown that amphetamine and cocaine strongly maintained the conditioned place preference of cocaine above the level measured for saline treatment. Haloperidol and pentobarbital, which do not share similar stimulus properties with cocaine, did not enhance the conditioned place preference, but actually blocked it, and thus possibly expedited the extinction process. These data suggest that $MgCl_2$ shares stimulus properties with cocaine as other drugs having stimulant properties do.

From these data it appears that the drug state of the animal affects place preference in the test situation. There have been only a few studies comparing CPP testing in a drugged vs. nondrugged state. For example, Mucha and Iverson (31) showed no difference between the morphine-induced CPP of animals tested in drugged or nondrugged state. In an experiment by Bozarth which used a procedure very similar to the present procedure (5), rats were conditioned by confining them to their nonpreferred side for three daily 30-minute sessions after being injected with either 0.3 mg/kg heroin or saline. On the first day after the conditioning phase, all rats were tested for a change in the amount of time spent in the conditioned side. Rats conditioned with saline showed no change in the amount of time spent on that side, though animals conditioned with heroin showed a significant increase in time spent on that side. On the following day both groups of animals were given injections of saline or heroin and then tested for preference. Those animals given acute heroin injections spent significantly more time on the conditioned side than the previous day, and those given saline showed no difference. Thus, it was found that testing animals under drug conditions following CPP conditioning in a within-groups design produced an increase in the magnitude of the conditioned place preference. This postconditioning effect was interpreted to be due to the additional presence of internal discriminative stimulus cues from the drug which were also present during conditioning, but not present on the first postconditioning test day when no drugs were given. The reinstatement of stimulus cues associated with drug reinforcement has also been used to explain the recovery of extinguished responding in an operant paradigm using cocaine (9) and heroin (10).

The present data further demonstrate that haloperidol can block cocaine CPP when the haloperidol is administered after conditioning to peripherally injected cocaine. This is in contrast to studies showing that cocaine CPP is not easily blocked by haloperidol when the haloperidol is administered at the time of conditioning (25,40). In the present study, there were no reductions in the number of visits from the day 3 baseline which indicates that the

drug treatments did not produce any sedative effects. This is important, particularly with respect to haloperidol which can reduce locomotion (14) and aggression (19) with the dose tested. It is interesting that with the treatments (including saline) that did enhance the conditioned effect of cocaine, there were more visits to the preferred side than nonpreferred side. The ability of $MgCl_2$ to enhance cocaine CPP in a manner similar to cocaine and amphetamine on both the preference and visit measures indicates again that $MgCl_2$ may share stimulus properties with cocaine.

This would be predicted from previous data from our laboratory showing stimulant-like properties of $MgCl_2$. Deficiencies of Mg^{2+} and high excess doses of $MgCl_2$ reduce agression, whereas low excess doses of $MgCl_2$ enhance it (17,18). Since tolerance develops to this agression-enhancing effect of $MgCl_2$ (17), and catecholamine function is enhanced by excesses (20) and reduced by deficiencies (18), a stimulant action of Mg^{2+} is suggested. Further support of this is indicated by the ability of 30 mg/kg $MgCl_2$ to enhance the behavioral facilitating action of cocaine on mouse aggression (19). The ability of Mg^{2+} to enhance cocaine CPP is related to dose of Mg^{2+} where an optimum effect is observed with 30 mg/kg. Doses of 15 mg/kg and 125 mg/kg did not enhance the cocaine CPP above saline levels. Since 125 mg/kg $MgCl_2$ has been shown to inhibit aggressive behavior (17), and to potentiate the inhibitory effects of cocaine on mouse aggression (18), these data may reflect a loss of stimulant- or reward-like effects at this dose. The 15 mg/kg dose of $MgCl_2$ may not be potent enough to sustain the conditioned cocaine effect, although it has potent stimulant-like properties in other paradigms (17).

The results of this study support the suggestion that $MgCl_2$ may share stimulus properties with cocaine. Whether it is reinforcing and/or discriminative stimulus properties that are shared remains to be specifically determined. The curious behavioral effects of $MgCl_2$ seen in these studies suggest that a careful analysis of the interaction of $MgCl_2$ and cocaine on drug reinforcement and drug discrimination is warranted in follow-up studies.

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