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Magnesium-Induced Conditioned Place Preference in Mice[1]

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LAWLEY, S. I. AND K. M. KANTAK. *Magnesium-induced conditioned place preference in mice.* PHARMACOL BIOCHEM BEHAV **36**(3) 539–545, 1990.—A conditioned place preference procedure was used in mice to test the hypothesis that magnesium possesses reinforcing properties. Mice were conditioned to the nonpreferred end of a three-compartment straight shuttle box with $MgCl_2$ injections alternating with saline injections on the preferred end. Dose of $MgCl_2$ was varied (0, 15, 30, 125 mg/kg) as well as number of conditioning trials (8 or 16). On the day after the first postconditioning test, animals were given acute injections of 5 mg/kg cocaine, or other test drug, to determine if the conditioned effect on behavior would be potentiated, maintained or blocked by these test drugs. Results demonstrated that 15 mg/kg $MgCl_2$ induced the greatest amount of conditioning and that increasing the number of $MgCl_2$/place pairings did not enhance the amount of conditioning, but rather, it decreased it. Amphetamine potentiated $MgCl_2$-induced place preference; cocaine and pentobarbital maintained it; and haloperidol blocked it. These data indicate that $MgCl_2$ has some primary reinforcing properties in mice and that $MgCl_2$ shares stimulus properties with other stimulants and reinforcing substances.

Amphetamine Cocaine Conditioned place preference Haloperidol Magnesium chloride Pentobarbital Reinforcement Stimulants

VARIOUS behavioral experiments have demonstrated stimulant-like properties of magnesium and interactive effects of magnesium with stimulants. This has been shown for mouse aggression (8, 10, 11), and drug-induced motor behaviors (12). In a previous report (13), it was demonstrated via conditioned place preference (CPP) in mice that postconditioning injections of magnesium chloride ($MgCl_2$) potentiated cocaine-induced CPP in a manner similar to amphetamine. Cocaine-induced CPP was blocked by postconditioning nonsedating low doses of haloperidol and pentobarbital which indicated that cocaine and $MgCl_2$, and cocaine and amphetamine, share stimulus properties The following study was conducted to determine if mice could be conditioned with $MgCl_2$ to change their preference for a previously nonpreferred location. Such changes might be indicative of primary reinforcing properties of $MgCl_2$ in mice.

Wise and Bozarth (23) have advanced a theory postulating that a common denominator in reinforcing drugs is their ability to cause psychomotor activation. They propose that stimulants, opiates and other drugs have effects in the dopaminergic system of the brain that lead to the reinforcing behavioral effects. Magnesium is abundant in the central nervous system and is a cofactor for reactions which take place in the dopaminergic system (5, 7, 20, 21). Thus, there is behavioral, pharmacological and biochemical evidence to suggest primary reinforcing properties of $MgCl_2$.

METHOD

Animals

The materials and methods used in this study were similar to those reported by Lawley and Kantak (13). Adult male CFW strain mice (42 days old) from Charles River Breeding labs, Portage, MI, were housed in groups of 2 or 3 and 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 ($72 \pm 4°F$). All subjects had free access to tap water and standard Purina Lab Chow.

Apparatus

Conditioning and testing of animals took place in conditioning chambers described elsewhere (13). Three cues (visual, olfactory, and tactile) were associated with the end chambers. The two end chambers were alternately black and white, the black side being swabbed with a 2–3% apple cider vinegar solution. Also the black floor was covered with a wire-mesh screen to provide a tactile cue. The white end 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.

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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 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 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.

Procedure

Experiment 1: Eight-day conditioning period. The purpose of the following experiments was to determine if $MgCl_2$ had reinforcing properties, and to determine those conditions under which those properties were maximally expressed. In the first experiment, animals were given three days to explore all three sections of the apparatus, and on the third day they were tested for side preference. On all three days the subjects were allowed 15 minutes exposure. On the first test day (day 3) the number of visits to each side was measured as well as side preference and total time in the ends. Side preference was established by determining that side on which the animal spent greater than 50% of the total time spent in either end.

The conditioning period of this experiment lasted 8 consecutive days. On the first day after the first test day (day 3), subjects were given one of four doses of $MgCl_2$ injections and then confined to the side which was determined to be nonpreferred for 30 minutes. The four doses were 0, 15, 30, 125 mg/kg. On the next day animals were given saline injections and confined to the preferred side for 30 minutes. Animals were thus given four nonpreferred side-drug (CS-UCS) pairings alternating with four preferred side-saline pairings.

Following this conditioning, animals were tested for shift in side preference. Animals were given a sham injection and then given free access to all sections of the apparatus for 15 minutes. This test for conditioning occurred on day 12. As on day 3, the number of visits in addition to side preference and the total time in the ends were measured. On the next day (day 13) the test procedure was repeated except that animals were given injections of cocaine (5.0 mg/kg) prior to the test to determine how cocaine influenced $MgCl_2$ conditioned behavior. Visits, side preference and total time spent in the ends were measured as on previous test days.

Experiment 2: Sixteen-day conditioning period. The habituation or preconditioning phase of this experiment was identical to the previous experiment. The second phase differed, however, in that the subjects were conditioned in 30-minute sessions on alternate days with either saline or $MgCl_2$ for a 16-day conditioning period. Animals were given one of four doses of $MgCl_2$ as in Experiment 1 (0, 15, 30, or 125 mg/kg) on drug conditioning days. They were given the drug on days 4, 6, 8, 10, 12, 14, 16, and 18, and saline on alternate days. Thus, the nonpreferred side was paired with the drug twice as many times as in Experiment 1 above.

Animals were tested on the first day following conditioning, day 20, for 15 min. Subjects were given a sham injection immediately prior to the test, and side preference, total time spent in the ends and number of visits were measured. On the next day (day 21), injections of 5.0 mg/kg cocaine were given immediately prior to the test period, and behavior was measured in the same way as before.

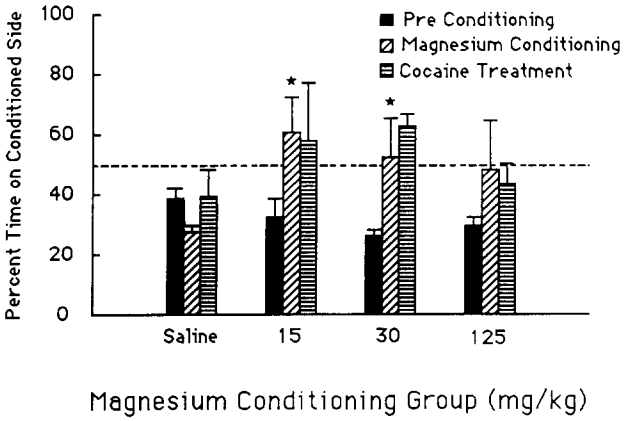

FIG. 1. Mean ± S.E.M. percent time spent on the conditioned side on day 3 (Preconditioning), day 12 ($MgCl_2$ Conditioning) and day 13 (Cocaine Treatment). Animals were conditioned with $MgCl_2$ (0, 15, 30, 125 mg/kg) using four place pairings and treated with cocaine (5.0 mg/kg) on day 13. $\star p<0.05$ compared to day 3 preconditioning percent time.

Experiment 3: Postconditioning drug effects on $MgCl_2$ CPP. The purpose of this experiment was to determine if various drugs had an influence which was similar to or different from that produced by $MgCl_2$ conditioning. The preconditioning and conditioning phases of this experiment were identical to those used in Experiment 1. During conditioning, animals were alternately administered 15 mg/kg $MgCl_2$, and saline. The $MgCl_2$ was paired with the nonpreferred side. Animals were tested on days 3, and 12 in the same way. On day 13, only animals which showed shifts in preference were randomly assigned to one of five groups and given injections prior to testing of either cocaine (5.0 mg/kg), d-amphetamine (1.0 mg/kg), $MgCl_2$ (15 mg/kg), pentobarbital (10.0 mg/kg), haloperidol (0.25 mg/kg), or saline.

Statistics

Data were analyzed by either one-way or two-way analysis of variance with repeated measures. Multiple comparisons among group means were made with the Duncan Multiple Range test.

RESULTS

Experiment 1: Eight-Day Conditioning Period

An analysis of variance of the percentage data showed a significant effect due to test day, $F(2,28)=4.78$, $p<0.05$. Overall, days 12 and 13 differed significantly from day 3 ($p<0.05$). Although there was no significant interaction, comparisons between preconditioning day 3 and conditioning day 12 revealed a significant change in preference following 15 and 30 mg/kg $MgCl_2$, $p<0.05$ (Fig. 1). The dose of 15 mg/kg was also significantly different from 0 mg/kg, $p<0.05$, on day 12. There was no conditioning effect of 0 mg/kg (saline), or 125 mg/kg. A postconditioning injection of cocaine maintained the conditioned effect of 15 and 30 mg/kg $MgCl_2$. In groups that did not show any conditioning, 0 and 125 mg/kg, a postconditioning injection of cocaine did not alter preference.

Following conditioning with 30 mg/kg $MgCl_2$, animals spent an increasing amount of time in the ends as opposed to the central

TABLE 1

EIGHT-DAY CONDITIONING WITH MAGNESIUM

(n)	Saline (5)	15 (4)	30 (4)	125 (5)
Day 3 Preconditioning				
Total Time (sec)	489.2 ± 52.9	458.0 ± 86.9	445.0 ± 56.6	547.6 ± 44.1
Visits				
Preferred	21.6 ± 2.7	12.3 ± 1.8	26.5 ± 2.9	22.2 ± 4.2
Nonpreferred	18.0 ± 2.4	15.0 ± 2.2	19.5 ± 3.9	20.0 ± 4.1
Day 12 Magnesium Conditioning				
Total Time (sec)	424.4 ± 23.3	628.3 ± 37.6	558.8 ± 73.1	586.6 ± 47.1
Visits				
Preferred	27.6 ± 3.5	15.8 ± 1.9	19.0 ± 3.8	30.8 ± 4.9†
Nonpreferred	20.8 ± 3.5	15.8 ± 3.2	17.0 ± 5.4	19.4 ± 6.0
Day 13 Cocaine Treatment				
Total Time (sec)	487.0 ± 31.8	720.0 ± 30.5	728.0 ± 37.5*	600.4 ± 85.6
Visits				
Preferred	54.2 ± 8.8†	28.3 ± 4.8†	38.8 ± 12.1*	38.8 ± 14.0*
Nonpreferred	38.2 ± 6.0	17.3 ± 5.0	20.3 ± 5.0	29.0 ± 11.8

Values are the mean ± S.E.M. Doses of magnesium used during conditioning were saline, 15, 30, and 125 mg/kg. Significant differences when compared with nonpreferred visits and total time on day 3 are indicated by *$p<0.05$ and †$p<0.01$.

area of the test apparatus over the three test days (Table 1). A significant difference was found on day 13 compared to day 3, $p<0.05$. In all other $MgCl_2$ conditioning groups, there was a tendency for the total time spent in the ends to increase over test days, but these differences were not significant.

Analysis of the total number of visits to the ends revealed a change in the number of visits over test days, $F(2,6)=13.73$, $p<0.01$. A significant increase from day 3 to day 13 was found in the 0 mg/kg group, $p<0.01$ (Table 1). Furthermore, in the 0, $F(1,4)=16.53$, $p<0.05$, 30, $F(1,3)=14.66$, $p<0.05$, and 125, $F(1,4)=21.42$, $p<0.01$, mg/kg groups, there was a significantly greater number of visits to the preferred end on all three test days. In the 15 mg/kg group, a significantly greater number of visits to the preferred end was found on day 13 only, $F(2,6)=11.71$, $p<0.01$.

Experiment 2: Sixteen-Day Conditioning Period

There was a significant effect due to test day, $F(2,30)=4.33$, $p<0.05$, where overall days 20, $p<0.05$, and 21, $p<0.01$, differed significantly from day 3 on the percentage of time spent on the conditioned side. There was a significant interaction between conditioning groups and test day, $F(6,30)=2.42$, $p<0.05$ (Fig. 2). Following conditioning with saline, 30 and 125 mg/kg $MgCl_2$, there were no differences between days 3, 20 and 21. In the 15 mg/kg injected group, although there was no significant change in preference on day 20 which did reach 50%, there was a significant change in preference on day 21 following the cocaine injection when compared to day 3, $p<0.01$, and day 20, $p<0.05$.

Animals in the saline-conditioned group spent increasing amount

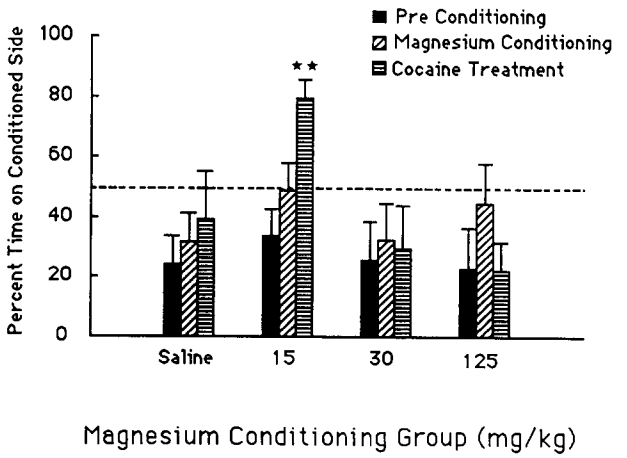

FIG. 2. Mean ± S.E.M. percent time spent on the conditioned side on day 3 (Preconditioning), day 20 ($MgCl_2$ Conditioning) and day 21 (Cocaine Treatment). Animals were conditioned with $MgCl_2$ (0, 15, 30, 125 mg/kg) using eight place pairings and treated with cocaine (5.0 mg/kg) on day 21. **$p<0.01$ compared to day 3 and day 12.

TABLE 2

SIXTEEN-DAY CONDITIONING WITH MAGNESIUM

(n)	Saline (5)	15 (5)	30 (5)	125 (4)
Day 3 Preconditioning				
Total Time (sec)	462.2 ± 60.8	492.2 ± 51.3	634.4 ± 25.2	654.5 ± 73.8
Visits				
Preferred	14.4 ± 2.5	24.0 ± 3.7	15.8 ± 2.4	14.5 ± 3.3
Nonpreferred	12.8 ± 1.8	21.0 ± 4.9	13.2 ± 2.3	12.8 ± 3.9
Day 12 Magnesium Conditioning				
Total Time (sec)	471.4 ± 55.5	541.6 ± 22.4	543.6 ± 70.3	572.8 ± 26.9
Visits				
Preferred	23.4 ± 2.2	26.2 ± 1.9	24.2 ± 3.8†	20.3 ± 2.8
Nonpreferred	20.6 ± 3.1	18.0 ± 2.5	13.2 ± 2.5	22.0 ± 6.5
Day 13 Cocaine Treatment				
Total Time (sec)	686.8 ± 47.0†	554.8 ± 51.9	662.2 ± 44.6	605.5 ± 31.7
Visits				
Preferred	34.0 ± 7.9	52.0 ± 8.3*	39.6 ± 1.7†	35.0 ± 2.9
Nonpreferred	23.6 ± 5.5	27.4 ± 8.7	19.0 ± 3.7	24.3 ± 2.3

Values are the mean ± S.E.M. Doses of magnesium used during conditioning were saline, 15, 30, and 125 mg/kg. Significant differences when compared with nonpreferred visits and total time on day 3 are indicated by *$p<0.05$ and †$p<0.01$.

of time in the ends of the testing apparatus, $F(2,8)=9.53$, $p<0.01$. Post hoc analysis showed that time spent in the ends on day 13 was significantly greater than that spent there on either day 3 or day 12, $p<0.01$ (Table 2). In the 15, 30 and 125 mg/kg conditioned groups there was no significant differences among the test days.

The total number of visits to the ends was significantly different for the 15 mg/kg group, $F(2,8)=5.8$, $p<0.05$, the 30 mg/kg group, $F(2,8)=12.29$, $p<0.01$, and the 125 mg/kg group, $F(2,6)=7.85$, $p<0.05$. Post hoc analysis for each group revealed that on day 13 there were more total visits to the ends than on either day 3 or day 13, $p<0.05$ (Table 2). Furthermore, following conditioning with 15 and 30 mg/kg, there were significant differences in the number of visits to the preferred and nonpreferred ends, $F(1,4)=8.44$, $p<0.05$, and $F(1,4)=16.65$, $p<0.05$, respectively; and following conditioning with 30 mg/kg there was a signficiant interaction between side preference and test day with respect to the number of visits, $F(2,8)=11.67$, $p<0.01$. There were more visits to the preferred end on day 12 and day 13, $p<0.01$, but not on day 3.

Experiment 3: Postconditioning Drug Effects on $MgCl_2$ CPP

For the six different drug treatments in this experiment, a two-way analysis of variance of the percent time showed that there were significant differences between groups, $F(5,20)=5.85$, $p<0.01$, test days, $F(2,40)=56.34$, $p<0.01$, and interaction of groups with test day, $F(10,40)=3.88$, $p<0.01$. Post hoc analysis showed that a change in preference was observed (Fig. 3) between days 3 and 12 in all groups, $p<0.01$, and that there were no differences among groups on day 3 (preconditioning test), and day

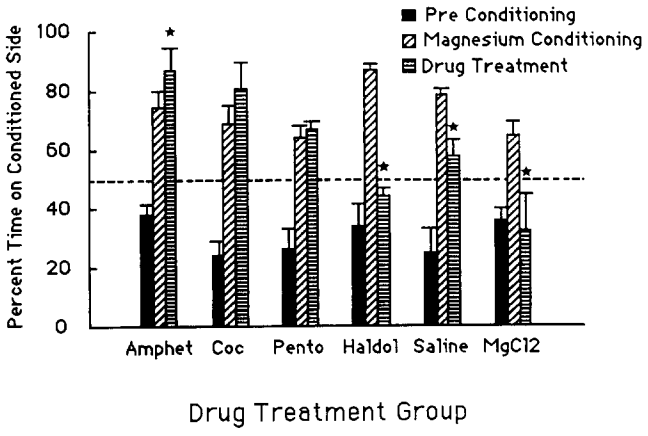

FIG. 3. Mean ± S.E.M. percent time spent on the conditioned side on day 3 (Preconditioning), day 12 ($MgCl_2$ Conditioning) and day 13 (Drug Treatment). The drug treatments were 1 mg/kg amphetamine (Amphet), 5.0 mg/kg cocaine (Coc), 10.0 mg/kg pentobarbital (Pento), 0.25 mg/kg haloperidol (Haldol), saline, 15.0 mg/kg magnesium chloride ($MgCl_2$). *$p<0.05$ compared to day 12 or to the day 13 saline treatment.

TABLE 3

POSTCONDITIONING DRUG EFFECTS ON MAGNESIUM

(n)	Amp (6)	Coc (6)	Pento (4)	Hal (5)	Sal (4)	Mg^{2+} (4)
Day 3 Preconditioning						
Time (sec)	465.5 ± 31.8	627.7 ± 92.7	502.8 ± 55.6	474.6 ± 73.8	585.2 ± 96.0	539.2 ± 55.6
Visits						
Pref.	20.3 ± 3.6	14.2 ± 2.0	18.8 ± 3.2	20.2 ± 2.9	19.2 ± 3.8	21.5 ± 2.2
Nonpref.	17.2 ± 3.5	10.2 ± 2.7	15.2 ± 3.3	17.8 ± 3.3	19.2 ± 2.8	19.0 ± 2.0
Day 12 Magnesium Conditioning						
Time (sec)	530.5 ± 34.4	550.0 ± 77.6	419.2 ± 80.3	470.8 ± 35.2	532.5 ± 108.1	436.0 ± 33.5
Visits						
Pref.	20.7 ± 4.2	15.7 ± 1.5	25.2 ± 4.5	28.8 ± 2.4†	26.0 ± 5.9†	23.8 ± 3.4
Nonpref.	15.2 ± 3.0	11.3 ± 2.6	20.2 ± 2.5	9.0 ± 2.5	13.5 ± 5.0	21.5 ± 2.2
Day 13 Drug Treatment						
Time (sec)	668.0 ± 98.3	619.0 ± 114	508.8 ± 79.2	541.6 ± 51.4	609.0 ± 100.4	556.8 ± 113.1
Visits						
Pref.	19.3 ± 3.7	21.2 ± 6.7†	47.5 ± 4.5†	17.4 ± 2.4	16.0 ± 2.7	22.0 ± 4.2*
Nonpref.	14.2 ± 6.9	10.3 ± 3.3	33.0 ± 4.8	19.0 ± 1.3	17.5 ± 3.2	14.0 ± 2.1

Values are the mean ± SEM. Dose of magnesium chloride used during condition was 15 mg/kg and number of compartment/drug pairings was 4. Drugs and doses used on day 13 were: 1.0 mg/kg d-amphetamine (Amp), 5.0 mg/kg cocaine (Coc), 10 mg/kg pentobarbital (Pento), 0.25 mg/kg haloperidol (Hal), saline (Sal), and 15 mg/kg $MgCl_2$ (Mg^{2+}). Significant difference when compared with nonpreferred visits are indicated by *$p<0.05$ and †$p<0.01$.

12 (conditioning test). Therefore, the conditioning effect of the $MgCl_2$ was equally effective for all animals selected for further study of drug effects on day 13. Significant differences were apparent between groups on day 13 (Fig. 3). A significant decrease in the day 12 conditioned effect of $MgCl_2$ was observed on day 13 following saline treatment, $p<0.05$. Amphetamine treatment significantly potentiated the conditioned effect compared to saline treatment on day 13, $p<0.05$. Cocaine and pentobarbital maintained the conditioned effect at a level comparable to day 12. Administration haloperidol and $MgCl_2$ on day 13 significantly attenuated the conditioned effect, $p<0.01$, and preference returned to that measured on day 3.

There was a tendency for all groups to spend more time in the ends of the test apparatus over the three test days, though there were significant differences for the cocaine treatment group only, $F(2,4)=13.13$, $p<0.05$ (Table 3). The time spent in the ends increased significantly from day 3 to day 12, $p<0.05$, and from day 3 to day 13, $p<0.01$.

There were differences in the total number of visits (Table 3) over test days in the pentobarbital group, $F(2,6)=16.85$, $p<0.01$, and the $MgCl_2$ group, $F(2,6)=10.96$, $p<0.01$. Animals in the pentobarbital group made significantly more visits to the ends in the drugged condition on day 13, $p<0.01$, than on either day 3 or day 12. Animals in the $MgCl_2$ treatment group made significantly more visits to the ends on day 12, $p<0.01$, than on day 3 and day 13.

There was also a tendency for all groups to visit the preferred end more often than the nonpreferred end. This tendency was significant as an interaction for the haloperidol treatment group, $F(2,8)=40.20$, $p<0.01$, and as a main effect for the $MgCl_2$ treatment group, $F(1,3)=12.77$, $p<0.05$. In the haloperidol group there was a greater number of visits to the preferred end on day 12, $p<0.01$, but not on day 3 or day 13. In the $MgCl_2$ treatment group there was a greater number of visits to the preferred end on day 13, $p<0.05$, but not on day 3 or day 12.

DISCUSSION

The purpose of Experiment 1 was to show the effects of conditioning with $MgCl_2$ at various doses. It is known that in the conditioning of rats with cocaine to a place, middle range doses (5.0 mg/kg) are more effective than low (0.625 to 2.5 mg/kg) or high (10 to 20 mg/kg) (18). A similar inverted U-shaped function is evident in the ability of d-amphetamine to induce place preference (19). When animals were given $MgCl_2$ as the conditioning drug, there were significant differences between day 3 (preconditioning) and day 12 (postconditioning). There were no differences when animals only received saline on conditioning trials. When 15 mg/kg $MgCl_2$ was administered to animals four times out of eight days on their nonpreferred side, significant changes relative to saline were measured. The administration of 30 or 125 mg/kg $MgCl_2$ appeared to induce changes in preference though these changes were not as robust as those induced by 15 mg/kg. Of all the animals from the three experiments that were

conditioned with 15 mg/kg, approximately 50% of those tested showed strong conditioned effects comparable to conditioning with cocaine (13). Thus, $MgCl_2$ may have some primary reinforcing ability, but because its conditioning is not as ubiquitous as conditioning with cocaine, its abuse potential is most likely considerably lower.

It has been previously reported (15) that increasing the number of drug/place pairings will lead to greater magnitude of CPP or conditioned place aversion. In these studies, Mucha and Iversen paired morphine and saline with opposite sides of a test apparatus in rats for one to four times. The difference between time spent on the morphine-paired side and time spent on the saline-paired side increased as a function of the number pairings. These differences were not significant after two pairings but became so after three pairings. They obtained a similar effect on place aversion by increasing the number of naloxone/place pairings. Experiment 2 of the present study was conducted to determine if a greater behavioral effect could be obtained by increasing the number of $MgCl_2$/place pairings from four to eight. Results from this experiment indicated the contrary, in that there was a decrease in the strength of the conditioned effect.

Carr *et al.* (4) discuss variables affecting the efficacy of drug/compartment pairings in the CPP paradigm. They suggest that the procedure is maximally efficient when the animal is exposed to the apparatus at the same time that the drug is having a reinforcing effect. In the resident-intruder model of mouse aggression, tolerance to the effects of chronic administration of $MgCl_2$ were seen after 14 days of administration of 15 mg/kg and after 4 days of administration of 30 mg/kg (8). Thus, the animals may, through chronic exposure of $MgCl_2$ during the 16-day conditioning cycle, have become tolerant to the reinforcing properties of $MgCl_2$, and the animals' natural preference was maintained on days 20 and 21 as it was following conditioning with saline. In this respect, $MgCl_2$ conditioning differs from cocaine conditioning. Chronic administration of cocaine does not produce a tolerance to the reinforcing effects of cocaine (16). Also, it has been demonstrated in a place preference paradigm that preexposure to cocaine intensifies the reinforcing effects of cocaine (14).

In Experiments 1, 2 and 3, cocaine was administered to animals which had been conditioned to their nonpreferred sides with $MgCl_2$. In all cases where conditioning with various doses of $MgCl_2$ produced at least a 50% shift in preference, injections of cocaine maintained that preference. Otherwise, there were no effects of cocaine on the preference of the animal. When saline was given on day 13, some strength of the conditioning was lost. Though these data are not conclusive, it appears that cocaine given after magnesium conditioning may reinstate cues associated with place preference. Others have found that testing animals under drug conditions following CPP conditioning produced an increase in the magnitude of the conditioned place preference compared to testing in the nondrugged state or to a group receiving saline (2,13). This postconditioning effect is thought 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. Apparently postconditioning cue reinstatement can ocur using the conditioning drug or one with similar properties to the conditioning drug. These present data suggest that $MgCl_2$ might share similar reinforcing and/or discriminative stimulus properties not only with cocaine, but also with amphetamine and pentobarbital, but not haloperidol. This profile differs slightly from that obtained following cocaine conditioning where stimulus properties of cocaine were shared by amphetamine and $MgCl_2$, but not pentobarbital and haloperidol (13). Besides having facilitating effects and interactions with other stimulants (8, 10–12), $MgCl_2$ has been shown to induce sleep (6) and to potentiate pentobarbital-induced sleep. Magnesium also has anticonvulsant properties (3). It is conceivable that stimulus properties could be shared between these two substances. No such generalization of stimulus properties has been found between stimulants and pentobarbital (24). Thus, this technique of administering drugs following conditioning to determine if drugs share stimulus properties appears to have pharmacological specificity.

The effect of $MgCl_2$ given on day 13 is surprising in light of its hypothesized stimulant-like effects. Half of the animals did strongly prefer the conditioned side, but the other half strongly reverted back to their unconditioned preference. Overall, this group showed a significant attenuation of their preference for the conditioned side. The development of tolerance to the stimulus or reinforcing effects of $MgCl_2$ by some animals but not others may account for these effects (8). If tolerance had developed, then extinction of the conditioned effect would occur because of the lack of a presence of stimuli associated with conditioning (1). This extinction of the conditioned effect might also explain the effects of haloperidol on day 13. Previous research has demonstrated that haloperidol blocks the reinforcing action of stimulant drugs (17), and that haloperidol blocks generalization of stimulant drugs during drug discrimination testing (9).

The pattern of activity of the animals in these $MgCl_2$ conditioning experiments did not reflect in a discernable way the preference data. For example, though the administration of cocaine on days 13 and 21 in Experiments 1 and 2 generally elevated the number of visits, there were no differences between these groups as there were in side perference. Also, in Experiment 3, where amphetamine elevated preference relative to saline, it did not elevate the activity of animals. Haloperidol decreased the $MgCl_2$-induced preference, but the number of visits in this group was not different from the saline, amphetamine, cocaine, or $MgCl_2$ treatment groups on day 13. As in Experiment 2, the activity of the animals (either conditioned or unconditioned) is independent of their side preferences. Also since visits were either elevated or remained at the frequency measured on day 3, no sedative effects of these drug treatments were in effect at the time of testing. Similarly, the lack of any suppressant effects by drugs on the total time spent in the ends indicates a lack of aversion to places associated with the conditioning and testing drugs. Such aversive effects would result in less time spent in the ends and more time spent in the neutral middle compartment.

Overall, these data indicate additional interesting behavioral effects of the Mg^{2+} ion. In light of evidence showing the involvement of Mg^{2+} in promoting dopamine receptor binding (5, 7, 20, 21), and evidence showing the mediation of the addictive properties of drugs by the DA system [reviews in (22,23)], further examination of the behavioral and pharmacological effects of this ion are warranted with respect to reinforcing and discriminative stimulus properties.

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