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# PIMOZIDE BLOCKS ESTABLISHMENT BUT NOT EXPRESSION OF COCAINE-PRODUCED ENVIRONMENT-SPECIFIC CONDITIONING

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## Summary

Two experiments were conducted to examine the effects of pimozide on cocaine-produced conditioning to a specific environmental context. On 8 treatment days, 12 rats were injected with cocaine (10 mg/kg i.p.) and 12 with saline prior to placement for 60 min into a test chamber outfitted with infrared emitters and detectors. Following each treatment session the saline group received cocaine in their home-cages and the cocaine group received saline. Cocaine produced a significant increase in vertical activity on treatment days. On test days all rats received saline. Significantly greater vertical activity was observed in the group previously receiving cocaine in the test environment. All rats then received 8 more treatment sessions. On saline test days, pimozide (0.4 mg/kg i.p.) pretreatment failed to antagonize expression of the conditioned effect. In experiment 2, pimozide was given prior to treatment and no evidence of conditioning was seen on saline test days. Thus, pimozide blocked the establishment but not the expression of cocaine-produced environment-specific conditioning. These results suggest that during conditioning, the effects of cocaine on dopaminergic neurons may have produced a change that subsequently influenced behaviour even when dopaminergic systems were blocked.

It has been shown that animals with a history of receiving psychomotor stimulants such as (+)-amphetamine in a specific environment show enhanced activity when injected with saline and placed there. Thus, Post et al. (1) conditioned rats with cocaine or saline in a specific environment. When tested with saline, animals which had experienced cocaine in the test environment had significantly higher activity scores than animals which had previously experienced saline in the same environment. Furthermore, sensitization to the stimulant effects of cocaine has been shown to occur in rats that received cocaine in an environment repeatedly paired with cocaine injections (1,2). Additionally, apomorphine and amphetamine have been shown to produce conditioned stereotyped behaviour such as head bobbing (3).

Recently, Beninger and Hahn (4) showed that (+)-amphetamine produced environment-specific conditioning and that the establishment but not the expression of conditioning could be blocked by the dopamine (DA) receptor blocker pimozide. This finding was of interest because it suggested that during conditioning dopaminergic neurons produced a change in the brain that subsequently influenced behaviour even when DA receptors were blocked (cf. 5).

It has been hypothesized that overfunctioning of dopaminergic systems may

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result in the development of schizophrenia (e.g., 6). However, DA receptor antagonists frequently used in the treatment of schizophrenia block DA receptors on their first administration although their therapeutic effectiveness usually is delayed (6). Beninger and Hahn (4) suggested that their observations may account for this paradox. Thus, in schizophrenics overactive DA may produce changes in nondopaminergic neurons that are only gradually undone when DA neurotransmission is decreased.

Cocaine has been shown to be a DA agonist in animal studies (7), to be psychotogenic in humans (8) and to produce environment-specific conditioning (1). Further understanding of the processes involved in cocaine-mediated conditioning in animals may contribute to the more effective treatment of cocaine effects in humans. The present study was carried out to test the hypothesis that conditioned activity seen in environments associated with cocaine, like that produced by amphetamine, is resistant to the effects of the DA receptor blocker, pimozide.

## Materials and Methods

Forty-eight male albino rats of the Wistar strain (Canadian Breeding Farms, St. Constant, Quebec) weighed 225-250 grams at the beginning of the experiment. They were individually housed in wire cages (18x20x25 cm) in a climatically controlled (21° ±1C) environment, kept on a 12 hour light-dark cycle (lights on at 0600 h), with food and water freely available.

Activity was monitored in six Plexiglas chambers (21x50x37 cm) each enclosed in a styrofoam-insulated wooden sound-attenuating box, illuminated by an overhead bulb (2.5w) and ventilated by a small fan that also provided constant masking noise. Each chamber was outfitted with 14 infrared emitters and detectors, eight being spaced at 10 cm intervals along the length of the chamber and six along the width at a height of 5 and 15 cm above the grid floor. This design allowed for an estimate of both horizontal activity, walking and running and vertical activity including rearing or jumping. Activity was monitored independently in each chamber with the use of a single board microcomputer (Cromemco) with its user interface being a screen or printing terminal. For further details see Beninger et al. (9).

Two experiments were conducted; in each 24 animals were randomly assigned to either an experimental group or a control group. In each experiment the general conditioning procedure was the same; sessions occurred at the same time each day, five days a week, for four weeks.

The purpose of experiment 1 was to test the hypothesis that cocaine would produce environment-specific conditioning and if so, to determine whether pimozide, a specific DA receptor blocker could antagonize this conditioned effect. On treatment days 1-4 (wk 1), 6-9 (wk 2), 11-14 (wk 3), and 16-19 (wk 4), 12 rats received cocaine hydrochloride (BDH Chemicals, Toronto) dissolved in distilled water in a dose of 10 mg/kg, i.p. and 12 rats received saline injections immediately before a 60 min session in the activity monitors. Following each treatment session the saline group received cocaine in their homecages and the cocaine group received saline. On test days 5 (wk 1) and 10 (wk 2), all rats received a saline injection prior to placement in the activity monitor and no injection upon return to their homecages. Test days 15 (wk 3) and 20 (wk 4) were like days 5 and 10, except that animals were pretreated with pimozide (0.4 mg/kg) four hrs prior to the saline test sessions. Pimozide (Janssen Pharmaceutica) was dissolved in boiling tartaric acid (40 umol/ml) and cooled to room temperature prior to injection.

The purpose of experiment 2 was to test the hypothesis that pimozide

would antagonize the establishment of environment-specific conditioning. On treatment days 1-4 (wk 1) and 6-9 (wk 2) the conditioning procedure was the same as in experiment 1 except that four hrs prior to each session in the activity monitor animals received i.p. injections of pimozide (0.4 mg/kg). On test days 5 (wk 1) and 10 (wk 2), all rats received one saline injection prior to placement in the activity monitor and no injections in the home cage. Treatment days 11-14 (wk 3) and 16-19 (wk 4) and test days 15 (wk 3) and 20 (wk 4) were a replication of the first 10 days of experiment 1.

For each 60 min session in the activity monitor each rat obtained 6 scores representing the cumulative number of horizontal activity counts and 6 scores representing vertical activity counts for six 10-min intervals. Statistical significance was evaluated with the use of analysis of variance (ANOVA).

## Results

The mean (±SEM) activity scores for ten min intervals of the 60 min session of each experiment collapsed over conditioning days, were averaged separately for horizontal and vertical activity as shown in Figs. 1-4.

For experiment 1 horizontal activity, cocaine in the activity monitor did not produce a significant increase during conditioning on wks 1 and 2 (p>

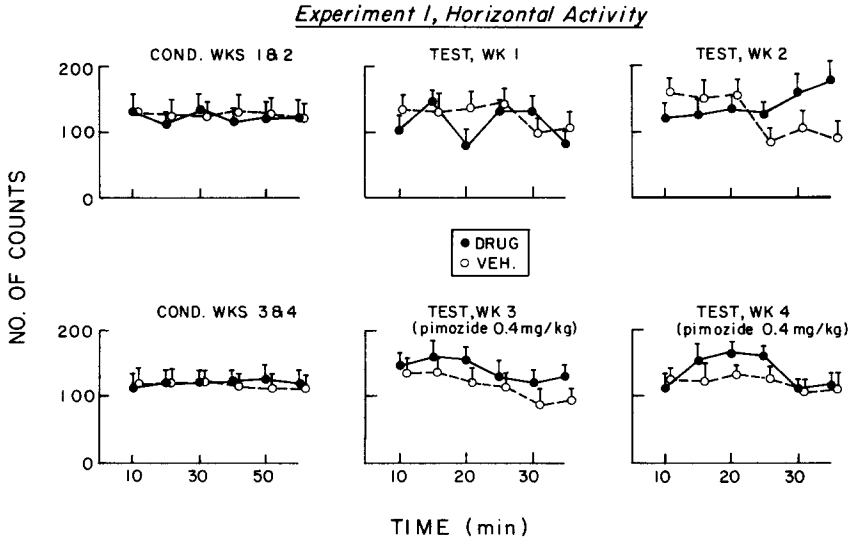


FIG. 1

Mean (±SEM) no. of horizontal activity counts following cocaine or vehicle (VEH) in experiment 1 averaged for conditioning (COND.) days of wks 1&2 and for conditioning days of wks 3&4. Test days without cocaine occurred at the end of wk 1 and wk 2. Pimozide (0.4 mg/kg) but not cocaine was given on test days at the end of wk 3 and wk 4.

0.05) or wks 3 and 4 ($p > 0.05$). On the saline test at the end of wk 1 and wk 2 and the pimozide challenge tests at the end of wk 3 and wk 4 there were also no significant group differences or interactions involving the group variable (see Fig. 1).

Vertical activity data of experiment 1 yielded quite different results. Significant group differences were found on conditioning days between the experimental and control animals during wks 1 and 2 ($p < 0.02$) and wks 3 and 4 ($p < 0.02$). The results comparing cocaine experimental animals to control animals on the test day at the end of wk 1 yielded nonsignificant group differences ($p > 0.05$); however, on the test day at the end of wk 2, the experimental animals exhibited significantly higher activity than their controls ($p < 0.01$). It was also observed that pimozide pretreatment on test days at the end of wk 3 and wk 4 failed to antagonize the expression of the conditioned effect, $p < 0.005$ and $p < 0.03$, respectively (see Fig. 2).

In experiment 2, horizontal activity of the two groups during conditioning wks 1 and 2, when pimozide pretreatment was given, was found to differ significantly ($p < 0.001$). However, horizontal activity on the saline test day at the end of wk 1 and at the end of wk 2 did not differ significantly for the two groups ($p > 0.05$ in both cases). During conditioning wks 3 and 4, groups differed in horizontal activity ($p < 0.001$). On the test at the end of wk 3 no significant group differences were found ($p > 0.05$); however, significant group differences were observed on the test day at the end of wk 4, $p < 0.01$ (see Fig. 3).

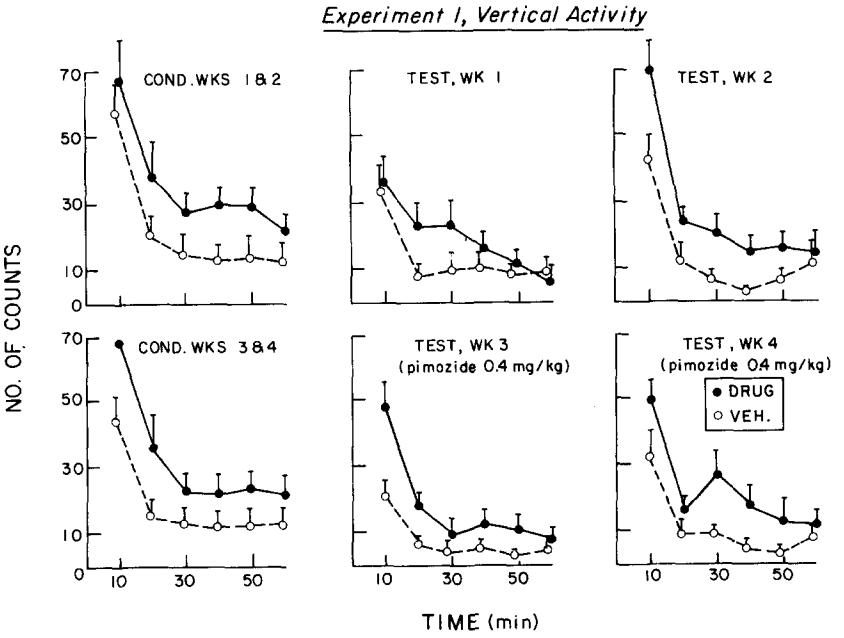


FIG. 2

Mean (±SEM) no. of vertical activity counts following cocaine or vehicle (VEH.) in experiment 1. For details see caption of Fig. 1.

The results for vertical activity were similar. During conditioning wks 1 and 2 when animals were pretreated with pimozide, groups differed ($p < 0.001$). However, the saline test days at the end of wk 1 and at the end of wk 2 group differences were not significant ($p > 0.05$ in both cases). During conditioning wks 3 and 4, cocaine produced significantly greater vertical activity counts ($p < 0.001$). On test days at the end of wk 3 and wk 4 significant group differences were observed, $p < 0.04$ and 0.01, respectively (see Fig. 4).

## Discussion

The results showed that cocaine reliably increased vertical activity while having a weak and inconsistent effect on horizontal activities such as running and walking. This observation is in excellent agreement with the results of Scheel-Kruger et al. (10) who reported that 10 mg/kg of cocaine produced weak motility changes mainly involving increased rearing and an alerting reaction whereas higher doses (25 and 35 mg/kg) induced high rates of activity although the greatest increase was still seen in rearing behaviour. During conditioning sessions of wks 1 and 2 of experiment 2, when both groups were pretreated with pimozide, significant horizontal and vertical activity differences between the cocaine and saline groups were seen. Possibly these differences reflect greater hypokinesia produced by pimozide in the saline treated animals than in those given cocaine prior to

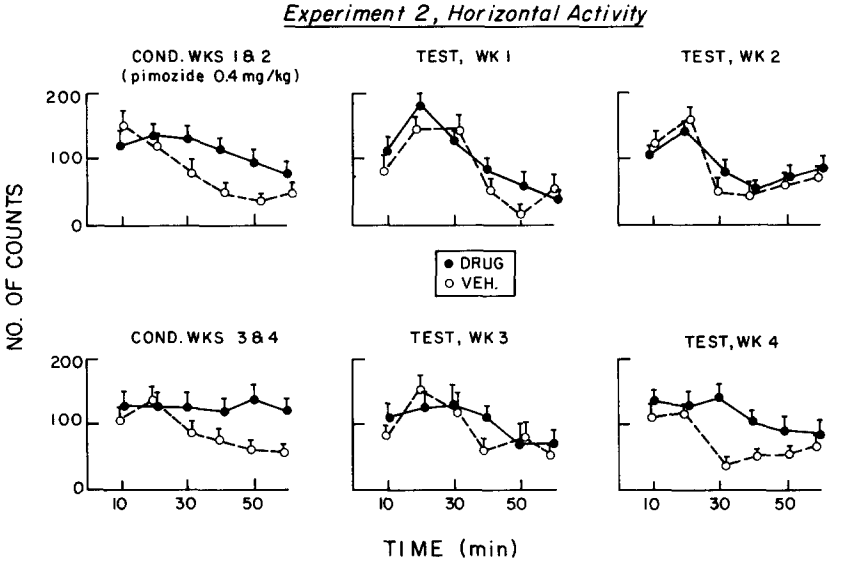


FIG. 3

Mean (±SEM) no. of horizontal activity counts for experiment 2. On conditioning (COND.) days of wks 1&2 animals received pimozide (0.4 mg/kg) 4 hrs prior and cocaine (10 mg/kg) or saline (VEH.) immediately prior to each session. On conditioning days of wks 3&4 animals recieved cocaine or saline prior to each session. Test days without cocaine occurred at the end of wk 1, wk 2, wk 3, and wk 4.

conditioning sessions. In spite of these differences, no significant environment-specific conditioning was seen when both groups were tested with saline at the end of wks 1 and 2.

The observation that rats repeatedly injected with cocaine in a specific environment were significantly more active than control animals when injected with saline and placed there is in good agreement with the previous results of Post et al. (1). The finding that pretreatment with the DA receptor blocker, pimozide prevented conditioning suggests that the establishment of cocaine-produced environment-specific conditioned activity may be related to cocaine's effect on dopaminergic neurotransmission. This finding is consistent with the previous report that the establishment of amphetamine-produced environment-specific conditioning also was blocked by pimozide (4).

It was observed that the same dose of pimozide that blocked the establishment failed to block the expression of environment-specific conditioning. This is in agreement with the similar finding that pimozide failed to block the expression of amphetamine-produced environment specific conditioning (4). These results suggest that during conditioning dopaminergic neurons produced a change in the brain that subsequently influenced behaviour even when DA receptors were blocked. This conclusion can be seen as consistent with many previous observations of a gradual decline in established operant responding following treatment with DA receptor blockers (5,11). These results invite further speculation that the delayed onset of action seen with neuroleptics in the treatment of schizophrenia may be understood with reference to this mechanism. Thus

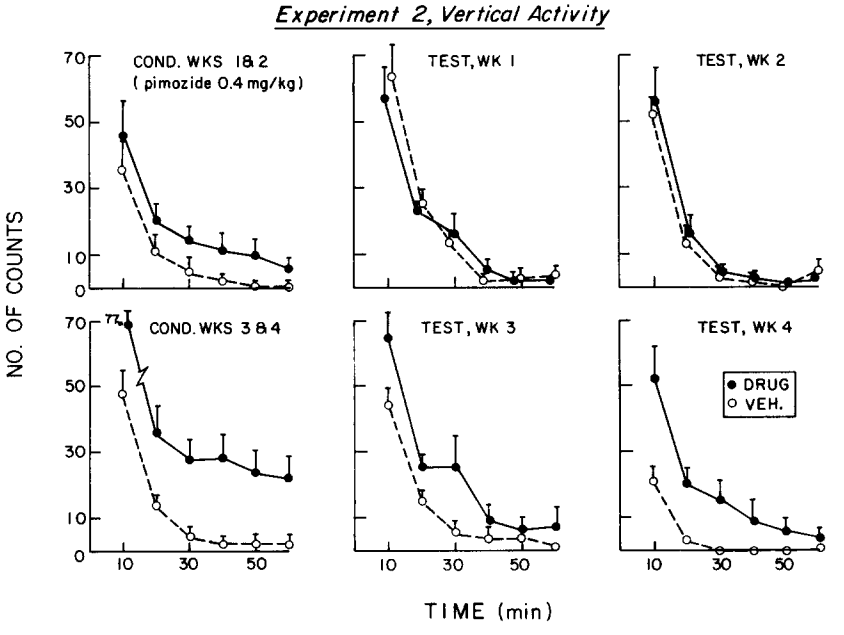


FIG. 4

Mean (±SEM) no. of vertical activity counts in experiment 2. For details see caption of Fig. 3.

during the development of the disease, hyperfunctioning DA neurons may mediate changes in the brain that are transiently refractory to treatment with DA receptor blockers.

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