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Phencyclidine Retards Autoshaping at a Dose Which Does Not Suppress the Required Response[1]

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Phencyclidine Autoshaping Exploratory behavior Rat

PHENCYCLIDINE is a member of the arylcyclohexylamine class of anesthetic agents developed in the 1950s incidental to the search for new analgetic drugs [9]. The re-emergence of widespread nonmedical use of PCP and related drugs in recent years, coupled with reports of disrupted cognitive and social behavior among users, underscores the need for controlled animal studies to further our understanding of these drugs.

The first paper [5] on the pharmacology of PCP in laboratory animals noted excitation, accompanied by ataxia, in rats given the drug. An increase in spontaneous motor activity was also evident in mice, but not in fish, pigeons, guinea pigs, dogs, cats or monkeys. The behavioral pharmacology of PCP has since been extended by studies using conditioned behavior (reviewed in [1]). Qualitative differences in the action of PCP across taxa are not pronounced with schedule-controlled operant behavior. Procedures involving repeated acquisitions of complex operant behaviors have been used to examine the actions of PCP upon learning in patas monkeys [11,12]. PCP, though less selective in this regard than dextroamphetamine, increased errors in acquiring a response sequence at doses lower than those which increased errors in performing an established response chain [11]. Likewise, PCP increased errors in acquiring a conditional discrimination in patas monkeys at doses lower than those increasing errors in performing a previously acquired discrimination, although responding was suppressed even at the lower doses [12]. Earlier, PCP had been shown to impair passive shock-avoidance acquisition in mice [6]. The effect was attributed to drug action upon memory "storage" as latencies to enter the shock compartment twenty-four hours following a single conditioning trial were shortened when PCP was given prior to the conditioning trial and not after. PCP effects upon escape latencies during the acquisition trials remains to be considered in order to appraise the role of performance variables in the observed impairment.

To our knowledge, no reports have ensued which address PCP action upon acquisition of conditioned responses by the laboratory rat, an animals with which much of the behavioral and biochemical research upon PCP is conducted. We report here an experiment in which PCP's effects upon learning in the rat are tested by a discrete-trial method which permits statistical treatment of the results and also allows assessment of acquisition by reference to individual-subject within-session data. We chose to study multiple behavioral effects of phencyclidine in a single automated apparatus: a small-

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animal operant chamber modified to detect selected unconditioned behaviors and to autoshape a lever-touch response. We asked whether the actions of PCP would be detected by our measures of spontaneous motor activity as predicted from the literature. In addition, we asked whether the acquisition of a conditioned response would be sensitive to PCP, and if so, for how long after a single injection of the drug would the acquisition process be affected. We believed that an autoshaping procedure, in which animals reliably "train themselves," would be ideally suited to study the effect of PCP upon learning, i.e., the acquisition of a conditioned response.

METHOD

Twenty experimentally naive male Long-Evans rats (Blue Spruce Farms, Altamont, NY) were housed individually in hanging wire mesh cages under conditions of controlled lighting (on 0700–1900 hrs) and temperature (21±1°C) and given free access to food and water for several weeks upon arrival. Their weights were gradually reduced to eight-five percent of the free-feeding weights and maintained by regulating daily food rations. Their final weights ranged from 320 to 420 g.

The animals were randomly assigned to one of four treatment groups (balanced for body weight) in which they would receive a single subcutaneous injection of 2, 4 or 8 mg PCP HCl/kg in 0.5 ml/kg isotonic saline or the saline vehicle fifteen minutes prior to behavioral testing on the first day.

Behavioral sessions were conducted in standard small-animal operant chambers (25 cm wide × 30.5 cm long × 27 cm high) each equipped with a retractable lever (BRS/LVE model KRP 11D) 3 cm wide and 1 cm in height which protruded 2 cm into the chamber when extended. It was installed such that its center was 4 cm above the floor and 9 cm from the center of the front panel. Also included was a stainless steel wall strip 8 cm wide and mounted with insulating hardware across the rear and one side wall such that the lower edge was 15 cm above the floor. The floor consisted of a series of parallel stainless steel rods running widthwise 0.5 cm in diameter and separated from each other by 2 cm, center-to-center. The operant chambers were enclosed in sound-attenuating outer boxes in which closed-circuit video cameras were installed to permit visual inspection of animal activity. Ventillation was supplied by exhaust fans in the outer boxes. Each chamber was illuminated by a light (G.E. 1820) centrally located 2 cm from the top of the front panel and a jewel-type cue lamp (G.E. 1819) centered 6 cm above the lever. Masking white noise was continuously present in the chambers. Data were recorded and events programmed with the aid of a NOVA 2/10 minicomputer (Data General Corporation, Southboro, MA) in conjunction with ACT software and INTERACT hardware (both BRS/LVE, Beltsville, MD). Solid-state touch circuits were calibrated to detect contacts with the lever or with the wall strip which completed an electrical circuit to the grid floor with an impedance less than 2.5 MΩ. Wall strip contacts were used as an index of rearing [16,17]. Lever contacts served as an additional simultaneous measure of spontaneous motor activity.

Contacts to wall strip and lever were recorded separately for an initial thirty-minute unconditioned behavior session. Immediately upon completion of this session, acquisition of a lever-touch response was studied using an autoshaping schedule [7] in which a randomly timed (range: 45–135 sec; mean: 90 sec) extension of the lever for fifteen seconds comprised a discrete trial. If no contact was made during the fifteen-second presentation, the lever retracted automatically and a 45 mg food pellet (P. J. Noyes Company, Lancaster, NH) was delivered in a food trough located centrally in the front panel 1.5 cm above the floor. If the animal made contact with the lever, it was retracted immediately and a food pellet was delivered. Thirty such trials comprised the session. At the end of each session, the chambers were swabbed with 70% ethanol and the food pellets delivered, but not eaten, were counted and removed. The autoshaping procedure was repeated the next two days for a total of three consecutive thirty-trial sessions.

Acquisition was assessed by the number of correct responses made in the session (i.e., the trials in which a lever contact was made) and the latency of each trial to make a lever contact. Trials when no responses were made were assigned the value of fifteen seconds each. Strip touches recorded during the autoshaping sessions were divided by the duration of the session in minutes.

Statistical treatment of the data included one-way analyses of variance for each measure made during the unconditioned behavior session. Latencies for each animal for the first autoshaping session were averaged in blocks of five trials for inspection. Responses per session were subjected to two-way analysis of variance adapted for missing data (four treatments by three days) with repeated measures on one factor. (Due to mechanical failure, the data for one animal in the 2 mg/kg group were lost for the third day of autoshaping.) Analyses of variance were followed by Duncan's New Multiple Range Tests. Data for food pellet consumption were analyzed by the Kruskal-Wallis test, followed by a rank-sum order test [3]. Strip touches recorded during autoshaping sessions were subjected to two-way nonparametric analysis [3], followed by rank-sum multiple comparisons testing (Duncan analogue) [18]. The missing datum for the 2 mg/kg group, third day, was supplied for this purpose by least-squares estimation. In all cases, $\alpha=0.05$.

RESULTS

The highest dose of PCP reduced the rate of lever touching during the unconditioned behavior session, $F(3,16)=16.89$, $p<0.05$ (Table 1, part A). In contrast, all three doses suppressed strip touching, $F(3,16)=3.34$, $p<0.05$ (Table 1, part B). The absence of a dose-related difference of PCP upon our measure of rearing suggests that the lowest dose was near maximal for this effect. Drugged animals displayed various degrees of ataxia which appeared more severe in those given higher doses, although no systematic attempt was made by observer rating to evalute quantitatively this or other unconditioned behavioral drug effect.

Lever contacts during the succeeding autoshaping session (Fig. 1, Day 1) show the same pattern of suppression by PCP that occurred with regard to the unconditioned behavior session. There was no difference in lever contacts between the groups given saline and the lowest dose of PCP. However, there was evidence that lever contacts made by PCP-treated animals did not reflect acquisition: PCP-treated animals consumed fewer food pellets delivered (see below); the latency to respond within the first session declined systematically in four of the five saline-treated rats, but fluctuated randomly in all rats given the low-dose (Fig. 2); and, while performance (lever contacts per session) of the saline group improved the next day as acquisition proceeded, the drugged groups' performances were similar to that of the control's first exposure (Fig. 1). Treatments: $F(3,16)=7.92$,

TABLE 1

EFFECT OF PHENCYCLIDINE ON EXPLORATORY BEHAVIORS

	Saline	2 mg/kg	4 mg/kg	8 mg/kg
A. Lever Contacts/min	3.91 ±0.83	3.70 ±1.09	1.85 ±0.30	1.26*† ±0.38
B. Strip Contacts/min	2.97 ±0.32	0.91* ±0.41	0.53* ±0.23	0.43* ±0.10

Mean ± S.E.M. (n=5).
*Significantly different from saline group.
†Significantly different from 2 mg/kg dose group.

$p<0.05$, Days: $F(2,31)=40.93$, $p<0.05$, Treatments × Days: $F(6,31)=2.45$, $p<0.05$; Simple main effects of: Control × Days: $F(2,32)=5.10$, $p<0.05$, 2 mg/kg Dose × Days: $F(2,28)=4.99$, $p<0.05$, 4 mg/kg Dose × Days: $F(2,32)=15.23$, $p<0.05$, 8 mg/kg Dose × Days: $F(2,32)=22.97$, $p<0.05$, Day 1 × Treatments: $F(3,46)=8.35$, $p<0.05$, Day 2 × Treatments: $F(3,46)=5.96$, $p<0.05$, Day 3 × Treatments: $F(3,46)=0.21$, n.s.

More food pellets remained at the end of the first session for all drug groups (medians for control, 2 mg/kg, 4 mg/kg and 8 mg/kg, respectively: 1, 16, 28 and 29, $H'=12.65$, $p<0.05$; Bradley rank-sum order test for the control group predicted as the lowest median yielded $p=0.013$). No animal in the control group left more than one food pellet, and one of the animals (#76) in the low-dose group ate all of the food pellets and made three responses; another (#73) left only one pellet and responded on twenty-one trials. All the animals ate essentially all food pellets delivered during the following two autoshaping sessions.

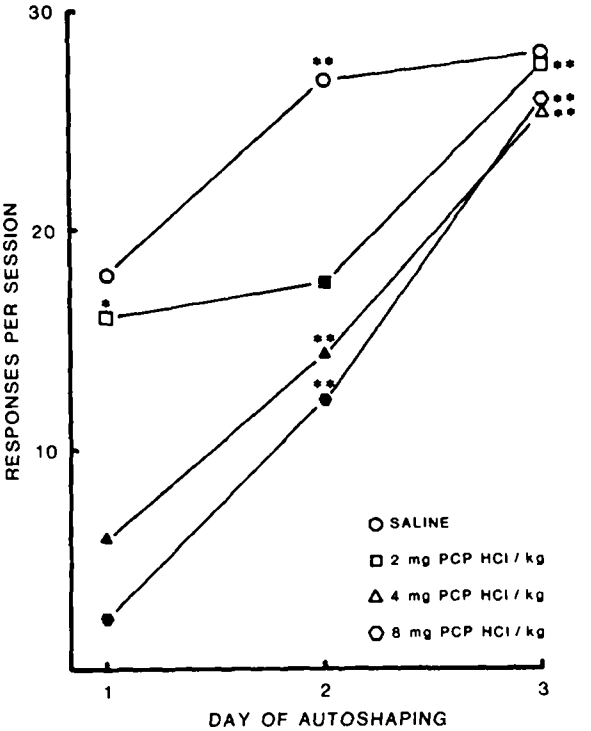

FIG. 1. Acquisition of autoshaped lever-touch response over three daily thirty-trial sessions. Closed symbols indicate values significantly different from saline group on the same day. *Significantly different from the other two drug groups on the same day. **Significantly higher than same group on the previous day.

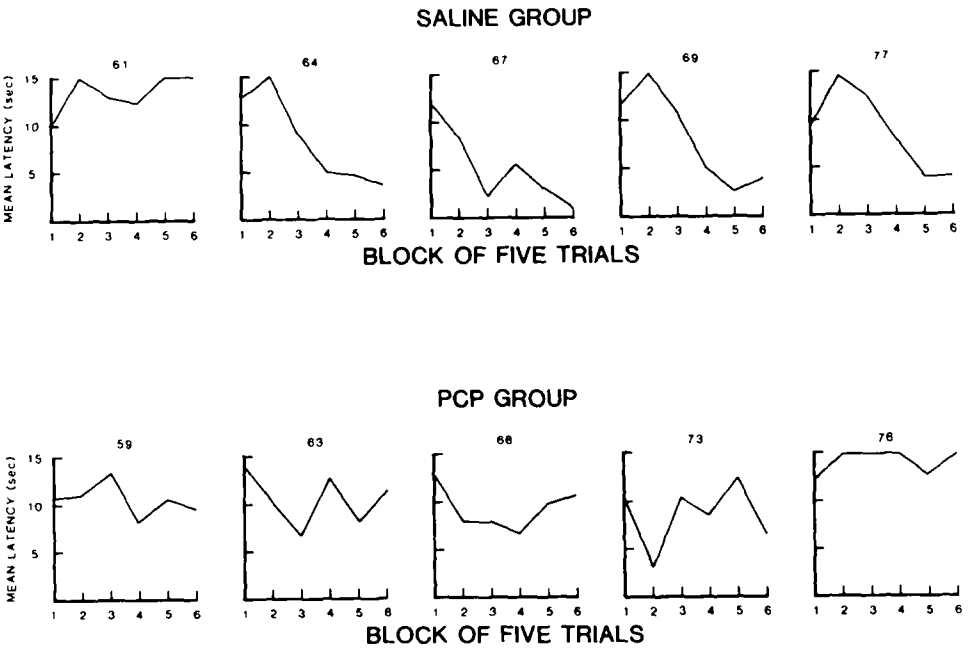

FIG. 2. Within-session latencies to respond during first autoshaping session—control group and 2 mg/kg group. Mean of each block of five trials for individual animals. Upper panels represent animals injected with saline, and lower panels represent those injected with 2 mg PCP HCl/kg. Rat numbers are given above the panels.

TABLE 2
REARING DURING AUTOSHAPING SESSIONS

Treatment Group	Day 1	Day 2	Day 3
Saline	0.26 (0.08–0.44)	0.86 (0.42–1.67)	0.52 (0.25–1.18)
2 mg PCP HCl/kg	0.20 (0.00–1.50)	1.04 (0.60–1.29)	0.81 (0.00–1.98)
4 mg PCP HCl/kg	0.07 (0.00–0.39)	0.76 (0.55–3.04)	0.74 (0.21–1.53)
8 mg PCP HCl/kg	0.06 (0.00–0.13)	0.82 (0.63–2.02)	0.92 (0.45–1.63)

Wall strip contacts recorded in each session were divided by session length in minutes, and expressed as the median (and range) of five observations, except Day 3 for the 2 mg/kg group, where 4 observations were recorded.

Strip touch frequency during the second and third autoshaping sessions (Table 2) were significantly different from that during the first, but did not differ among treatments. Treatment main effects: $H=0.26$, n.s.; Days main effects: $\chi_r^2=8.40$, $p<0.05$; Treatment × Days: $H=2.08$, n.s.

DISCUSSION

When Chen and his coworkers [5] employed a tilt cage to evaluate PCP's action upon spontaneous motor activity, they found the drug to be half as potent as methamphetamine in producing increased activity. PCP-induced increases in the locomotor activity in the rat have been confirmed using other apparatus [4] and are included in an observer rating scale for the drug [19]. In the present experiment, when PCP was given in the same dose and by the same route used by Chen and coworkers [5], the direction of change was opposite on both measures of spontaneous motor activity. Variables controlling spontaneous motor activity, for example food deprivational state, dimensions of the experimental space, its ambient noise and light, stock of rat, etc., [14] might have been sufficiently dissimilar to account for this discrepancy. However, it is more likely that the differences observed were due to the type of spontaneous motor activity studied.

Locomotor activity and exploratory activity are conceptually distinguished aspects of an animal's total spontaneous motor activity [14]. Whereas the tilt cage and other stabilimetric techniques are more commonly used to measure locomotor activity, the behaviors we measured, rearing and lever touching, were selected to reflect exploration of a novel and relatively complex environment. When tested in a conventional apparatus used to observe exploratory behavior, the open field arena, PCP has been reported to increase line crossing by rats. Yet the animals' activities were described as resembling "blind, compulsive forward locomotion that was often ataxic [13], p. 128)." An objective was to reduce the extent to which this kind of locomotor activity would affect our measure of exploration. Thus, the criterion for lever touching was designed to exclude incidental contact made by portions of the animal covered by insulating fur, and to include responses, such as sniffing, biting and manipulations with the forepaws, when they are strongly tied to the lever as a discrete stimulus.

As the stereotypic behavioral syndrome induced by PCP is reported to include rearing [19], we anticipated that at least one dose would result in an increase in rearing over controls. All doses suppressed rearing. PCP-produced ataxia can interfere with the expression of many components of the stereotypic syndrome induced by dextroamphetamine [2], and we presume this ataxia can impede similar stereotyped behaviors induced by PCP as well. The apparatus we used has features in its design which may be responsible for the increased sensitivity of rearing to ataxia as we measured it. First, the height of the wall strip in our apparatus precludes detecting instances of rearing except those in which the animal rears up at least 15 cm. Although the PCP-treated animal may rear more frequently, it may only rarely be capable of raising its forelimbs more than a few cm. Second, in order for the animal to rear in the apparatus employed, it must balance itself on steel dowels, not on a continuous surface of bedding as in [19]. The additional dexterity required to balance on steel rods may tend to make our measure more sensitive to ataxia. Final methodological differences appear to be critical: exploratory behaviors of our food-deprived subjects were measured during their first exposure to the chambers at a time when baseline frequencies of these behaviors are expected to be at their highest; animals in the study of Sturgeon, Fessler and Meltzer [19] were at their normal free-feeding weights and were allowed at least one hour's adaptation to the experimental environment before treatment and observation. Baseline rates of spontaneous activity are lower after adaptation and are lower when the animal is sated. The relatively higher initial values in our control animals enabled us to observe the suppression of innate exploratory behavior by PCP. Lower initial values of these same behaviors in their controls permits a more sensitive detection of the PCP-induced stereotypic behavioral syndrome, which includes behaviors topographically similar to, or even indistinguishable from, those in the animal's natural repertoire. That lever contacts were less sensitive to PCP than strip contacts attests to the greater resistance of floor-level activities to ataxia. Ultimately, the latter measure is also decreased as the dose is raised, producing greater degrees of ataxia and inducing other behaviors (e.g., stereotypies and ambulation) incompatible with investigation of the lever.

The finding of opposite actions upon locomotor activity and exploration which may be the case with PCP would not be unprecedented. Robbins and Iversen [15] found that dextroamphetamine reduced the time rats spent exploring novel discrete stimuli and increased their locomotor activity, which were behaviorally incompatible responses in the apparatus they utilized.

During the first autoshaping session, wall strip contacts made by the control group fell to an average of 0.23 per minute, down from 2.97 per minute during the preceeding unconditioned behavior session. It is difficult to tell whether

this drop reflected adaptation or resulted from the imposition of autoshaping contingencies. The number of contacts per minute rose for all groups the second day, even though acquisition of the autoshaped behavior was nearly complete for the control group. Rearing during autoshaping sessions was more frequent for the drugged groups on the second day, when acquisition was occurring for them, than for the control group during its first day's session.

Employing a discrete-trial behavioral analysis, we were able to demonstrate that PCP can retard learning at a dose which does not significantly reduce performance of the required motor response. Moerschbaescher and Thompson [11] studied effects of PCP upon learning in patas monkeys using a multiple schedule of operant behavior in which repeated acquisitions of response sequences were compared against performance decrements in a component for which the required response sequence did not vary. Although PCP in comparison with dextroamphetamine was less selective in increasing errors in acquisition components, each drug at some dose increased errors made in acquiring a new response sequence over those made in performing an established sequence. Disruption of acquisition was greatest in sequences unaccompanied by discriminative stimuli.

The resilience of conditioned behavior to drug disruption generally is greater when the behavior is under strong external stimulus control [8]. In the autoshaping procedure, the response to be conditioned is brought under the control of a stimulus which is paired with the delivery of food. Stimulus control exerted by the lever is weak at first, but the probability that the rat will make the response increases with repeated trials. We found that the acquisition of the autoshaped response was more sensitive to disruption by PCP than the performance of the required motor response. This was perhaps because the degree of stimulus control over the response was so low initially (in fact, it has to be gained as part of the conditioning process) and because the response requirement was undemanding. By lowering response requirements to a simple motor act and by beginning the acquisition tasks with small but accelerating degree of stimulus control, we were able to render greater divergence between learning and performance. In addition to the animal's capacity to make the response, acquisition of the autoshaped response depends upon pairing the presentation of the lever and the delivery of reinforcer. Although no animal in the low-dose group left all of the food pellets on Day 1, the increase in the food pellets remaining first suggested that learning may not have been occurring in that group. Food may not be as powerful a reinforcer in a fasted animal when given PCP. Anorectic action of the drug has been reported by users taking "typical 'street' doses" [9]. Two animals in the low dose group and one in the control group ate all or nearly all food pellets and yet failed to acquire the behavior in the first session. These animals may have failed to observe and consume the reinforcer soon enough in order for the close temporal pairing of lever presentation and reinforcement to occur. Analogously, failure to establish the magazine sound as a conditioned reinforcer also could have contributed to the retarded learning. Compared to either an organism's ability to respond or to consume the reinforcing stimulus, its ability to be brought under stimulus control may be the more sensitive aspect of acquisition. While the data clearly demonstrate that PCP impairs acquisition of a simple autoshaped behavior in the absence of significantly impaired responding, it remains to be determined if the reduction of reinforcer consumption as immediate consequences of responding is primarily responsible for the retarded learning.

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