Pharmacology Biochemistry & Behavior, Vol. 12, pp. 445–448. Printed in the U.S.A.

The Use of Conditioned Defensive Burying to Test the Effects of Pimozide on Associative Learning

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Received 28 July 1979

BENINGER, R. J., A. J. MACLENNAN AND J. P. J. PINEL. *The use of conditioned defensive burying to test the effects of pimozide on associative learning.* PHARMAC. BIOCHEM. BEHAV. 12(3) 445–448, 1980.—Rats shocked by a wire-wrapped prod mounted on the wall of the experimental chamber buried the prod with available bedding material when they were tested 24 hr later. Injection of the neuroleptic, pimozide (1.0 mg/kg) before conditioning and again before testing disrupted this conditioned defensive burying; however, a concomitant reduction in general activity suggested that this deficit in conditioned burying may have reflected a general motor impairment instead of a learning deficit. The observation that rats conditioned under the influence of pimozide but tested 24 hr later while undrugged did not display deficits in conditioned burying confirmed this view. Thus, neuroleptics appear to disrupt learned behavior by interfering with the performance of conditioned responses rather than by disrupting associative learning per se.

Pimozide Neuroleptics Dopamine Defensive burying Associative learning

NEUROLEPTIC drugs such as haloperidol and pimozide that in low doses produce a relatively specific blockade of dopamine (DA) receptors [4] have been used extensively to investigate the role of DA pathways in learning. For example, some investigators have found that rats under the influence of neuroleptics do not acquire a one-way avoidance response [2,3]; others have reported that neuroleptic-treated animals fail to learn a brightness discrimination [8]. However, because these studies employed procedures that involved animals drug-treated at the time of testing, the results cannot be interpreted unambiguously. Thus, any possible effects of these drugs on associative learning per se are confounded with the well documented disruptive effects of neuroleptics on motor activity [9].

One way to assess the relative merits of these two possible interpretations is to employ a design in which the conditioning and testing phases are separate. With such a design, the drug's effect on learning mechanisms can be measured unambiguously by conditioning one group of animals under the influence of the drug and another without the drug and then comparing the responding of the two groups in a later drug-free test session.

Pinel and Treit [5] recently have developed a conditioning paradigm that should prove to be a major addition to the behavioral techniques available for assessing the effects of various pharmacological and physiological manipulations on learning. In one of their experiments, every rat shocked once through a stationary, wire-wrapped prod mounted on the wall of a test chamber selectively buried the shock prod with commercial bedding material from the chamber floor although a comparable control prod was mounted on the opposite wall. An important feature of this conditioned defensive buying phenomenon from the standpoint of the present investigations is the excellent retention displayed by subjects after only a single conditioning trial. Rats removed from the apparatus immediately after the shock displayed significant levels of prod burying even when they were not returned to the apparatus until 20 days later [5], thus making it feasible to condition subjects under the influence of a drug and to test their retention at a later point in time when they are drug free.

Two separate experiments were conducted to assess the effects of the neuroleptic, pimozide on associative learning. In Experiment 1, pimozide was injected prior to conditioning and testing to establish that neuroleptics produce a deficit in conditioned burying. In Experiment 2, the effect of pimozide injected prior to conditioning on the subsequent conditioned defensive burying of undrugged rats was assessed; in this experiment, possible confounding of motor activity and learning was prevented by testing undrugged animals.

[1]This research was supported by a Natural Science and Engineering Research Council of Canada grant to John Pinel. R. J. Beninger is a Medical Research Council of Canada postdoctoral fellow. Requests for reprints should be sent to: Richard J. Beninger, Department of Psychology, University of British Columbia, Vancouver, B.C., Canada V6T 1W5.

EXPERIMENT 1

The purpose of the first experiment was to establish that neuroleptics produce a deficit in conditioned burying like that seen in more traditional avoidance paradigms in which the effects of neuroleptics on conditioning and testing typically have been confounded [2, 3, 8]. Thus, the experimental rats in Experiment 1 received an injection of pimozide both prior to the single conditioning trial and before the test of retention 24 hr later. In anticipation of deficits in conditioned burying, the effect of pimozide on general activity levels was assessed to determine whether deficits in conditioned responding could reasonably be attributed to general motor impairment.

METHOD

Subjects

The 48 experimentally naive, 350 to 450 g male, hooded rats (Canadian Breeding Farm and Laboratories, St. Constant, Quebec) were housed on a floor of bedding material made from ground corn cob (San-i-cel, Paxton Processing Co., Paxton, IL) in 50×40×20 cm polyethylene cages (five per cage) where water and Purina laboratory food pellets were available continuously.

Apparatus

The conditoning chamber was a 44×30×44 cm Plexiglas box, the floor of which was evenly covered with a 5 cm layer of San-i-cel. Affixed at right angles to the middle of one of the end walls 2 cm above the bedding was the experimental prod. It was constructed of a "square" (2×2 cm) hollow cardboard tube 6.5 cm in length and covered with black electrical tape. The shock was administered through two uninsulated wires wrapped around the tube. The control prod was constructed from a solid 0.5×0.5×6 cm Plexiglas rod wrapped first with masking tape and then with two uninsulated wires. The control prod was held by the experimenter during shock administration. Shocks were administered from an 800 V, 60 Hz AC source through an 8×10^4 Ω dropping resistor.

Procedure

All rats were exposed in groups of four to the test chamber for five 45-min habituation periods distributed over the first 8 days of the experiment. The experimental prod was not present in the chamber during these sessions. Each rat then was assigned randomly to either the Experimental group (n=24) or the Control group (n=24). Every subject received a single conditioning trial followed 24 hr later by the test session. Approximately 4 hr before conditioning each rat received an intraperitoneal (IP) injection of 1.0 mg/kg pimozide. This dose was employed because it has been shown to effectively block the acquisition of avoidance responding [1]. The drug was dissolved in a ratio of 1:6 in boiling tartaric acid and then cooled to about 45°C prior to injection. In all published studies of conditioned defensive burying, rats were shocked when they voluntarily placed a forepaw on the shock prod; however, pilot observations suggested that some rats under the influence of pimozide were so inactive that they would not contact the prod in a reasonable amount of time. Thus, both the experimental and control rats were shocked manually 30 sec after being placed into the conditioning chamber. Each of the rats in the Experimental group was picked up and held so that it faced the experimental prod with a forepaw resting on it. At this point the shock circuit was activated. To insure that any burying behavior directed at the experimental prod by the experimental subjects was not simply an unconditioned consequence of shock, control rats were shocked in a similar manner. These shocks were administered at the opposite end of the chamber by holding the animal and placing the control prod against its hindpaw. The shock durations in all cases were controlled by withdrawal reflex of the subjects; both the intensity and duration of each shock was monitored by an oscilloscope and recorded. Each rat was removed from the chamber 10 sec after the shock and returned to its home cage.

The Experimental and Control groups each were subdivided randomly into two groups (n=12) for the test phase. The rats in two of these groups (Pimozide groups) received an IP injection of pimozide (1.0 mg/kg) 4 hr prior to testing and those in the other two groups (Vehicle groups) received the tartaric acid vehicle. Thus the design was a 2×2 factorial with 12 rats in each of four groups: Experimental Pimozide, Control Pimozide, Experimental Vehicle and Control Vehicle.

Test sessions occurred 24 hr after conditioning and commenced when each rat was placed in the chamber facing the wall opposite the experimental prod. The control prod was absent from the chamber during these test sessions. Each rat was left in the chamber for a 15-min shock-free test period during which behavior was viewed via closed circuit television and recorded by a video taperecorder. The burying behavior of rats in this situation typically consists of a series of stereotyped sequences that begin with the rat facing the prod from a distant part of the apparatus [5]. The rat then moves toward the prod pushing and spraying bedding at the prod with shovelling movements of the snout and alternating pushing motions of the forelimbs. The durations of these bursts of snout and forelimb movements were recorded on an event recorder. In addition, the height of the bedding at the base of the prod was measured at the end of each test.

Each rat's activity level was measured during the 10 min immediately preceding its 15-min test for burying. The floor of a barren 44×30×44 cm Plexiglas chamber was divided into six equal-area rectangles (14.7×15.0 cm). Each rat was placed in a corner of the chamber and the number of squares traversed was recorded.

RESULTS

The fact that the experimental rats learned the association between the experimental prod and the shock is readily apparent from Fig. 1. Rats shocked by the experimental prod spent substantially more time burying it (Fig. 1A) and accumulated substantially higher piles at its base (Fig. 1B) than rats shocked by the control prod. The significance of these effects was confirmed by an examination of the appropriate main effects in two analyses of variance: duration, $F(1,44) = 4.66$, $p<0.05$; height, $F(1,44)=4.13$, $p<0.05$.

It also is obvious from Figs. 1A and 1B that pimozide had a substantial disruptive effect on conditioned defensive burying. The rats in the Experimental Pimozide group spent significantly less time burying the prod, $t(23)=2.67$, $p<0.02$, and accumulated significantly less bedding material at its base, $t(23)=2.57$, $p<0.02$, than did the rats in the Experimental Vehicle group. This difference also was reflected in the significant main effect of drug (Pimozide vs Vehicle) on both the duration of burying, $F(1,44)=9.92$, $p<0.003$, and the

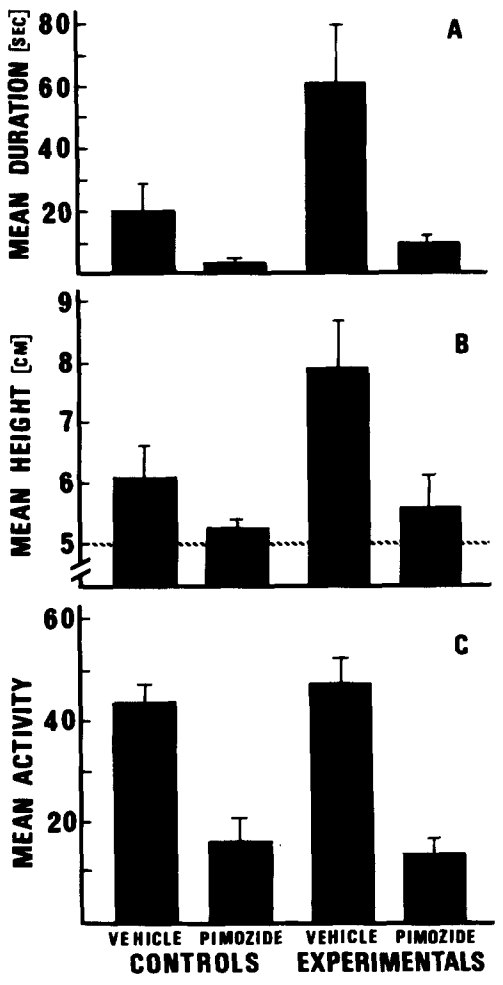

FIG. 1. Mean duration of burying (Panel A), height of bedding accumulated at the experimental prod (Panel B), and activity (Panel C) for each of the four groups (n=12) in Experiment 1. Vertical lines indicate SEMs. Although all the subjects received pimozide prior to shock only half received pimozide prior to the test; the others were injected with the vehicle. Half were shocked by the experimental prod and half by the control prod.

height of accumulated bedding, $F(1,44)=9.30$, $p<0.004$, in two independent analyses of variance.

Pimozide also produced a substantial reduction in locomotor activity (Fig. 1C). The significance of this effect was confirmed by an evaluation of the appropriate main effect in a two-way analysis of variance, $F(1,44)=53.17$, $p<0.001$.

The mean duration (±SEM) and amplitude (±SEM) of the shocks given to the Experimental and Control rats during the conditioning phase were 42.8 (±4.9) msec and 38.1 (±3.8) msec and 9.4 (±0.4) mA and 10.0 (±0.4) mA, respectively. There was no significant difference between the two groups on either measure: duration, $t(46)=0.47$, $p>0.05$; amplitude, $t(46)=0.01$, $p>0.05$.

DISCUSSION

The results of Experiment 1 clearly demonstrated that the defensive burying was controlled by the conditioned association between the experimental prod and the shock rather than being an unconditioned consequence of shock in the conditioning chamber. Rats shocked by the experimental prod buried it. Those shocked by the control prod also exhibited a small amount of burying of the experimental prod but this was significantly less than Experimental levels; possibly there was some generalization from the control to the experimental prod. However, the conditioned defensive burying of rats in the experimental condition was disrupted by an injection of pimozide prior to the test; rats injected with pimozide prior to both conditioning and testing buried significantly less than did rats injected with pimozide before conditioning and the vehicle before the test. In addition, pimozide significantly reduced open field activity. Thus although the disruptive effects of pimozide on conditioned behavior frequently have been attributed to its disruption of learning processes, e.g., [2,8], the results of Experiment 1 suggested that they simply may be a reflection of the effects of pimozide on locomotor activity.

EXPERIMENT 2

If the disruptive effects of pimozide on conditioned defensive burying are attributable to a general reduction in locomotor activity during the test rather than to an interference with associative learning, then rats conditioned while under the influence of pimozide but tested in its absence should bury as well as vehicle controls. Experiment 2 tested this prediction.

METHOD

The 48 experimentally naive, male 350 to 450 g hooded rats were purchased, housed, habituated, divided into groups, conditioned and tested as in Experiment 1. However, unlike Experiment 1, all rats were injected only before conditioning. Thus, each rat in two of the four groups (Experimental Pimozide and Control Pimozide) was injected with pimozide 4 hr before conditioning and each rat in the other two groups (Experimental Vehicle and Control Vehicle) was injected with the vehicle.

RESULTS

As in Experiment 1, rats shocked by the experimental prod spent significantly more time burying the prod (Fig. 2A), $F(1,44)=39.85$, $p<0.001$, and accumulated significantly higher piles of litter at its base (Fig. 2B), $F(1,44)=46.52$, $p<0.001$, than rats shocked with the control prod.

Pimozide injected prior to conditoning had no discernible effect on the amount of conditioned responding displayed during the drug-free test phase; rats under the influence of pimozide during conditioning did not display any significant decline in duration of burying, $F(1,44)=1.75$, $p>0.05$, or in the height of the pile of bedding material accumulated at the base of the prod, $F(1,44)=0.01$, $p<0.05$, in comparison to rats treated with the vehicle.

GENERAL DISCUSSION

In both experiments, the rats shocked by the experimental prod buried significantly more than rats shocked by the control prod. Thus, the burying was a consequence of the learned association between the experimental prod and shock rather than an unconditioned effect of being shocked in the conditioning apparatus.

The effects of pimozide on this conditioned defensive

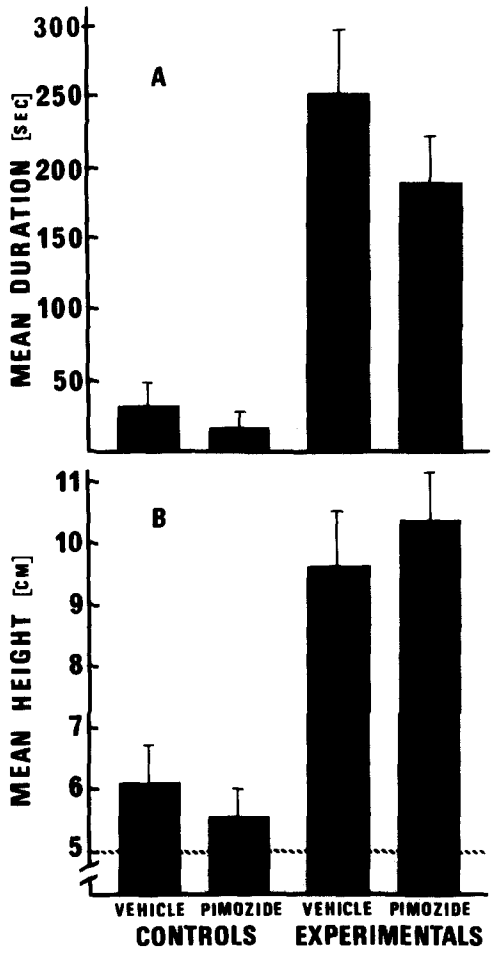

FIG. 2. Mean duration of burying (Panel A) and height of the bedding accumulated at the experimental prod (Panel B) for each of the four groups (n=12) in Experiment 2. Vertical lines indicate SEMs. Half of the subjects received pimozide prior to the shock and half received the vehicle, but no injections were administered prior to testing. Half were shocked by the experimental prod and half by the control prod.

burying can be summarized as follows. In Experiment 1, rats under the influence of pimozide during both conditioning and testing buried significantly less, and were less active, than rats that were conditioned under the influence of the drug and tested without it. In Experiment 2, rats conditioned under the influence of pimozide but tested later without the drug displayed no deficit in conditioned defensive burying in comparison to rats that were drug free during both conditioned and testing. These results indicate that pimozide does not affect significantly the associative or stimulus-stimulus (i.e., shock-prod) learning involved in conditioned defensive burying, but does reduce the conditioned responding that reflects this learning.

Numerous studies have indicated that low doses of pimozide are relatively specific in blocking DA receptors (see review in [4]). Thus, the present findings suggest that synaptic transmission of DA neurons is not required for associative learning to occur and support the view that DA modulates levels of general activity.

These views are consistent with the results of several recent studies. Beninger, Mason, Phillips and Fibiger [1], for example, found that rats injected with pimozide failed to acquire a one-way active avoidance response during five training sessions. When in an undrugged state these same animals were trained to lever-press for food on a random interval schedule until responding had stabilized. Then the tone that had signalled shock during avoidance training was presented. Significantly greater conditioned suppression was observed in the animals that had received avoidance training than in unshocked controls. This result indicated that drugged animals that usually failed to avoid during avoidance training in fact had learned the association between tone and shock.

It has been shown that the conditioned defensive burying phenomenon has a number of features that will facilitate its use in experimental investigations of learning, memory, and defensive behavior [6,7]. Among them are its reliability, generality, stability, and simplicity. However, the present experiments illustrate a previously undiscussed feature of conditioned defensive burying that is particularly relevant to its application to pharmacological investigations of learning. Conditioned defensive burying is an unambiguous instance of stimulus-stimulus learning. Once an animal has learned the association between the shock and its source, it reacts with a directed response already in its repertoire; it buries the source. Because response learning is not involved, it proved possible to condition quickly animals with motor disabilities by placing them in contact with the source before shocking them. In both of the present experiments, rats rendered hypoactive by pimozide were conditioned successfully by placing them on the experimental prod rather than waiting for them to make voluntary contact. In addition to clarifying the effects of neuroleptics on conditioned responding, the present experiments comprise the first instance in which the conditioned defensive burying paradigm has been used to investigate pharmacological factors in learning.

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