Pharmacology Biochemistry & Behavior, Vol. 10, pp. 37–41. Printed in the U.S.A.

Possible Involvement of Serotonin in Extinction[1]

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(Received 9 August 1978)

BENINGER, R. J. AND A. G. PHILLIPS. *Possible involvement of serotonin in extinction.* PHARMAC. BIOCHEM. BEHAV. 10(1) 37–41, 1979.—In Experiment 1, rats were trained to lever-press for continuous reinforcement with food; half were then intubated with the serotonin synthesis inhibitor parachlorophenylalanine (PCPA: 400 mg/kg) and half with water. In extinction the PCPA-treated rats responded at a higher rate. In Experiment 2, rats were trained on a random interval schedule and then assigned to two groups, treated as in Experiment 1, and tested in extinction. There was no significant difference in the resistance to extinction of the two groups. In Experiment 3, the responding of rats trained in a punished stepdown response paradigm and then given an intragastric injection of PCPA took longer to recover than the responding of water-injected controls. These observations suggest that serotonergic neurons might play a role in extinction processes.

Serotonin Extinction PCPA

SINCE THE discovery of serotonergic neurons originating in the mid-brain raphe nuclei [1, 4, 5], there has been considerable interest in determining the role of these systems in behavioral processes. A commonly used method for manipulating serotonin levels is the injection of parachlorophenylalanine (PCPA) a drug which inhibits the syntheis of serotonin. Koe and Weissman [6] reported that a single injection of PCPA (316 mg/kg) produced a 90% depletion of serotonin in 72 hr following administration.

Using this method, Conner, Stolk, Barchas and Levine [3] reported that PCPA-treated rats took longer to habituate to an auditory signal; these authors also observed that once the PCPA-treated rats had habituated they were still hyperreactive to altered environmental stimuli (e.g., a new auditory signal) when compared to similarly habituated controls. Brody [2] has reported similar observations. Solomon, Kiney and Scott [15] recently demonstrated that latent inhibition [8] failed to occur in rats with serotonin depleted during preexposure to the stimulus and suggested that the serotonergic system may be involved in "tuning out" nonreinforced or irrelevant stimuli. The data of Conner *et al.* [3] and Brody [2] are consistent with this suggestion that serotonin neurons are involved in the process which underlies a reduction in responding to certain stimuli.

The extinction paradigm would appear to provide a direct test of the hypothesis that serotonergic mechanisms mediate changes in the organism's response to non-reinforced stimuli. According to this hypothesis, a reduction in serotonergic activity following pretreatment with PCPA should increase resistance to extinction. The following experiments were designed to test this hypothesis in tasks involving either appetitive or aversive stimuli.

EXPERIMENT 1: EXTINCTION AFTER CONTINUOUS REINFORCEMENT

This experiment was designed to compare the resistance to exinction of PCPA-treated and control rats which had a history of continuous food reinforcement for lever-pressing. If serotonin neurons are involved in the extinction process, the serotonin-depleted rats should be more resistant to extinction.

METHOD

Animals

Thirty-seven experimentally naive male albino rats of the Wistar strain with ad lib feeding weights of 250 to 400 g, were housed individually in a climatically controlled colony room, on a 12 hr light/dark cycle. All rats were deprived to 80% of their free-feeding body weights and were maintained at those weights throughout the experiment by daily feeding with measured rations.

Apparatus

The experimental environment consisted of a cubicle (23.4×20.4×19.5 cm) with Plexiglas sides and top, aluminum plate ends and a grid floor. In the middle of one of the ends at a height of 5.5 cm was a lever (Scientific Prototype) that was 5.0 cm wide and had a force requirement of about 0.11 N. To the left of the lever at a height of 1.5 cm was a feeder cup. The cubicle was located in a small illuminated room. Lever-presses were recorded on an impulse counter.

[1]The authors wish to thank Fred Lepaine for assistance with data collection and Don Wilkie for helpful comments on the manuscript. This research was supported by a grant from the National Research Council. R. J. Beninger is an MRC post-doctoral fellow. A. G. Phillips is a Killam Senior Research Scholar.

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Procedure

Twenty-one rats were trained for five 30-min sessions over five consecutive days at about the same time each day. During each session a continuous reinforcement (CRF) schedule was in effect, i.e., every lever-press produced the delivery of one 45 mg food pellet (Noyes). No attempt was made to shape responding on the lever. Those rats that reached a criterion of 200 to 300 lever presses on each of the last two training sessions were placed into one of two test groups. Several hours following the last training session, one of these groups (n=8) was lightly anaesthetized with ether and given an intragastric injection of PCPA (400 mg/kg); the PCPA was prepared as a suspension in water with a few drops of polysorbate (Tween) added. The other group (n=8) was similarly anaesthetized and intubated with water. Three days following intubation, each rat received one 50-min extinction session during which lever-press responses did not produce food pellets. The dependent variable, i.e., number of lever-presses, was recorded every 10 min during this session.

As a control for possible non-specific effects of PCPA on lever-pressing, the remaining 16 rats received the following training. To determine the operant rate of lever-pressing, each rat was given three 30-min sessions in the cubicle during which lever-presses had no prearranged consequences. These rats were matched for total number of lever-presses over these three sessions; one group (n=8) was intubated with PCPA as described above and the other (n=8) with water. Three days following intubation each rat received one additional 30-min session in which lever-presses again had no prearranged consequences.

RESULTS

Sixteen of the 21 rats trained on CRF reached criterion. These rats formed the PCPA (n=8) and water (n=8) groups. The mean (± SEM) lever-press rates of these two groups during the last two CRF training sessions did not differ significantly being 8.5 (± 0.3) and 8.6 (± 0.4) responses per min, respectively. The respective mean 80% weight of the PCPA and control groups was 303.1 (± 21.1) and 259.2 (± 15.3) g; on the extinction test day the mean weights were 295.0 (± 19.0) and 259.6 (± 14.8) g, respectively.

The mean lever-press rates of the PCPA and water groups during each 10-min block of the extinction sessions are shown in Fig. 1. A two-way analysis of variance with repeated measures on the blocks variable revealed a group difference, $F(1,14)=8.51$, $p<0.01$, a significant effect of 10-min blocks, $F(4,56)=35.55$, $p<0.001$, and no significant interaction, $F(4,56)=1.72$, $p>0.05$. Tests of simple main effects revealed that the two groups differed in the first block, $F(1,68)=10.86$, $p<0.01$ and in the second block, $F(1,68)=5.46$, $p<0.05$ but the differences were insignificant for the remaining three blocks. From this analysis it can be concluded that during the first 20 min of the test session the PCPA-treated rats were more resistant to extinction than the water group.

The mean (± SEM) operant lever-press rates (resp. per session) of the PCPA and water rats for the 3 baseline sessions of the non-reinforced control condition were 4.7 (± 4.7) and 4.5 (± 4.7), respectively. Three days after PCPA or water treatment these lever-press rates were 2.9 (± 3.6) and 2.8 (± 3.9), respectively. These data indicate that there was no difference in the number of responses

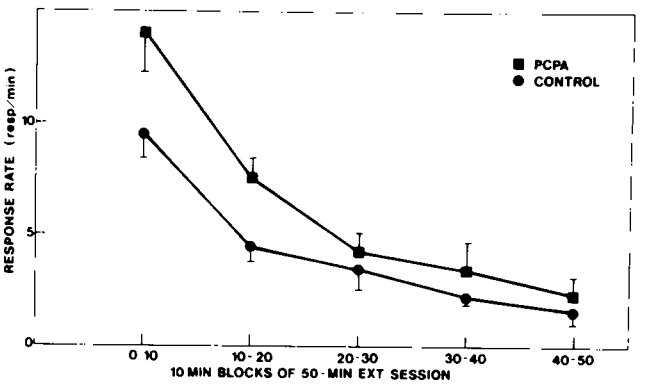

FIG. 1. Mean lever-press rates per min of PCPA (n=8) and control (n=8) groups during each 10-min block of the 50-min extinction session which followed CRF training in Experiment 1. Error bars are SEMs.

made by the two groups during the test sessions prior to or following intubation. The mean 80% weight of the PCPA group before intubation was 232.0 (± 10.6) g and 3 days after PCPA treatment was 233.0 (± 10.4) g. For the control group, these values were 231.0 (± 8.8) and 233.8 (± 9.5), respectively.

EXPERIMENT 2: EXTINCTION AFTER PARTIAL REINFORCEMENT

It is well known that animals trained on a partial reinforcement schedule will display increased resistance to extinction [9]. Therefore, it was of interest to determine whether rats pretreated with PCPA would again show increased resistance to extinction relative to undrugged control animals.

METHOD

Animals

Sixteen experimentally naive male albino rats of the Wistar strain, with ad lib feeding weights of 255–360 g and housed as described in Experiment 1, were deprived to 80% of their free-feeding body weights and were maintained at those weights throughout the experiment by daily feeding with measured rations.

Apparatus

Three cubicles identical to the one described in Experiment 1 were each located in a ventilated sound-attenuating box illuminated with an overhead light. Schedules of reinforcement and data collection were controlled by a Data General Nova 3 computer.

Procedure

At about the same time each day for three days each rat received a 40-min session of CRF with reinforcement consisting of the presentation of one 45 mg food pellet (Noyes). The schedule was then changed to random-interval (RI) 64-sec, i.e., response contingent reinforcement was made available at random with a 64 sec mean. Eight rats received 5 sessions on this schedule and eight received 7 sessions. Fol-

lowing these training sessions, each subgroup of eight was divided into four matched pairs of rats. One member of each pair was assigned to the PCPA group and the other 8 rats constituted the water group. Several hours following the last session of RI training the groups were intubated with PCPA or water as described in Experiment 1. Three days after intubation each rat received one 90-min extinction session. Number of lever-presses was recorded every 10 min.

RESULTS

The mean (± SEM) response rates of the PCPA (n=8) and water (n=8) groups for the last two sessions of training were 20.5 (± 1.6) and 19.4 (± 1.6) responses per min and did not differ significantly. The respective mean 80% weight of the PCPA and control groups was 231.3 (± 3.5) and 233 (± 1.5); on the extinction test day the weights were 223.5 (± 4.9) and 231.3 (± 2.2) g, respectively.

The mean response rates during each 10-min block of the extinction session are shown in Fig. 2. A two-way analysis of variance with repeated measures on both variables revealed no significant difference between groups, $F(1,7)=1.99$, $p>0.05$, an effect of 10-min blocks, $F(8,56)=166.44$, $p<0.001$, and no significant interaction, $F(8,56)=1.35$, $p>0.05$. From this analysis it can be concluded that the extinction of lever-pressing after RI 64-sec training is not affected by PCPA.

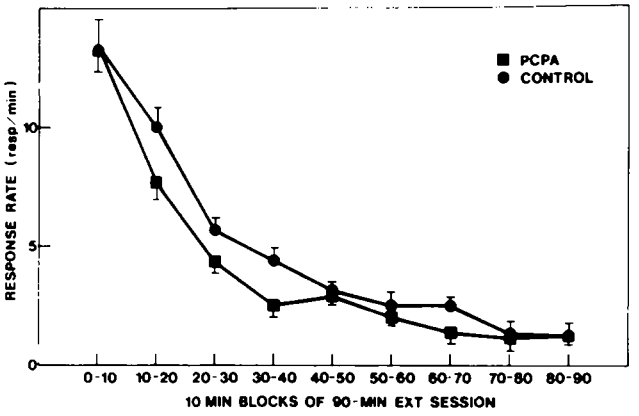

FIG. 2. Mean lever-press rate per min of PCPA (n=8) and control (n=8) groups during each 10-min block of the 90-min extinction session which followed RI training in Experiment 2. Error bars are SEMs.

EXPERIMENT 3: EXTINCTION OF A PUNISHED STEPDOWN RESPONSE

The results of Experiments 1 and 2 indicate that extinction of lever-pressing is affected by serotonin depletion after CRF training but not after experience with partial reinforcement. In an attempt to gain further insight into the possible role of serotonin systems in extinction processes, the following experiment examined the effect of PCPA on extinction (or recovery from suppression) of a punished stepdown response. This task was selected because it differs in a number of important ways from the procedures employed in Experiments 1 and 2. The major difference is the use of shock to suppress a prepotent response.

In this stepdown paradigm the removal of punishment (i.e., extinction) should lead to a recovery of the response, whereas removing the reinforcement contingencies in appetitive situations results in a weakening of the previously reinforced response. On the basis of this distinction, it should be possible to determine whether disruption of serotonergic activity increases resistance to extinction as a consequence of an elevation in general activity (although the results of Experiment 2 are opposed to this idea) or whether it reflects a more specific role for serotonin in the modification of response patterns after non-reinforcement; this modification, for example, could involve tuning out previously relevant stimuli. If the increased resistance to extinction observed in Experiment 1 is mediated by an elevation in general activity, rats pretreated with PCPA should show faster step-down latencies in the extinction phase of the punished stepdown response paradigm. Conversely, if serotonergic neurons are involved in modifying responding after changes in response contingency, animals in the PCPA group should take longer to recover the suppressed step-down response.

METHOD

Animals

Eighteen experimentally naive male albino rats of the Wistar strain weighing from 270 to 300 g and housed as described in Experiment 1, had food and water available ad lib.

Apparatus

The experimental environment consisted of a Plexiglas box (25×29×40 cm) outfitted with a platform (25×7 cm) extending across one wall at a height of 7.5 cm above the grid floor. The grid floor could be electrified with a scrambled 1.0 mA current.

Procedure

Punished stepdown response training was carried out in one session for each rat. The session began by placing the rat on the narrow platform and was terminated when the rat remained on the platform for a criterion period of 120 consecutive sec. During this session the grid-floor was electrified. Dependent variables included latency to the first descent, number of descents before reaching criterion and total time to criterion. Four hours following acquisition training each rat was randomly assigned to either the PCPA (n=9) or control (n=9) group and intubated as described in Experiment 1.

Following training and intubation, each rat was given five test sessions during which the grid was not electrified. These sessions occurred, 2, 3, 4, 7, and 8 days after training. The dependent variable during these sessions was latency to the first descent; each session was terminated when the rat either failed to step down for 180 sec or remained on the grid for 20 consecutive secs. This 20-sec criterion was used to minimize any possible effects of handling on the step-down response.

Maximum serotonin depletion occurs 72 hr after intubation [17]. Thus, the treatment and control groups were not expected to differ in their latencies to the first descent during the first test session (approximately 48 hr after PCPA). A group difference might be expected by test sessions 2 (72 hr after PCPA) and 3 (96 hr after PCPA) and might also be expected to begin to disappear by test sessions 4 or 5 (168

and 192 hr after PCPA) since serotonin levels would almost be recovered by that time [17].

RESULTS

For the pre-drug training session, the mean (± SEM) latencies to the first descent for the PCPA and control groups were 7.0 (± 2.2) and 5.0 (± 1.5) sec., respectively, and did not differ significantly, $t(17)=0.75$, $p>0.05$. The mean number of descents before reaching criterion was 1.78 (± 0.2) for the PCPA group and 2.33 (± 0.2) for the control group and the mean time to criterion was 232.8 (± 14.3) and 228.6 (± 15.3) sec, respectively. The difference between each of these pairs of means was not significant, $t(17)=2.0$, $p>0.05$ and $t(17)=0.02$, $p>0.05$, respectively.

The median latencies to the first descent for each group for each post-drug test session are shown in Fig. 3. The distribution of latencies was skewed for each group as can be seen from Table 1; the relatively small numbers of rats falling into the 60 to 120 sec interval suggests that in this task the rats tended to either remain on the platform for most of the criterion 180-sec test period or step down in the first 60 sec. Mann-Whitney U Tests revealed that the median latency of the PCPA group was longer than that of the control group on test sessions 3 and 4, $U(9,9)=21$, $p<0.05$ and $U(9,9)=17.5$, $p<0.05$, if a directional null hypothesis (i.e., a one-tailed test) is used. On the basis of the results of Experiments 1 and 2 we were expecting extinction of the punished stepdown response to take longer in the PCPA group, especially because Experiment 2 seemed to rule out an interpretation based on changes in general activity; therefore, the one-tailed tests were used. The median latencies of the two groups did not differ significantly in test session one, $U(9,9)=29$, $p>0.05$, two $U(9,9)=28.5$, $p>0.05$, or five, $U(9,9)=24$, $p>0.05$.

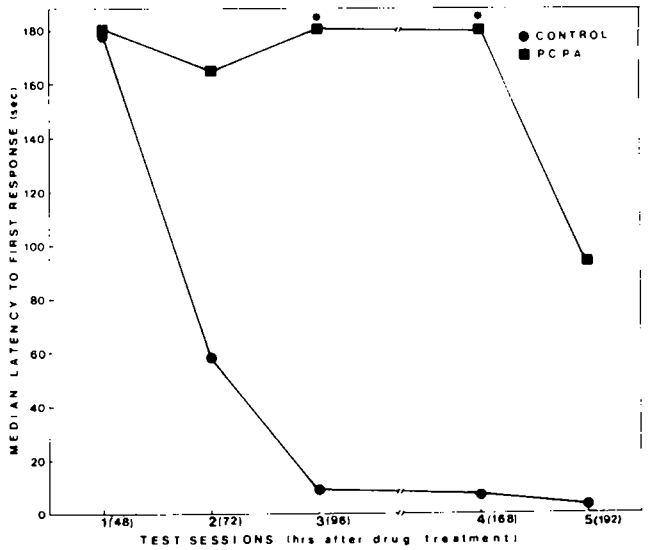

FIG. 3. Median latencies to the first descent of PCPA (n=9) and control (n=9) groups during each of five test sessions following punishment of the stepdown response in Experiment 3. The numbers in parenthesis indicate the number of hours elapsed since PCPA treatment for that session; asterisks indicate significant ($p<0.05$) group differences for these sessions.

TABLE 1

NUMBER OF RATS FROM THE PCPA AND CONTROL GROUPS OF EXPERIMENT 3 THAT STEPPED DOWN IN THE FIRST, SECOND, AND THIRD MINUTE OF EACH TEST SESSION

Test Session	Latency to Stepdown in 60-Sec Class Intervals		
	0–60	60–120	120–180
	PCPA Group		
1	–	1	8
2	3	–	6
3	3	1	5
4	4	–	5
5	4	1	4
	Control Group		
1	–	2	7
2	5	1	3
3	6	1	2
4	7	1	1
5	7	–	2

GENERAL DISCUSSION

The results of Experiment 1 showed that PCPA-treated rats made more lever-presses in extinction than did control rats and that this increased responding was probably not related to a general elevation in activity. Experiment 2 showed that there was no significant effect of PCPA treatment on extinction following RI training; this observation provided further evidence that PCPA did not produce some general increase in activity. Experiment 3 also contradicted the possibility of a general change in activity since PCPA-treated rats remained on the narrow platform longer than control rats. These observations are in good agreement with a recent report that PCPA-treatment does not alter activity [7].

These effects cannot be attributed to state dependent learning [14]. This theory treats the drug state as an additional stimulus condition that can effect the animal's behavior. Accordingly, animals trained on an appetitive task in a nondrugged state and then given extinction testing while drugged should respond *less* than nondrugged controls because of enhanced generalization decrement. That is, the extinction conditions plus drug stimuli are more different from training conditions than the extinction conditions alone. The observation of *increased* responding by the PCPA group in extinction after CRF training is opposite to the predictions that would be made by theories of state dependent learning. Similarly, theories of state dependent learning would predict more rapid recovery from punishment in animals trained in a nondrug state and then tested in a drug state; the results of Experiment 3 failed to confirm this prediction.

Some of the effects observed in serotonin-depleted animals appear similar to those reported for norepinephrine-depleted animals or animals with lesions of the dorsal noradrenergic bundle. For example, responding in extinction after CRF training is enhanced in norepinephrine-depleted animals [13,16]. Mason and Iverson [13] have shown that this effect was due to the loss of central norepinephrine. Responding during extinction after partial reinforcement is un-

changed by dorsal bundle lesions [10]. Dorsal bundle lesioned animals also show slower recovery of a punished stepdown response [11]. In all of these studies, however, norepinephrine depletion preceded training whereas in the experiments reported here, serotonin depletion occurred following training and before extinction tests. Mason and Fibiger [12] recently have reported that enhanced responding during extinction after continuous reinforcement fails to occur in animals trained while intact and then lesioned and tested. From these data it appears that serotonin depletion produces its effects on extinction via a different mechanism than lesions of the dorsal bundle.

Solomon *et al.* [15] have suggested that serotonin neurons play a role in "tuning out" nonreinforced or irrelevant stimuli. This conclusion was based on the observation that PCPA-treated animals failed to show latent inhibition; in nondrugged animals, preexposure to a tone retarded acquisition of a two-way avoidance response whereas PCPA-treated rats given the same preexposure failed to show this effect. Extinction also can be viewed as a similar process of "tuning out" stimuli that previously signalled reinforcement or punishment. The index of this tuning out process would be a decrease in previously reinforced responding or an increase in previously punished responses. According to this line of reasoning a reduction in serotonergic activity would prolong the influences of salient sensory stimuli on behavior.

Although it is difficult to specify the precise nature of the stimuli which signalled reinforcement or nonreinforcement in the extinction paradigm, the results of Experiment 2 indicate that prior experience with non-reward can offset the effect of reduced levels of serotonin on resistance to extinction. This, in turn, would suggest that once an intact animal has formed the appropriate associations between stimuli that predict reinforcement and nonreinforcement, it can utilize the relevant stimuli to modify behavior during extinction despite a substantial disruption of serotonergic activity. If this admittedly speculative analysis should prove to be correct, serotonin would appear to serve an important role in information processing, particularly as it relates to stimulus-response associations.

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