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Effects of Parachlorophenylalanine and Amphetamine on Habituation of Orienting

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FILE, S. E. *Effects of parachlorophenylalanine and amphetamine on habituation of orienting.* PHARMAC. BIOCHEM. BEHAV. 3(6) 979–983, 1975. – Parachlorophenylalanine significantly reduced the orienting response to the first presentation of a tone but did not alter the size of responses to subsequent tone presentations nor the rate of habituation. In contrast amphetamine did not alter the orienting response but significantly impaired habituation. It was concluded that there was little evidence for a serotonergic involvement in behavioral habituation, although a role for the catecholamine system could not be excluded.

Habituation Orienting PCPA Amphetamine

SEVERAL studies [4,15] have indicated that a reduction in brain levels of 5-hydroxytryptamine (serotonin: 5-HT) is accompanied by a sensitivity to sensory stimulation. It has also been suggested that 5-HT is involved in the modulation of behavioral habituation [2]. However, the evidence to support this is not convincing and should be subject to further examination.

Rats treated with parachlorophenylalanine (PCPA), a depletor of brain serotonin [12], had a lower startle response to the first stimulus presented each day, followed by a period of increased startle amplitude, but habituation of this response was not prevented [3]. Carlton and Advokat [2] also found a lower startle amplitude for the first few stimulus presentations, followed by a period of enhanced responsivity. No habituation was found in the PCPA treated rats after 30 trials, but this is too few trials to conclude that habituation of startle was prevented. A third study [5] found that PCPA did not affect startle amplitude or the rate of habituation.

Results from lesion studies do not support a serotonergic role in startle habituation. No difference was found in startle amplitude or the rate of habituation between medial forebrain lesioned rats (with a 58 percent depletion of 5-HT) and control animals [20]. Similarly the size of startle to the first stimulus was unaffected by lesions of the raphe nucleus (resulting in about 50 percent depletion of 5-HT) and the rate of habituation was normal [4]. However, the lesioned group did show an enhanced sensitization effect to the stimulus repetition and Davis and Sheard [4] suggested that independent neural systems underly habituation and sensitization and that 5-HT is only involved in the latter.

These experiments have all investigated habituation of startle responses and it is possible that the effects of PCPA were influenced by the aversive nature of the stimulus. Certainly the only study finding an impairment in habituation following PCPA used a relatively low intensity stimulus, although the dB level was not reported. Experiment 1 was designed to extend investigations of PCPA to the orienting response and its habituation. A distraction measure of orienting was used as in previous studies [7], distraction being measured by the interruption in base-line activity, the justification for this being that one of the characteristics of the orienting response is that it involves cessation of other activities [17].

It has been suggested [11] that the catecholamine system is involved in the exploration of novel objects but this suggestion has been based on changes in arousal and spontaneous activity produced, for example, by amphetamine. While there may be an interdependence between orienting and exploration and states of arousal this does not mean that the latter can be treated as synonymous with the former. Supporting this distinction is the result that while amphetamine (1 mg/kg) and amylobarbitone (20 mg/kg) increased and decreased general activity respectively they did not alter the amplitude of the startle response. However, there was some evidence of an augmented startle response at 3 mg/kg (+) -amphetamine [10]. Also in support of a distinction between activity and exploration are Kumar's results [13,14] that whereas both (+) -amphetamine (0.25, 2.0 mg/kg) and amylobarbitone (7.5 mg/kg) increased locomotor activity, trough exploration was decreased by the former and unchanged by the latter drug.

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The decrease in motor activity over trials was also impaired by amphetamine [14]. This may suggest that amphetamine would also impair habituation of orienting responses, if Berlyne's [1] classification of orienting, locomotor activity and investigation responses as all being categories of exploratory behavior is accepted. Experiment 2 extended investigation of the effects of amphetamine to the orienting response and its habituation, since different mechanisms may be involved in the production of orienting and startle and/or their subsequent habituation [7].

EXPERIMENT 1

METHOD

Animals

Thirty female hooded Lister rats, supplied by the Medical Research Council, Mill Hill, and 24 male hooded Lister rats, supplied by Olac (Southern) Limited, Bicester, were used. All rats weighed 300–350 g at the start of the experiment. They were housed in groups of 6 per cage in an 11 hr light, 13 hr dark cycle at 25°C. Food was available ad lib but water was available only in the test chamber or immediately following an experimental session, in sufficient quantity to maintain a constant body weight throughout the experiment.

Apparatus

The test chamber was 19 cm high with a grid floor 19 × 26.5 cm and was enclosed in an acoustically insulated box. A slit in the end wall gave access to a water spout and a drinkometer recorded the rat's licking. Experimental events were automatically programmed using standard transistor-transistor logic. The source of tones was a Weinbridge oscillator which fed into an RS components 5 W audio amplifier and then to a loudspeaker, (RS components 8 ohm Tweeter) positioned in the lid of the chamber at the water spout end. The tones used were 9 and 7 KHz, 75 dB (re 0.0002 dynes/cm^2) for 9 sec.

A standard black plastic cage 129 cm square and 12 cm high, was adapted for measuring spontaneous activity by placing 9 touch sensitive plates, 5.5 cm square, in the floor. The output from the amplifier on each plate was connected to an individual counter.

Procedure

On Day 1 of the experiment 16 females and 12 males received 200 mg/kg IP injection of PCPA (methyl ester). The control animals, 14 females and 12 males received equal volume injections of saline. On Day 2 the injections were repeated, bringing the total dose to 400 mg/kg PCPA. On Day 3 all animals were water deprived for a period of 48 hr. On Day 5 onwards all animals received water in the experimental chamber and immediately afterwards, in sufficient quantity to maintain a constant body weight. On Day 5 all animals were given 10 min in the chamber or until they had made at least 1,000 licks. Testing took place 4 and 5 days after the final injection because at this time it was expected that the whole brain 5-HT levels were depleted to about 35 percent of normal whereas the noradrenaline levels had recovered to about 80 percent of normal levels [16].

On Day 6 each animal was placed in the activity cage and its activity recorded for 10 min prior to being placed in the test chamber. When each animal was placed in the test chamber, its 100th lick switched on a control period of 9 sec in which the number of licks made (A) was counted. After this period the next 100th lick switched on a tone stimulus for 9 sec and the number of licks made during this tone presentation (B) was recorded. A ratio of $\frac{A-B}{A}$ was calculated and if this was ≤ 0.10, i.e. if the animal did not distract to the tone, then the animal's next 100th lick restarted the previous circuit. In this case the number of licks occurring during a control and a tone period was again scored. The animal was considered to have reached habituation criterion when two successive tone presentations produced ratios of ≤ 0.10, a criterion established in previous experiments [5]. If the ratio of $\frac{A-B}{A}$ was >0.10 the animal's 100th lick switched on a second circuit which presented a 9 sec tone stimulus at 1 min intervals. After 3 automatic presentations the first circuit was activated and again the number of licks made in a control period was recorded and then the number made during the tone presentation. In this way if the ratio did not reach criterion level the distraction to every fourth stimulus presentation was recorded. The session was terminated when habituation criterion was reached or if no records of licking could be obtained over a period of 1 min. Animals not reaching criterion on Day 6 continued with tone presentations in the same way on Day 7. Testing was terminated after 30 presentations if habituation criterion had not been reached.

In order to assess the specificity of habituation under PCPA and to compare this with the specificity found in previous experiments [8] the male rats received a second phase in the experiment. When they had reached habituation criterion to the first tone presented (9 KHz) the frequency of the tone was changed. Thus the PCPA and saline injected males all received 3 presentations of a 7 KHz tone, and the number of licks made in control periods and during the tone presentations were recorded.

RESULTS

Table 1 shows the mean distraction ratios ($\frac{A-B}{A}$) to the first tone presentation, for each of the 4 groups tested. The saline male rats showed a significantly higher distraction to this tone than did the PCPA treated males ($t = 2.2$, $d.f. = 22$, $p<0.025$). Similarly the mean distraction for saline female rats was significantly greater than that for the PCPA treated females ($t = 1.88$, $d.f. = 28$, $p<0.05$) and thus certainly PCPA lowered the initial orienting response. Table 1 also shows the mean number of trials to reach habituation criterion to the tone, for all 4 groups tested.

However, neither the male nor the female rats differed significantly from the controls in their rate of habituation (Mann-Whitney U, $p>0.05$). So. although PCPA reduced the initial orienting response, it did not impair the subsequent habituation. Further, the reduction in the size of the initial distraction was not followed by a generally lowered orienting response from the PCPA treated animals. Two of the PCPA treated males and 3 of the females that failed to distract to the first tone, produced a sizeable distraction to the second stimulus. Thus, it seems that PCPA has only a short-term effect on lowering the very first orienting response but, thereafter, does not affect orienting or its subsequent habituation.

TABLE 1

EFFECTS OF PCPA ON INITIAL DISTRACTION AND HABITUATION

		Initial Distraction	Trials to Habituate
Males	PCPA	0.25 ± 0.07	11.75
	Saline	0.51 ± 0.08	14.67
Females	PCPA	0.36 ± 0.09	15.06
	Saline	0.60 ± 0.08	12.64

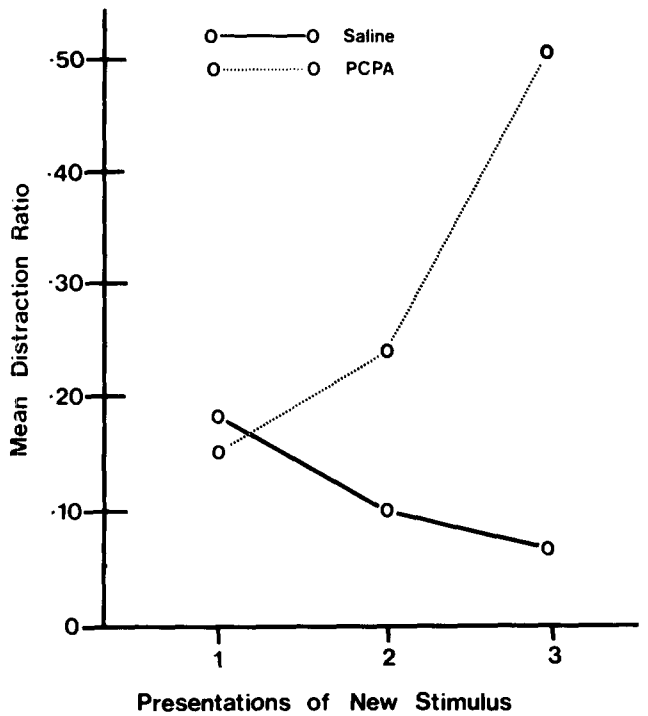

FIG. 1. Dishabituation to stimulus change.

It is possible that while the PCPA treated animals were able to habituate to the tone the specificity of their habituation would be poorer than that of the controls. It has been found [8] that 8/12 undrugged rats distracted to a change in tone frequency from 9 to 7 KHz, the mean distraction being 0.21 and the rats requiring a mean of 2.8 trials to habituate to the new frequency. Figure 1 shows the mean distraction ratio for the saline males, to each of the 3 presentations of the 7 KHz tone. From this it can be seen that the mean distraction to the stimulus change was close to that found previously, and 7/11 rats distracted (i.e. had ratios >0.10). Thus the results from the saline rats are very similar to those from previous experiments. The mean distraction to the changed stimulus was 0.15 for the PCPA animals, but this value was heavily influenced by one rat with a distraction ratio of 0.90. Without this animal the group mean would have been 0.08. However, this lower response to the stimulus change was followed by a significant increase in orienting to the 7 KHz tone on its two subsequent presentations ($Z = 1.87$, $p = 0.03$, non-parametric trend test [6]). In contrast the saline animals showed a significant decrease in distraction to the new tone (Wilcoxon $T = 2.5$, $N = 8$, $p<0.05$). Thus once more the initial orienting of the PCPA animals was reduced but this effect did not persist through subsequent tone presentations and the PCPA animals showed a greater dishabituation to the change in tone.

Finally, the measurements of spontaneous activity, prior to exposure to tones, revealed no differences due to PCPA treatment. The PCPA males had a mean count of 112.8 and the saline males a mean of 128; the PCPA females a mean of 103.1 and the saline females a mean of 112. Thus any differences in initial distraction to the tone cannot be attributed to different levels of spontaneous activity, and hence probability of pausing from licking, between the drugged and undrugged animals.

EXPERIMENT 2

METHOD

Animals

Thirty male hooded Lister rats, supplied by Olac (Southern) Limited, and housed as in Experiment 1.

Apparatus

Both the test chamber and the activity cage were those used in Experiment 1.

Procedure

All animals received a period of 48 hr water deprivation and then on Day 1 of the experiment were placed in the test chamber for 10 min or until they had made 1,000 licks. On Day 2 the rats were randomly assigned to 3 groups, with 10 in each group. One group received IP saline injections 25 min before being placed in the test chamber, the second group received 2 mg/kg (±)-amphetamine and the third 4 mg/kg (±)-amphetamine. Fifteen min after injection each rat was placed in the activity cage and its activity recorded for 10 min. The rat was then placed in the test chamber and given a series of tone presentations as in Experiment 1. However, this pattern of tone presentations had to be abandoned for several of the 4 mg/kg group which either failed to lick at all, or failed to start licking again after the first tone presentation. When it was necessary animals in this group were given 30 tone presentations at intervals of 1 min. Animals that had not reached criterion on Day 2 were replaced in the chamber and tone presentations continued on Day 3.

When habituation criterion had been reached, or thirty stimulus presentations had been given, the rats entered the second phase of the test. The following day they were placed in the test chamber and tone presentations given as in Experiment 1, but they were now tested in a different drug state from the first phase of the experiment. Animals first habituated undrugged were now tested after injection with 2 mg/kg amphetamine and animals habituated under either of the amphetamine doses were now tested undrugged. Tone presentations continued until habituation criterion was again reached.

TABLE 2

EFFECTS OF AMPHETAMINE ON INITIAL DISTRACTION AND HABITUATION

	N	Initial Distraction	No. at Criterion After 30 Trials
Saline	10	0.52 ± 0.09	10
Amphetamine			
2 mg/kg	9	0.44 ± 0.12	4
4 mg/kg	6	0.42 ± 0.15	3
State change to saline			
(after 2 mg/kg)	10	0.46 ± 0.09	10
(after 4 mg/kg)	10	0.43 ± 0.11	10
State change to amphetamine			
2 mg/kg	10	0.24 ± 0.06	7

RESULTS

Since the amphetamine caused sufficient adipsia completely to stop some of the rats from drinking it could have also caused alterations in the baseline licking rate. Although this is to some extent accounted for in the use of a ratio to assess distraction, nevertheless interpretations of the effect of amphetamine would be altered if the baseline were affected. The mean number of licks made in the control period (A) was 45.5 for the saline control animals, 45.0 for the 4 mg/kg amphetamine animals and 41.2 for the 2 mg/kg amphetamine animals. Thus, although amphetamine totally prevented licking in some animals, those that continued did so at the same rate as the control animals.

Table 2 shows the mean distraction ratios to the first stimulus presentation for all 3 groups, and also the number of animals contributing to that score in each group. There was no significant difference between the ratios for the saline and the 2 mg/kg amphetamine groups ($t = 0.55$, $d.f. = 17$, $p > 0.05$). Table 2 also shows the number of animals at criterion after 30 trials, because of the large number not habituating by this time a value for the mean trials to habituate for the drug groups could not be computed. Significantly fewer of the 2 mg/kg amphetamine group had reached habituation than the saline group (Fisher-Yates, $p = 0.01$). The mean trials to habituate for the saline group was 14.1 which is very similar to the rate of habituation of the male rats in Experiment 1.

Table 2 also gives the initial distraction to the first tone presentation after the rats had changed state. For the rats previously habituated under amphetamine, but now tested undrugged, the initial distraction ratios were lower than for the rats tested undrugged in the first habituation series, but this failed to reach significance ($t = 0.65$, $d.f. = 18$, $p > 0.05$ comparing saline with saline after 4 mg/kg amphetamine). It is not possible to interpret these results in terms of transfer between states because of the small number of rats reaching habituation criterion under amphetamine. The number of rats failing to lick under 4 mg/kg amphetamine also prevented any testing of a state-constant group i.e. animals habituated under 4 mg/kg and then retested in the same state. More marked was the transfer of habituation from the saline condition to the drugged state of 2 mg/kg amphetamine, although this just failed to reach significance ($t = 1.53$, $d.f. = 17$, $p < 0.10$, comparing 2 mg/kg amphetamine with this dose following saline). A saline-saline state constant group was not run in this experiment, but from previous studies [5] it is known that once two successive criterion trials are reached undrugged rats remain at criterion on subsequent test trials. The results of this experiment therefore suggest that a certain amount of dissociation took place between the undrugged and drugged state.

The results of this experiment suggest that amphetamine does not alter the size of the orienting response but does significantly retard the rate of habituation. Amphetamine also significantly increased the amount of spontaneous activity from a saline level of 123.4 to 162.4 with 2 mg/kg and 150.3 with 4 mg/kg ($U = 26$ and 27 respectively $p = 0.05$).

DISCUSSION

Both male and female rats were tested in Experiment 1 because of observations of the difference in their general behavior following treatment with PCPA. Male rats become typically jumpy, aggressive and hypersexual [20], whereas females become extremely docile, and these differences in general behavior were also noticed in this experiment. In

spite of these differences the results show that in the test situation their behavior was identical with a reduction in orienting to the first stimulus presentation, but thereafter no significant differences from saline injected controls. These results support Davis' conclusions [4] that serotonin is not involved in habituation, and extend the evidence to the orienting response. Davis found that PCPA led to an increased sensitization, this being a process independent of habituation, leading to an increase in responsivity to the 2nd and a few subsequent presentations of high intensity stimuli. In Experiment 1 the distraction ratios showed gradual decrement throughout the test session and there was no evidence of sensitization occurring in this test situation. The PCPA treated animals were more dishabituated by the stimulus change (after its first presentation) and this is in agreement with the effects of PCPA on dishabituation of startle [9]. If it is accepted that dishabituation is a form of sensitization [9] then the results of Experiment 1 might support a role of serotonin in sensitization.

Amphetamine increased the level of spontaneous activity and this may have been another reason for failing to obtain steady licking in the 4 mg/kg group. The results of Experiment 2 showed that amphetamine did not alter the size of the initial distraction ratio, but it is not certain that this can be interpreted as a reflection of orienting in the drugged animals. Several of the 4 mg/kg group moved back suddenly to the tone and this response may have been indicative of startle. If this was so it would be interesting to explore the mechanisms through which a stimulus normally eliciting only orienting could come to elicit a startle response in amphetamine injected animals. Animals with the 2 mg/kg dose did not seem to produce this exaggerated response and it is more likely that their response reflected orienting. The main effect of amphetamine was to retard the rate of habituation so that few animals had reached criterion after 30 trials. However, if it was really habituation of startle that was being monitored in the drugged animals 30 is too few trials to conclude that startle habituation is impaired. The performance of the saline controls in Experiment 2 was very similar, both in initial distraction and trials to habituate, to the saline controls in Experiment 1 and therefore the results from amphetamine can in no way be attributed to differences in the batches of rats.

In conclusion there is no evidence from the results of these experiments and little from those on the startle response to support the suggestion [2] that the serotonergic system is involved in behavioral habituation, although the involvement of the catecholamine system cannot be excluded.

It has been suggested [19] that the cholinergic system is involved in habituation of exploratory responses but not in habituation of startle responses. The role of the cholinergic system has yet to be assessed in habituation of distraction responses, as used in these experiments, and therefore at this stage its involvement cannot be excluded.

In view of the complexity of the CNS, suggestions that one particular transmitter is involved in habituation are unlikely to be supported. It is unlikely that only one transmitter will be involved and even less likely that changes in whole brain levels will be crucial. At the very least the critical neuroanatomical site or sites should be specified, but even then depletion of, say, serotonin at all synapses is unlikely to have a uniform effect.

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