Pharmacology Biochemistry & Behavior, Vol. 6, pp. 151–156.

Effects of Parachlorophenylalanine and Amphetamine on Habituation of Exploration[1,2]

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(Received 15 September 1976)

FILE, S. E. *Effects of parachlorophenylalanine and amphetamine on habituation of exploration.* PHARMAC. BIOCHEM. BEHAV. 6(2) 151–156, 1977. – Parachlorophenylalanine (PCPA, 400 mg/kg) retarded the habituation of exploration in the rat recorded over 3 successive days. A more detailed analysis showed that this was not due to any retention deficit, but was secondary to an impairment of habituation within each session. PCPA also disrupted the response of rats to a change of stimuli. (+)amphetamine (4 mg/kg) prevented exploration in the rat and a 2 mg/kg dose reduced exploration and impaired within-session habituation. (+)amphetamine (1 mg/kg) in mice impaired within-session habituation, without significantly reducing exploration. The nature of the deficits produced by these 2 drugs is discussed.

Habituation Exploration PCPA (+)amphetamine Stimulus analysis

HABITUATION is the decrement of an unconditioned response to repeated stimulus presentations. In studies of behavioural habituation three types of responses have been used – startle, distraction and exploration. It has been suggested [20] that different neuropharmacological mechanisms may be involved in the habituation of different response systems. However, it is possible that any differences that are found can be attributed to differences in stimulus analysis. Both distraction and exploration have been considered as measures of orienting [1, 9, 13] but these two situations differ both in the responses involved and in the extent to which stimulus presentations are under the experimenter's control. It is therefore possible that drugs will affect habituation differently in the two situations, even though both are considered to be measures of a single process – orienting.

Williams *et al.* [20] suggested that central muscarinic cholinergic mechanisms are involved in the habituation of exploration. However, recent studies have thrown doubt on the role of a cholinergic system in the habituation of exploration [9], and one purpose of this experiment was to re-examine the role of serotonin in habituation of exploration. Its involvement might be indicated by studies showing habituation of exploratory responses in preweanling rats from Day 15 [2,18] a time at which the serotonergic system is functional [16] but before the cholinergic system has fully matured [3].

Parachlorophenylalanine (PCPA), a depletor of serotonin [14] has been reported to reduce the distraction response to the first stimulus presentation, but not to affect distraction to subsequent presentations nor the rate of habituation; PCPA also disrupted the distraction to a change in auditory stimuli [8]. Similarly PCPA causes an increase in startle response to presentations of the startle stimulus immediately following the introduction of a novel stimulus [5]. (+)Amphetamine was found to significantly retard habituation of orienting, measured by distraction responses [8]. In the present study the effects of these two drugs on habituation of exploration were investigated using a holeboard apparatus [12]. Two extra days with new stimulus objects were included in the test for PCPA rats to determine their response to stimulus change.

METHOD

Animals

Male hooded rats (supplied by Olac Ltd, Bicester) 300–350 g were housed in groups of six and male mice of Tuck No. 1 strain, 30 g in weight, were housed in groups of ten. All animals were kept in an 11-hr light–13-hr dark cycle (lights on at 08.00 hr), in rooms maintained at a constant temperature of 25° C. They were allowed food and water ad lib.

[1] This work was supported by a Science Research Council grant to Dr. M. J. Neal.

[2] The PCPA injected rats displayed no increased aggression, which may have been because all the animals were thoroughly handled and were very tame before any injections were given.

Drugs

Twenty-five rats to be tested in the holeboard were pretreated with p-chloro-DL-phenylalanine (methyl ester HCL, from Koch-Light Laboratories Ltd) by injecting 200 mg/kg PCPA IP on two successive days. An additional six animals, to be used in the biochemical assay, were similarly pretreated . The drug was dissolved in saline to a concentration of 100 mg/ml. Twenty-four control rats received equal volume injections of saline. Two clear days were left after the second PCPA injection before testing began, in order to obtain whole brain serotonin levels depleted to about 35% of normal, but norepinephrine levels recovered to about 80% of normal [17]. Levels of 5HT were assayed on the first, third and fifth day of testing to provide a measure of depletion in this experiment.

(+)Amphetamine sulphate (Sigma Chemical Co.) was dissolved in saline to a concentration of 1 mg/ml for the rat injections and a concentration of 0.05 mg/ml for the mice.

Apparatus

Rats were tested in a holeboard [12] which was a wooden box with walls 45 cm high and a floor 55 × 55 cm. In the floor were four equally spaced holes, 3.8 cm in diameter. The floor of the holeboard was 12 cm above the base of the box and when objects were placed under the holes they came to 2 cm below the top of the holes. Mice were tested in a holeboard 27 cm high and a floor 40 × 40 cm. The holes were 3 cm in diameter. All the objects were supported below the floor by glass funnels and were designed to smell and feel different from each other. The objects used were a brass rod, a rolled piece of suede, a rubber bung and a cork. When the objects were changed in the PCPA experiment the second set of objects were matches, plasticene, an eyebrow brush and a copper fork.

The illuminance on the floor of the holeboard was 25 scotopic lux, measured with a photocell calibrated with respect to the C.I.E. scotopic curve and to the C.I.E. standard radiator.

Procedure

Since the time of day affects the level of head-dipping [11] rats were tested only between 10.00 and 12.00 hr with the order of testing randomised between the drug and control animals, and the mice were tested only between 14.00 and 16.00 hr.

Twelve PCPA-treated and 12 control rats received three ten-minute trials in the holeboard, in the absence of objects, each trial separated by 24 hr. Similarly, a further 12 PCPA and 12 control rats received three trials in the presence of objects, and then an additional two trials with a new set of objects. An additional PCPA rat was tested in the holeboard on Day 1 and then sacrificed for 5-HT determination, and one of the rats tested for 5 days was killed after testing on Day 5. The other 6 PCPA-treated rats were not tested in the holeboard but 3 were killed on the equivalent of Day 1 of testing and 3 on Day 3.

Thirty rats were randomly allocated ten each to saline, (+)amphetamine (2 mg/kg) and (+)amphetamine (4 mg/kg) groups. They received four 10-min trials in the holeboard, in the presence of objects, separated by 24 hr. Similarly 30 mice were randomly allocated ten each to saline, (+)amphetamine (0.5 mg/kg) and (+)amphetamine (1 mg/kg) groups and received two 10-min trials in the holeboard, in the presence of objects, separated by 24 hr. All injections were given IP 30 min before testing.

Each animal was placed in the centre of the holeboard and its behaviour was observed on a monitor in an adjacent room. A head-dip was defined as when the animal reached into the hole at least as far as its ears. The frequency of head-dips (number/10 min) and the duration of each dip (to the nearest sec) were scored. The number of rears made was also counted. The scoring was divided into four 2.5 min periods to give a measure of within-session habituation. At the end of each trial any boluses were removed and the floor was wiped with water and dried, to minimise any traces of the path taken.

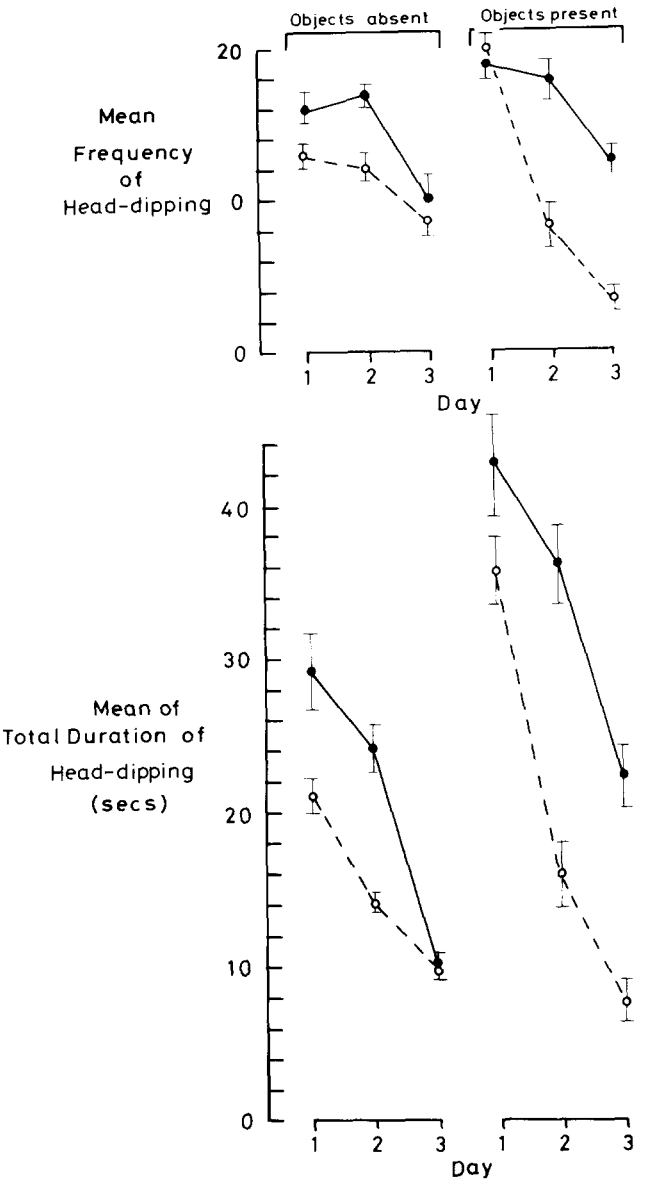

FIG. 1. Between-session habituation in the absence of objects and in their presence. Frequency = no. of head-dips/10 min. Duration is the mean total time spent head-dipping during each 10 min test session. Each point is the mean from 12 rats. ○----○ controls ●——● PCPA (400 mg/kg).

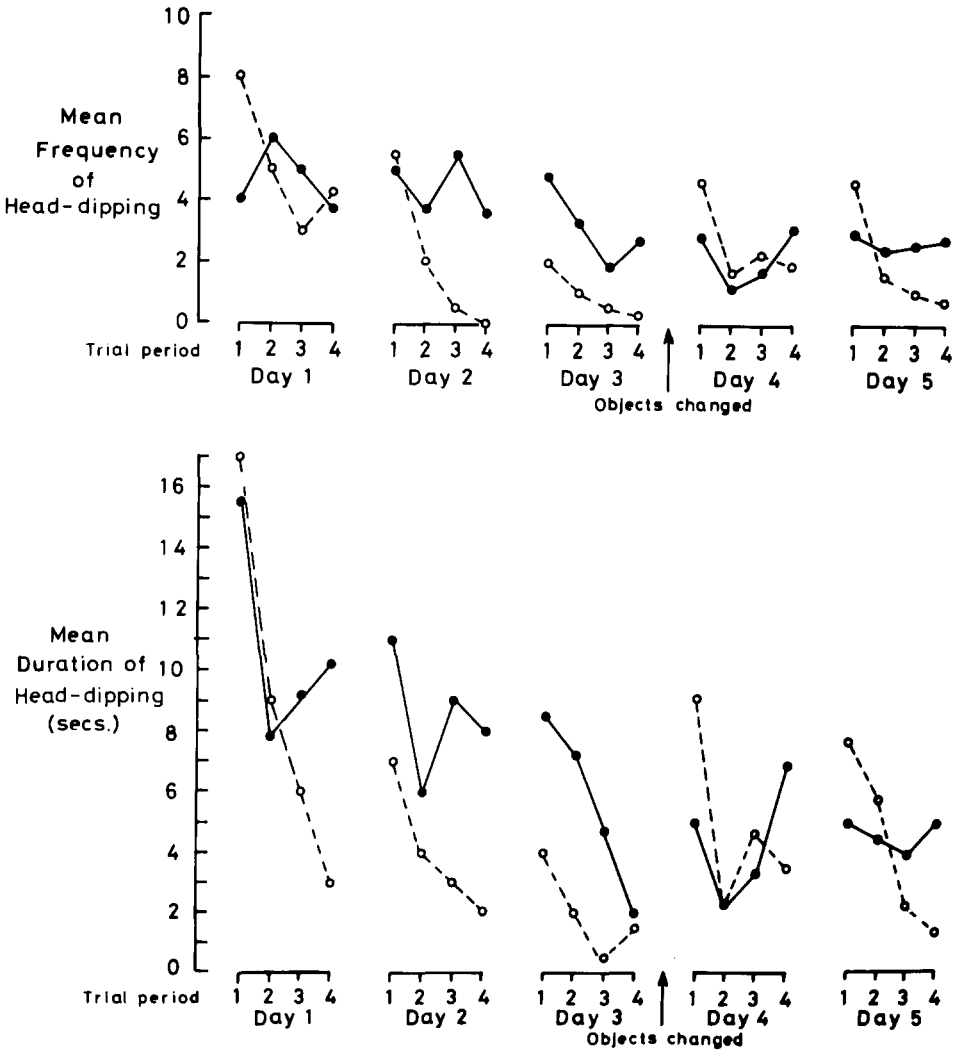

FIG. 2. Within-session habituation in the presence of objects. Frequency = no. of head-dips/2.5 min. Duration is the mean time spent head-dipping during a 2.5 min period. o------o controls ●————● PCPA (400 mg/kg).

5HT Assay

On the first, third and fifth day of testing, 5HT determinations were made. A control brain was assayed on each day. The rats were stunned with a blow to the head, their necks broken and the brains quickly dissected out. The extraction and estimation of 5HT followed the method of Curzon and Green [6]. Known amounts of 5-HT were added to the homogenate and these internal standards were run to correct for recovery.

RESULTS

Statistics

The results were subjected to a 3-way split-plot analysis of variance. The between subjects factor (drug) was at two levels (PCPA and control), and there were two within-animals factors, days (at 3 levels) and trial periods (at 4 levels). A significant drug × days interaction would indicate a drug-induced impairment of between-session habituation; and a significant drug × trial periods interaction would indicate an impairment of within-session habituation.

Effects of PCPA

Figure 1 shows the pattern of between-session habituation for the PCPA and control rats. When rats were tested in the absence of objects both the frequency and the duration of head-dipping was increased by PCPA ($F(1,22) = 4.01$ and 4.77 respectively, $p<0.05$), but PCPA did not significantly retard the rate of between-session habituation ($F(2,44) = 2.60$ and 2.40 for frequency and duration, $p>0.05$). All rats showed significant habituation of head-dipping ($F(2,44) = 17.08$ and 22.98 for frequency and duration, $p<0.001$). When rats were tested in the presence of objects the PCPA animals showed a significantly slower decline in head-dipping over days, shown by the days × drug interaction ($F(2,44) = 19.58$ and $F = 5.57$ for frequency and duration, $p<0.001$ and $p<0.01$ respectively). PCPA did not significantly alter the number of rears made. On Day 1 the control rats made a mean of 30.0 ± 2.8 rears in the absence of objects and 22.0 ± 3.7 rears in the presence of objects, the scores for the PCPA rats were 28.0 ± 2.9 and 17.0 ± 2.1 respectively.

Figure 2 shows the pattern of within-session habituation

for the PCPA and control rats tested in the presence of objects. On Day 1 the control animals showed a significant linear trend over the four trial periods in both the frequency and the duration of head-dipping (F (1,36) = 15.6 & 18.38 respectively, $p<0.001$), but the PCPA animals showed no significant linear or quadratic trend. Similarly, on Days 2 and 3 the controls showed a within-session linear trend in the frequency and duration of head-dipping (F (1,36) = 11.66 and 7.5 on Day 2, $p<0.01$; F = 5.10 and 5.01, $p<0.05$ on Day 3) whereas the PCPA rats showed no significant trends until Day 3 when the frequency and duration of head-dips showed linear decreases (F (1,36) = 5.04 and 5.18, $p<0.05$). Thus the typical pattern in the control animals was a within-session decrease in head dipping, followed by some, but not complete, spontaneous recovery overnight. The PCPA rats showed a different pattern with less within-session decrease in head-dipping, and for the duration of head-dipping less spontaneous recovery overnight. Thus the slower between-session habituation shown by the group of PCPA rats cannot be due to an impaired ability to retain the information over a 24 hr period, but is more a reflection of an impaired within-session habituation. When the objects were changed on Day 4 the control rats showed a significant rise in the duration of head-dipping from their Day 3 level ($t(11)$ = 2.08 $p<0.05$), but the PCPA animals did not show an increase ($t(11)$ = 0.92, $p>0.05$). The control animals again showed linear decreased in head-dipping within trials on Days 4 and 5, but the PCPA rats showed no significant trends.

5HT levels

The PCPA rat tested in the holeboard on the first day of testing and then sacrificed had a mean whole brain 5HT level of 0.204 μg/g, giving a depletion to 27% of the control brain level. The other PCPA animals sacrificed at the same time after injection had a mean whole brain level of 0.222 ± 0.004 μg/g, compared with a control level of 0.760 μg/g, giving a depletion to 29% of the control level. The PCPA rats killed 5 days after injection (i.e. Day 3 of testing) had a mean 5-HT level of 0.250 ± 0.005 μg/g, the control level was 0.756 μg/g, thus the depletion was down to 33% of the control level. The PCPA rat tested for 5 days & then sacrificed had a 5-HT level of 0.381 g/g compared with a control level of 0.760, a 50% depletion. The recoveries of 5-HT averaged 59%. Recoveries of 5-HT are typically lower than those of catecholamines and in the routine assays conducted in our laboratory the range of recoveries is 55–69%.

Effects of (+)amphetamine

Figure 3 shows the means of the total time spent head-dipping on 4 successive days, and a more detailed breakdown into the mean time spent head-dipping during each 2.5 min period of each 10 min trial for saline and (+)amphetamine (2 mg/kg). The 4 mg/kg dose of (+)amphetamine resulted in zero head-dips and these data have not been plotted. These rats showed constant walking around the edge of the holes (rather than keeping to the sides of the box), but never head-dipped. From Fig. 3 it can be seen that (+)amphetamine (2 mg/kg) greatly reduced the level of head-dipping on Day 1 ($t(18) = 2.75$, $p<0.02$), and also impaired between-session habituation. Thus there was no significant effect of the drug on the level of head-dipping on all 4 days, but the poor habituation is reflected

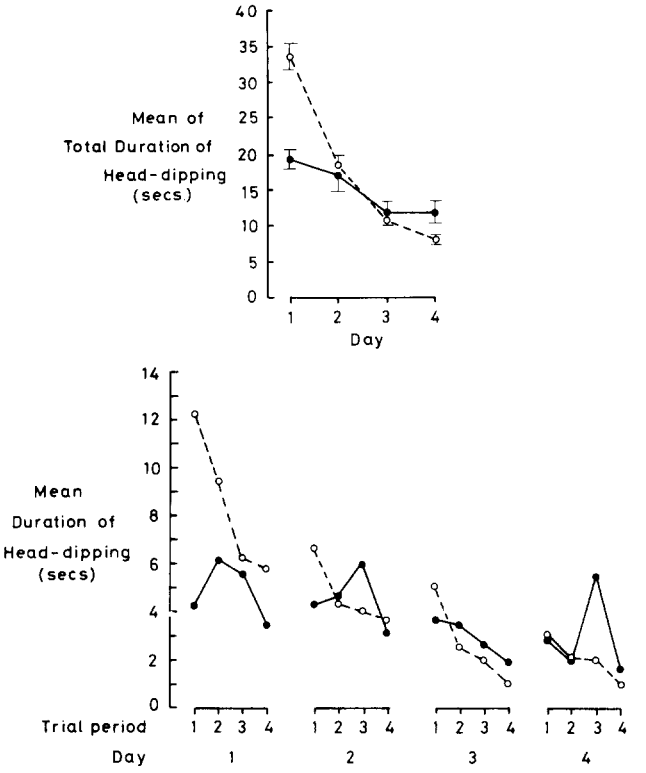

FIG. 3. The upper figure shows the mean of the total time spent head-dipping during a 10 min test period in the presence of objects, on four successive days. The lower figure shows the mean time spent head-dipping during each 2.5 min period of each trial. All points are the mean from 10 rats. o------o saline, ●————● (+)amphetamine (2 mg/kg).

in a significant drug × days interaction, F (3,54) = 8.04, $p<0.001$. Once more the more detailed breakdown shows that the between-session impairment is not due to a retention deficit but is secondary to an impaired pattern of within-session habituation, reflected in a significant drug × trial period interaction, F (3,216) = 6.30, $p<0.001$. Because of the zero scores made in some trial periods by amphetamine animals the data were subjected to a $\sqrt{x+1}$ transform before analysis of variance was conducted.

The impaired within-session habituation might have been a direct effect of amphetamine, or it might merely have been secondary to the reduced level of exploration produced by this drug. The effects of lower doses of (+)amphetamine were therefore studied and mice were used since these animals have a higher level of head-dipping than rats [12]. Thus the chances of getting too low a level of responding to be able to study subsequent habituation were reduced.

Figure 4 shows the means of the total time spent head-dipping on each of two days, and the breakdown into the mean time spent head-dipping in the four 2.5 min periods, for mice injected with saline, 0.5 and 1.0 mg/kg (+)amphetamine. From this it can be seen that the lower dose affected neither the level of head-dipping nor the pattern of habituation. The higher dose reduced head-dipping on trial 1 but not significantly (t (18) = 1.56, $p>0.05$). The between-session habituation was not impaired by (+)amphetamine (drug × day interaction F (2,27) =

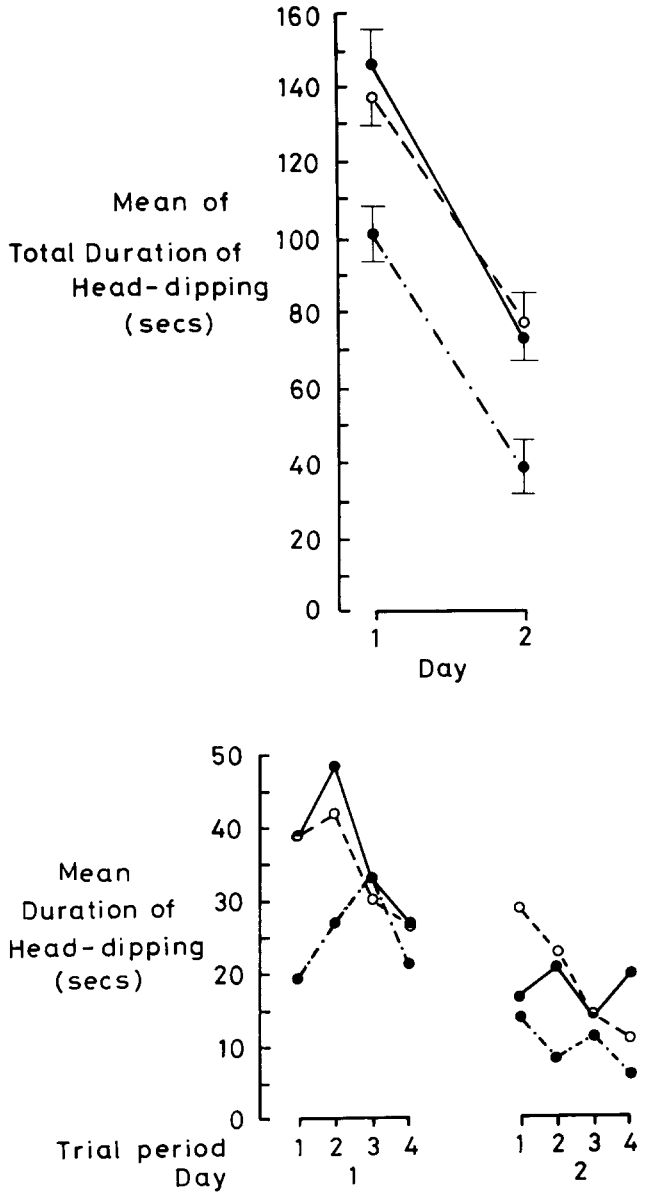

FIG. 4. The upper figure shows the mean of the total time spent head-dipping by mice tested for 10 min in the presence of objects on two successive days. The lower figure shows the mean time spent head-dipping during each 2.5 min period of each trial. All points are the mean from 10 scores. ○-----○ saline, ●————● (+)amphetamine (0.5 mg/kg), ●—·—·—● (+)amphetamine (1 mg/kg).

0.16) but the within-session habituation was impaired (drug × trial period interaction $F(6,162) = 3.0$, $p<0.01$).

Amphetamine did not significantly increase the number of rears made by mice on Trial 1. The saline controls made a mean of 46.6 ± 5.4 rears and (+)amphetamine (1 mg/kg) produced a mean of 57.62 ± 9.0 (t (18) = 1.10, $p>0.05$). However the higher doses of amphetamine significantly increased rearing on Trial 1 in rats from a control level of 25.0 ± 3.76 to 53.0 ± 3.13 for amphetamine (2 mg/kg) (t (18) = 2.91, $p<0.01$) and to 121.0 ± 2.99 for amphetamine (4 mg/kg) (t (18) = 4.71, $p<0.001$). In this study rearing is not used as a measure of exploration. Whilst it is not denied that there is an exploratory component to rearing, this response may also reflect the level of general motor activity and/or attempts to escape. The situation is further complicated when amphetamine is used since, certainly at the higher dose, this can produce stereotyped rearing. Thus the drug as well as increasing this response may also be changing its nature.

DISCUSSION

The impairment in habituation produced by PCPA was most apparent when animals were tested in the presence of objects, a result which has been found with other drugs [9]. The detailed analysis of head-dipping showed that the impairment of between-session habituation was not due to any failure of 24 hr retention, but was secondary to an impaired pattern of within-session habituation on Days 1 and 2. It is unlikely that the linear decrease on Day 3 can be attributed to a recovery of serotonin levels since these were still depleted to 33% of the control level, and the within-session decrease was again disrupted on Days 4 and 5.

The PCPA rats showed a reduced frequency and duration of head-dipping to the objects during the first trial period on Day 1. This parallels results from distraction [8] and startle responses [4,5]. The impaired response to stimulus chnage also parallels results in other situations [7,8]. Thus PCPA has very similar effects on exploration and distraction and taking the two results together the impairment seems primarily to be in the animals' responses to stimulus change. Whether this deficit is best described in terms of dishabituation or sensitisation rather than habituation [7] requires further investigation. It should be remembered that these effects of PCPA might be secondary to other PCPA induced changes, e.g. in arousal, sleep patterns or blood pressure.

(+)Amphetamine also had its primary effect on the within-session habituation of exploration. For rats this impairment was accompanied by , and possibly secondary to, a reduced level of exploration. In mice, although 1 mg/kg reduced head-dipping this reduction was not significant whereas the impaired within-session habituation was. In general where measures of exploration are not heavily dependent on the level of motor activity (+)amphetamine has been found to reduce exploration [15, 19, 21]. The results of this experiment are consistent with this and also show an habituation deficit. This may be secondary to changes in stimulus analysis and once more the results are similar to those found in a distraction task where amphetamine impaired habituation, but where this was possibly secondary to a response change [8]. Thus, although amphetamine produces increased motor activity, and in higher doses (4 mg/kg) stereotyped walking around the holes it impairs the animal's exploration of its environment and the habituation deficit may well be the result of poor stimulus sampling.

Amphetamine appears to increase the release of dopamine, mainly by blocking its re-uptake; many modes of action have been suggested for the effects of amphetamine on norepinephrine, but these all seem to result in increased norepinephrine at the receptor. Since it is possible for drugs to interact with habituation in several ways [10] it is not surprising that both increases in catecholamines and a reduction in 5HT can impair performance in an habituation task. The former is perhaps due to changes in the nature of the response (distraction to startle or exploration to

hyperactivity) and hence an impairment in stimulus sampling and the latter to a direct or indirect reduction in channel capacity. Although habituation may be a process particularly sensitive to disturbances in stimulus sampling the impairments produced by PCPA and amphetamine should be reflected in other learning situations.

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