Pharmacology Biochemistry and Behavior, Vol. 1, pp. 657–663. ANKHO International Inc., 1973. Printed in the U.S.A.

Attenuated Habituation Due to Parachlorophenylalanine

PETER L. CARLTON AND CLAIRE ADVOKAT

Department of Psychiatry, CMDNJ - Rutgers Medical School, Piscataway, New Jersey 08854

(Received 2 July 1973)

CARLTON, P. L. AND C. ADVOKAT. *Attenuated habituation due to parachlorophenylalanine.* PHARMAC. BIOCHEM. BEHAV. 1(6) 657–663, 1973.–The effect of parachlorophenylalanine (PCPA), and the presumed depletion of serotonin due to it, was studied for its effect on the habituation of a startle response in rats. Parachlorophenylalanine was found to increase overall levels of startle amplitude. Detailed examination of the data from individual animals indicated that this effect was entirely due to an attenuation of the rate of habituation.

Habituation of startle PCPA Brain serotonin Inhibition

THE phenomenon of habituation has attracted considerable interest because it reflects a simple kind of nervous system plasticity that may be more amenable to analysis than more complex kinds of plasticity. One kind of habituation that has previously been investigated is the waning of a startle response elicited by the presentation of a stimulus.

Although the phenomenon of habituation has received considerable interest, it has been rarely analyzed from a pharmacological point of view. One exception to this generality is an early study [18] in which it was found that scopolamine had no effect on the course of habituation of startle. This result has recently been replicated and extended [19] in a study in which it was found that, although scopolamine did not alter the habituation of startle, it did profoundly retard the course of habituation of exploratory behavior. This result indicates that, at a pharmacological level at least, these two classes of habituation cannot be simply equated.

A second compound that has been studied for its effect on habituation of startle is parachlorophenylalanine (PCPA). It has been reported [4] that PCPA, and the depletion of endogenous serotonin (5-HT) that this compound produces, retards the course of habituation of startle. Similar data were reported in an independent, but only preliminary, study of the relationship between evoked cortical responses and the startle response [15]. In contrast to these reports, it has also been found [1] that PCPA does not alter the course of habituation itself, although it does reverse the dishabitutory effect of direct stimulation of the Raphé system.

The conflicting results of these studies on the course of habituation itself may lie in the fact that the first studies involved very intense stimuli which were almost certainly aversive to the rat (c.f., [2]), whereas the third experiment involved stimuli that were presumably weaker (auditory clicks of unspecified intensity). Thus, the difference in these studies may inhere in the relative aversiveness of the stimuli used. This possibility is made more likely by the fact that PCPA has been found to attenuate the suppressive effects of punishment [7, 13, 14, 17, 20].

Another problem in the consideration of these reports on the effects of PCPA is that in the experiment in which PCPA retarded the course of habituation [4], neither the course of habituation in individual animals nor the rate of habituation were analyzed. Accordingly, we undertook a study of the effects of PCPA on the habituation of startle using a relatively weak stimulus (the click of a relay closure) and a technique that in preliminary experiments we had found to provide data of sufficient reliability to allow for analysis of the rate of habituation in individual animals.

METHOD

The animals were 12 naive, male albino rats obtained from Sprague-Dawley, Madison, Wisconsin, that weighed approximately 175–200 g at the start of the experiment. All animals were housed individually with continuous access to food and water; they were maintained in continuous room illumination.

The apparatus is shown in Fig. 1 and is a modified Gerbrands student demonstration box. The basic modification involved mounting the chamber itself on a Plexiglas plate which was then drilled to accommodate bolts that had been secured to the wooden board on which the chamber had been originally mounted.

The Plexiglas sheet holding the chamber rested on springs and was held in place by nuts loosely threaded on to the bolts and held in position by Epoxy cement. Although

[1]Supported by NIMH Grant MH-08585 to Peter L. Carlton.

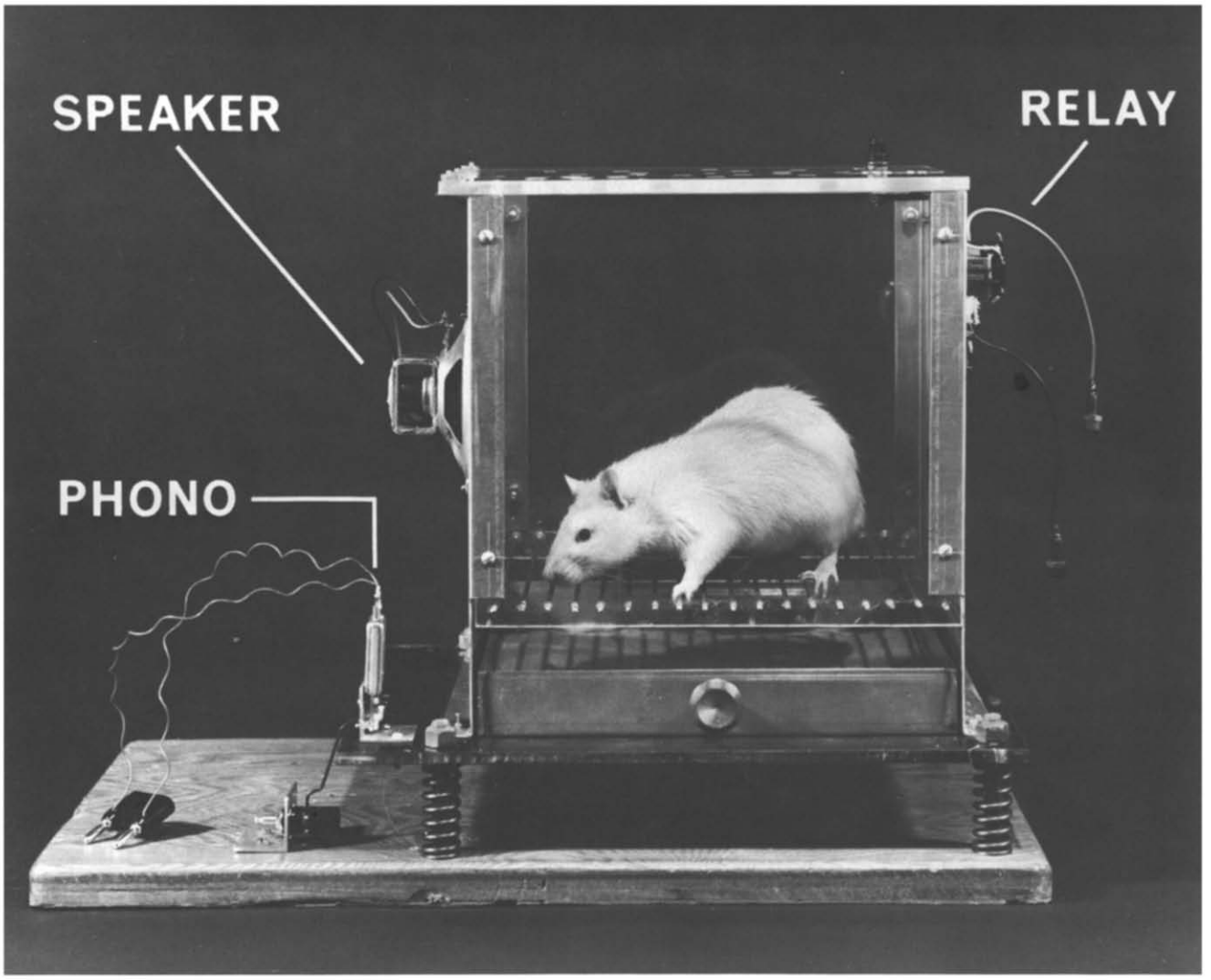

FIG. 1. Startle apparatus.

the tension of the springs is not critical, we have found that best results are obtained when they are of a rigidity such that startle of the animal does not result in an observable movement of the chamber.

The startle stimulus was provided by closure (from a Grason-Stadler E783 F pulse former) of the relay mounted on one wall of the chamber. The startle response of the animal was registered by an Astatic N4-2 (Model 16) phonograph cartridge mounted on the Plexiglas plate; a piece of heavey gauge wire was inserted into the socket of the phonograph cartridge that would normally have accommodated the phonograph needle. The other end of this connector was held immovable by a General Radio plug rigidly mounted to the wooden base plate.

The output of this cartridge was fed into a digital voltmeter which, in turn, was connected to a Beckman Printer (CMMP-3). Not all output from the cartridge was fed into the voltmeter; rather, voltage was recorded for only a brief period that was initiated coincident with the operation of the relay so as to provide a sampling "window" of 0.5 sec. Furthermore, only the peak voltage that occurred during this interval was recorded by the voltmeter. The printer was triggered at the end of this sample period so as to provide a permanent record of startle amplitude.

White noise from a Grason-Stadler Type 901B White Noise Generator (with the meter set at 15 dB) was delivered through the speaker mounted on the wall opposite to that holding the relay. This produced an ambient noise level of 94.0 dB measured with a sound level meter (Type 1558-BP) in the geometric center of the chamber.

The entire apparatus was housed in a larger refrigerator shell ventilated by a fan; this shell as well as the cables necessitated by its use are not shown in Fig. 1.

On Day1, all animals were randomly assigned to one of two groups. Group 1 received a single injection of PCPA, 100mg/kg, ip, on each of four successive days. PCPA was prepared by suspension in saline containing 2 3 drops of Tween. Group 2 received a singly control injection on each of the four days.

On Day 5, 24 hr after the last injection, each rat was weighed and placed in a startle apparatus, with the white noise on, for a 5 min foreperiod. This was followed by 30

presentations of the startle stimulus at a fixed intertrial interval of 10 sec. These sessions were conducted between 1:00 and 4:00 p.m. Because of an apparatus failure, the data from one animal given PCPA were lost.

RESULTS

The effect of repeated PCPA treatment on body weight is summarized in Table 1. On Day 5, there was no overlap of the individual body weights; i.e., the highest body weight in the PCPA group was below that of the lowest in the control group.

TABLE 1

DAILY BODY WEIGHTS (G)

Group	Day				
	1	2	3	4	5
*p*CPA					
1	194	201	199	205	204
2	174	182	179	180	188
3	189	197	195	204	202
4	192	197	199	208	216
5	185	188	177	178	177
6	188	191	190	197	200
Mean	187.0	192.7	189.8	195.3	197.8
Control					
7	200	206	212	225	234
8	201	206	206	210	217
9	183	189	205	216	221
10	194	201	205	210	217
11	194	202	207	212	224
12	188	193	201	212	217
Mean	193.3	199.5	206.0	214.2	221.7

Figure 2 summarizes the data for each of the animals in the two groups. Each bar represents a single rat and denotes the amplitude of startle accumulated over all 30 stimulus presentations. As is indicated by the horizontal dashed line, there is only one instance of overlap between the two groups ($p<0.02$ by two-tailed Mann-Whitney U-test). Figure 3 shows the amplitude of startle for two individual animals cumulated over each of the 30 stimulus presentations; the records of these two rats were selected because their total amplitudes were closest to the mean amplitudes for their respective groups. As the figure indicates, the control animal showed a reasonably orderly decline in startle amplitude, whereas the PCPA animal showed much more variable amplitudes over the course of the stimulus presentations and little, if any, waning of startle.

In order to provide a simple index of rate differences like those shown in Fig. 3, the amplitude accumulated in successive thirds of the session were calculated for each animal. Thus, for example, if an animal accumulated a total startle amplitude of 100 in the first 10 presentations, 200 by the 20th and 250 by the 30th, its scores would be 100, 100 and 50. Because stimuli were presented on a fixed interval, these successive differences can be read as reflecting rate in successive thirds of the session; i.e., amplitude/100 sec. Therefore, for each animal, the sum of these three amplitudes (obtained from Fig. 4), when multiplied by 100, will equal the total amplitude for the session (presented in Fig. 2).

These rate values are shown for individual animals in Fig. 4. As the figure indicates, there was no difference between the groups over the first ten trials. On the other hand, there was only minimal overlap between the groups in subsequent portions of the session ($p<0.02$ in both cases). Thus, the data in Fig. 3 and 4 clearly indicate that PCPA retards the rate of habituation but that this effect is reliable only in the later part of the session.

Figure 3 also suggests that the variability of PCPA animals was greater than that of the controls. However, because of the non-linearity of the recording system, this effect may be partly, if not entirely, artifactual. That is, the nature of the system was such that the voltage readout would necessarily be less variable at low startle amplitudes than at high. Because of this apparatus fault, response variability due to PCPA, if any, cannot be systematically analyzed.

DISCUSSION

Our results replicate and extend the generality of the effects of PCPA [4,15] to a situation involving a less intense startle stimulus; precisely why the earlier study [1] failed to obtain an effect of PCPA itself remains unclear. Furthermore, the effect of PCPA reported here may represent an underestimate of the magnitude of the phenomenon in that control rats were uniformly heavier than those receiving PCPA (see Table 1). That is, the heavier animal would, if anything, be expected to generate larger voltages because of the larger mass moved. Thus, the side-effect of weight loss, like that on food intake reported elsewhere [12], may have attenuated the differences between experimental and control animals.

A second factor, in addition to that of body weight, that could have affected our data is the activity of the animal in the experimental chamber. There are four reasons why this factor is unlikely to have been a contaminant.

First, unsystemic preliminary observations indicated that movement in the response chamber was minimal. Second, gross locomotion had marginal effects on the phono cartridge because, with the relatively rigid mounting employed, the system was primarily responsive only to ballistic movements of the animal. Third, in other preliminary experiments we found that blank presentations (i.e., triggering of the apparatus without relay operation) uniformly failed to produce voltages equal to the minimal voltage produced by an animal given the startle stimulus. Fourth, and most germane, the effects of activity would bear on our data only if there were differential levels of such activity due to treatment; rats given PCPA are less active than controls [17] and this would have, if anything, attenuated the differences observed here. On balance it seems most reasonable to con-

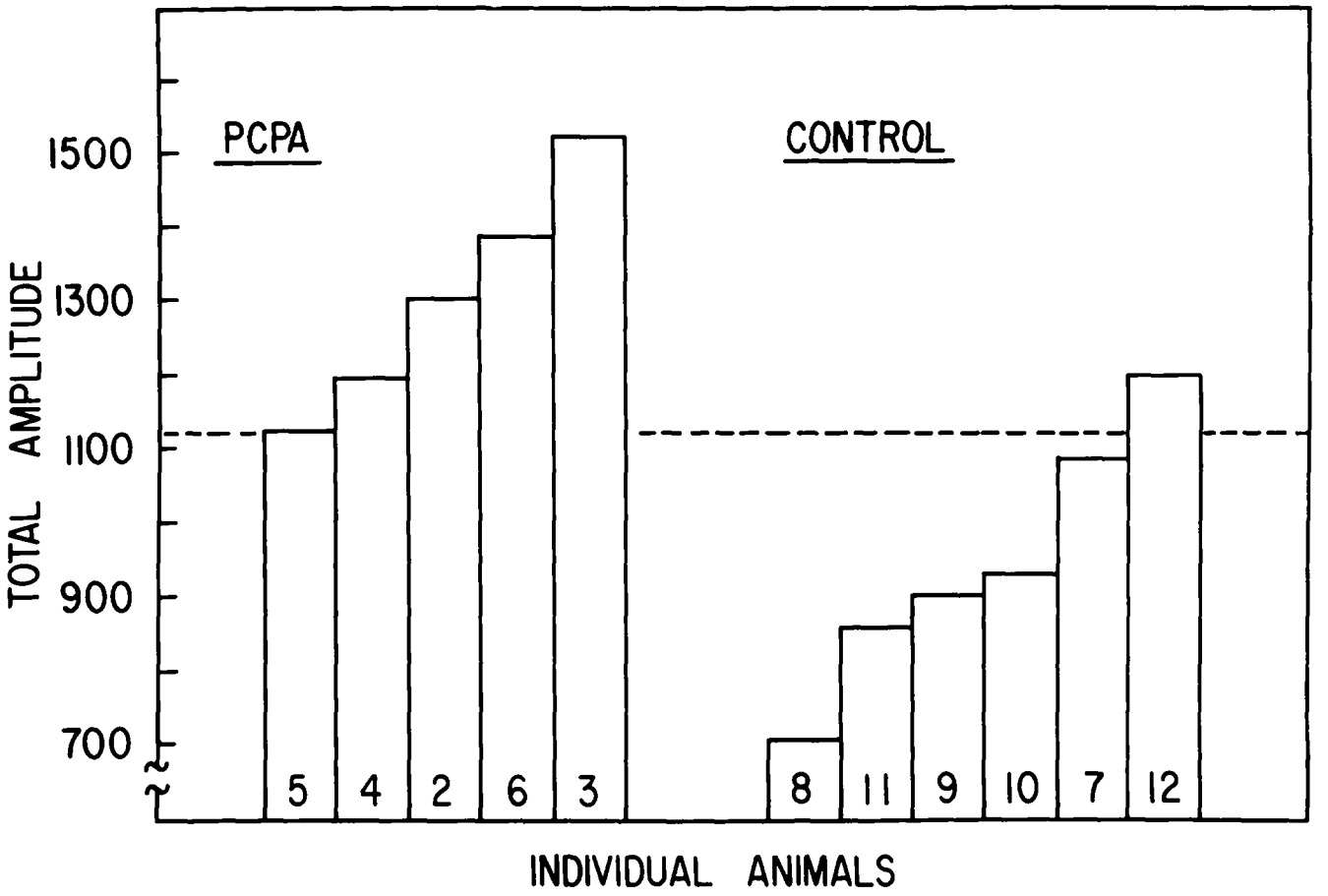

FIG. 2. Total amplitude of startle for each animal accumulated over all 30 stimulus presentations. Individual animal numbers are given at the bottom of each bar.

clude that activity played no significant role in determining these data.

More important than the possible contaminants of body weight and activity is that our data provide single-subject reliability sufficient to permit analysis of rates of habituation in the individual rat. This analysis indicates that the effect of PCPA is not evident in the initial trials but only develops during the course of stimulus presentations. This finding is important because it bears on various interpretations of the effect of PCPA.

There are two basic interpretations of the effects of PCPA that have been offered. The first of these [10, 11, 17] is that 5-HT attenuates reactivity to stimuli and that PCPA therefore increases such reactivity (i.e., decreases jump thresholds to electric shock). A simple application of this interpretation to our data would imply that PCPA, by increasing general reactivity, would effectively add a constant to startle amplitudes independent of number of stimulus presentations. The fact that PCPA interacted with number of presentations to produce a divergence of rates thus argues against this interpretation, at least in its direct application to our data. This lack of initial difference cannot be artifactual because the amplitudes generated by both groups were well below the maximum imposed by the recording system.

The second interpretation [7, 13, 14, 17, 20] is that 5-HT modulates the suppression of behavior and that PCPA therefore antagonizes punishment-induced suppression. Application of this interpretation to our data implies that suppression due to the aversiveness of the stimulus develops with repeated presentations of the startle stimulus and that PCPA would be expected to attenuate this development. The obtained divergence of rates would therefore be expected. We will return to the possible significance of this result in a later paragraph; first, a consideration of more general problems is in order.

These two interpretations of the general effects of PCPA are mutually contradictory in one sense: How can the drug increase reactivity to an aversive stimulus like shock and also decrease reactivity to a conditioned aversive stimulus? It is our view that the contradiction is more apparent than real, that 5-HT modulates the inhibition of behavior. PCPA is therefore disinhibitory in effect, antagonizes the conditioned suppression due to punishment and, not unexpectedly, increases reactivity to a stimulus that elicits a particular response (e.g., jump in the earlier experiments).

In this view, the effect of PCPA on startle is to be expected. That is, repeated stimulus presentations eventuate in response decrement, presumably an instance of accumulated inhibition. Thus, PCPA should act as a disinhibitor, which it does; it should also interact with the accumulation of inhibition by antagonizing its development and therefore

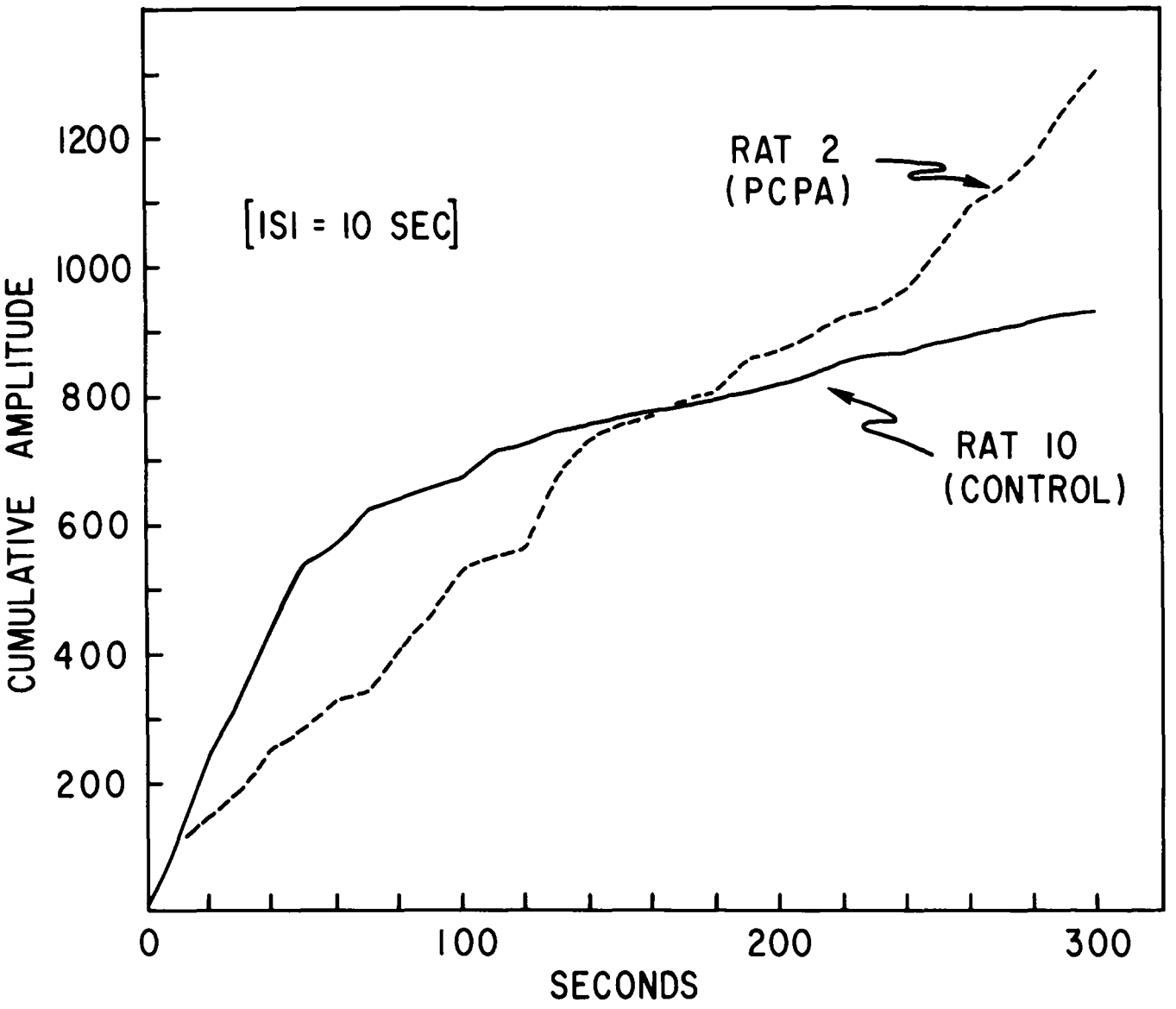

FIG. 3. Cumulative amplitude of startle for one experimental (PCPA) and one control animal over each of the 30 stimulus presentations.

produce divergent rates, which it also does. Unfortunately, one cannot determine whether a similar divergence in jump thresholds was obtained because in the relevant experiments [10, 11, 17] only data pooled over total trials were presented.

This view, similar to that favored by others [4], supposes that PCPA cannot act on inhibition until it has accumulated and that, therefore, rate divergence should occur. This supposition does not, however, rule out the possible involvement of suppression due to the aversiveness of the stimulus.

If an interaction with such suppression is to be invoked as an account of the effects of PCPA, then it must be assumed that suppression and rate of habituation are positively correlated. That is, PCPA could reduce suppression and thereby reduce rate of habituation. If this were the case, then other manipulations affecting the aversiveness of the stimulus should have congruent effects. One such manipulation is stimulus intensity; increased intensity should increase aversiveness, therefore increase suppression and thereby increase rate of habituation. Just the opposite has, however, generally been found [5,6].

Other pharmacological data bear on the point. The drug scopolamine has generally been found to antagonize suppression due to conditioned aversion [3] but has no effect on habituation of startle [18,19]. Conversely, amphetamine has been found to increase suppression [8] but, in a comparable dose range, to have no effect on startle [9].

It would seem, therfore, that an interaction of PCPA with aversion can, at best, account for only a fraction of the effect of that drug on habituation of startle. This is not to say, however, that PCPA does not affect aversion; it is to say that conditioned suppression and the habituation of startle are largely independent instances of behavioral

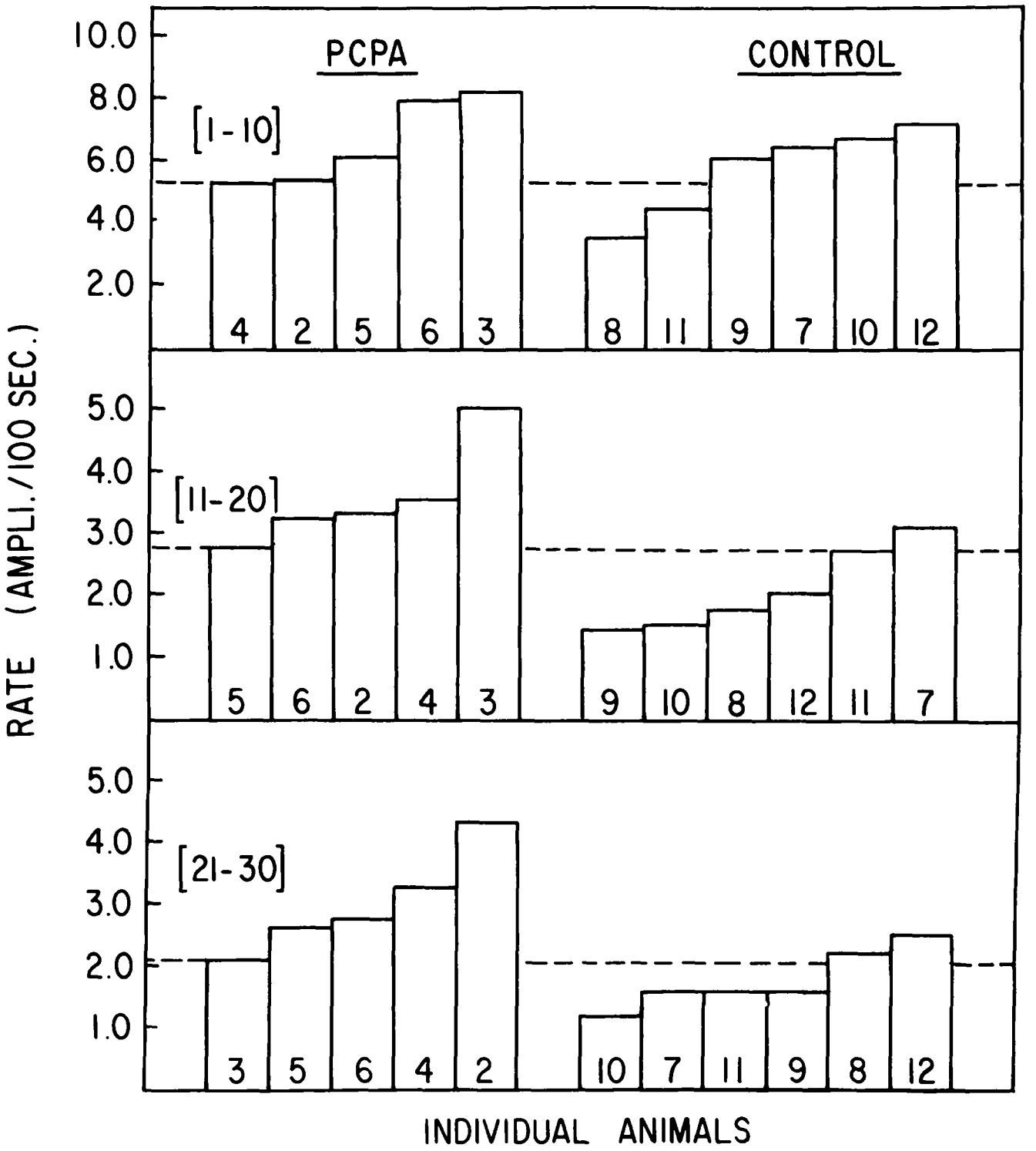

FIG. 4. Rate of habituation for each subject in successive thirds of experimental session. Individual animal numbers are given at the bottom of each bar.

inhibition, both of which are affected by the disinhibitory effect of PCPA.

This conclusion suggests, by inference, that 5-HT is involved in the modulation of behavioral inhibition, a view in agreement with that recently advanced [16] on the basis of detailed analysis of the effects of a variety of pharmacological manipulations on habituation of exploratory behavior.

REFERENCES

1. Aghajanian, G. K. and M. H. Sheard. Behavioral effects of midbrain Raphé stimulation—dependence on serotonin. *Communs behav. Biol.* **1**: 37–41, 1968.
2. Campbell, B. A. Auditory and aversion thresholds of rats for bands of noise. *Science* **125**: 596–597, 1957.
3. Carlton, P. L. and B. Markiewicz. Behavioral effects of atropine and scopolamine. In: *Pharmacological and Biophysical Agents and Behavior*, edited by E. Furchtgott. New York: Academic Press, 1971, pp. 345–373.
4. Conner, R. L., J. M. Stolk, J. D. Barchas and S. Levine. Parachlorophenylalanine and habituation to repetitive auditory startle stimuli in rats. *Physiol. Behav.* **5**: 1215–1219, 1970.
5. Davis, M. and A. R. Wagner. Habituation of startle response under incremental sequence of stimulus intensities. *J. comp. physiol. Psychol.* **67**: 486–492, 1969.
6. Davis, M. and A. R. Wagner. Startle responsiveness after habituation to different intensities of tone. *Psychon. Sci.* **12**: 337–338, 1968.
7. Geller, I. and K. Blum. The effects of 5-HT on para-chlorophenylalanine (p-CPA) attenuation of "conflict" behavior. *Eur. J. Pharmac.* **9**: 319, 1970.
8. Geller, I. and J. Seifter. The effects of meprobamate, barbiturate, d-amphetamine, and promazine on experimentally induced conflict in the rat. *Psychopharmacologia (Berl.)* **1**: 482–492, 1960.
9. Kirkby, R. J., D. S. Bell and A. C. Preston. The effects of methylamphetamine on stereotyped behavior, activity, startle and orienting responses. *Psychopharmacologia (Berl.)* **25**: 41–48, 1972.
10. Lints, C. E. and J. A. Harvey. Altered sensitivity to footshock and decreased brain content of serotonin following brain lesions in the rat. *J. comp. physiol. Psychol.* **67**: 23–31, 1969.
11. Lints, C. E. and J. A. Harvey. Drug induced reversal of brain damage in the rat. *Physiol. Behav.* **4**: 29–31, 1969.
12. McFarlain, R. A. and J. M. Bloom. The effects of para-chlorophenylalanine on brain serotonin, food intake, and U-maze behavior. *Psychopharmacologia (Berl.)* **27**: 85–92, 1972.
13. Robichaud, R. D. and K. L. Sledge. The effects of p-chlorophenylalanine on experimentally induced conflict in the rat. *Life Sci.* **8**: 965, 1969.
14. Stevens, D. A. and L. D. Fechter. The effects of p-chlorophenylalanine, a depletor of brain serotonin, on behavior: II. retardation of passive avoidance learning. *Life Sci.* **8**: 379, 1969.
15. Swonger, A. K., W. F. Chambers and R. H. Rech. The effects of alterations in brain 5-HT on habituation of the cortical evoked response and the startle response in rats. *The Pharmacologist* **12**: 207, 1970.
16. Swonger, A. K. and R. H. Rech. Serotonergic and cholinergic involvement in habituation of activity and spontaneous alteration of rats in a Y maze. *J. comp. physiol. Psychol.* **81**: 509–522, 1972.
17. Tenen, S. S. The effects of p-chlorophenylalanine, a serotonin depletor, on avoidance acquisition, pain sensitivity and related behavior in the rat. *Psychopharmacologia (Berl.)* **10**: 204–219, 1967.
18. Warburton, D. M. and P. M. Groves. The effects of scopolamine on habituation of acoustic startle in rats. *Communs behav. Biol.* **1**: 289–293, 1968.
19. Williams, J. M., L. W. Hamilton and P. L. Carlton. Pharmacological and anatomical dissociation of two types of habituation. *J. comp. physiol. Psychol.*, in press.
20. Wise, C. D., B. D. Berger and L. Stein. Evidence of α-noradrenergic reward receptors and serotonergic punishment receptors in the rat brain. *Biol. Psychiat.* **6**: 3–21, 1973.