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Neurochemical and Behavioral Consequences of Mild, Uncontrollable Shock: Effects of PCPA

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EDWARDS, E., J. JOHNSON, D. ANDERSON, P. TURANO AND F. A. HENN. *Neurochemical and behavioral consequences of mild, uncontrollable shock: Effects of PCPA.* PHARMACOL BIOCHEM BEHAV **25**(2) 415–421, 1986.—The present experiments examined the role of the serotonergic system in the behavioral deficit produced by uncontrollable shock. In Experiment 1: Establishment of model, the behavioral potential of the Sprague-Dawley rat was defined. When exposed to mild uncontrollable stress such as a 0.8 mA electric footshock, a significant percentage of rats developed a shock escape deficit which was evident when subsequently placed in a shock escape paradigm. Serotonin depletion was produced by chronic treatment with p-chlorophenylalanine. Biogenic amine levels and 5-HT levels were monitored in various brain areas using HPLC. Following chronic treatment with PCPA, the shock escape capability of the Sprague-Dawley rat was assessed. The severe depletion of 5-HT in various brain regions was highly correlated with a dramatic improvement in the shock escape scores. Thus, the detrimental effects of exposure to a mild course of inescapable shock can be prevented by chronic treatment with PCPA. These experiments implicate the serotonergic system as a possible mediator of the "learned helplessness" phenomenon.

Stress Uncontrollable shock Shock escape behavior Open-field behavior Serotonin release PCPA (p-chlorophenylalanine)

SELIGMAN and his associates demonstrated that exposure to uncontrollable shock produces a behavioral deficit described as "learned helplessness" [39]. This phenomenon transfers across different aversive training and testing contexts and the apparent development of an escape deficit seems to depend on factors such as the nature of the aversive stimulus [20,44], the parameters of the presentation of that stimulus [1,38] and the nature of the escape response [39,48].

The mechanism by which exposure to uncontrollable shock produces this behavioral deficit remains undefined. A great deal of experimental attention has been devoted to investigating the role of catecholamines in the modulation of behavioral effects of exposure to inescapable shock [4, 23, 45, 46]. Alpha-methyl-p-tyrosine, a tyrosine hydroxylase inhibitor, mimics the behavioral effects of inescapable shock while substances such as L-dopa, which increase catecholaminergic activity, reverse the escape deficits typically observed after exposure to inescapable shock [5].

The evidence implicating catecholamines in the modulation of the behavioral effects of uncontrollable shock does not preclude the possibility that other neurotransmitter systems may be involved. Numerous studies indicate that stress also affects the activity of serotonergic neurons. Thierry [42] reported that the synthesis of 5-HT is moderately increased following exposure to stress. Serotonin metabolism was shown to be accelerated in the central nervous system after stressful conditions [10,22]. Specific stressors, such as inescapable shock seem to produce an enhanced functional activity at serotonergic receptor sites as evidenced by an increase of 5-HIAA [23] in addition to increases of the levels of brain serotonin [26].

Petty and Sherman [33] also measured serotonin levels in cortical perfusate of rats exposed to inescapable shock and reported decreased 5-HT levels from rats that had developed a behavioral deficit after exposure to shock. This behavioral deficit was reversed by injection of 5-HT in frontal neocortex while no reversal was experienced after injection of norepinephrine, GABA, acetylcholine, glutamate and aspartate [40]. These authors also implicated serotonin (5-HT) in the mediation of the learned helplessness reversal by antidepressant drugs since chronic treatment of deficient rats with tricyclic antidepressants increased the calcium specific 5-HT release from cortical slices [40].

However, other stressors such as immobilization, fighting, or shock, also affect serotonin levels and turnover [4,16] suggesting that these systems may be important in the mediation of inescapable shock on subsequent behavior. This possibility is supported by evidence that manipulations which effect levels of serotonin modulate the acquisition and performance of aversively motivated behavior. Increasing the levels of serotonin by injections of L-tryptophan produces deficits in the performance of a conditioned avoidance task [18]. The administration of the precursors of 5-HT, D,L-5 hydroxytryptophan (5HTP) as well as

L-tryptophan to rats or pigeons working on certain schedules of food reinforcement resulted in a behavioral decrease in response rates [7,8]. This behavioral effect was apparently due to increased release of 5-HT [6,30]. Pretreatment with a monoamine oxidase inhibitor such as iproniazid or a 5-HT reuptake inhibitor as fluoxetine, enhanced the behavioral deficit induced by tryptophan [24] as well as by 5-HTP alone [7]. Pretreatment with para-chlorophenylalanine (PCPA), an inhibitor of tryptophan-5-hydroxylase (the enzyme governing serotonin synthesis), sensitized animals to the disruptive effects of 5-HTP [11,19] and resulted in improved performance in a shuttle avoidance task [41].

The present study seeks to establish further support for 5-HT involvement in the mediation of the effects of uncontrollable stress. We hypothesized that the development of a performance deficit following exposure to mild uncontrollable shock is mediated through an initial release of serotonin at specific synapses. If this purported increase of 5-HT following uncontrollable shock mediates the learned helplessness effect, then pretreatment with PCPA, followed by inescapable shock, should prevent the establishment of the stress-induced increase of serotonin as suggested by the results of Bliss [10], Aprison and Hingtgen [6,8] and hence prevent the development of the shock escape deficit.

Our laboratory has been studying a modified version of Seligman's "learned helplessness" model where the experimental animals are exposed to mild uncontrollable shock. After exposure to inescapable shock there have been numerous reports of a learning deficit which only show a degree of internal consistency within each investigator's paradigm. Researchers from various laboratories have agreed that there is a behavioral difference between response deficient rats and rats that have received identical shock and still respond to control levels [27]. There is a high degree of variability in obtaining the behavioral deficit after inescapable shock training, but in the present study a revised "learned helplessness" model was used. With this approach we have compared two distinct groups of rats which emerge when subjected to a mild course of inescapable shock: one group developed a performance deficit on a subsequent shock escape test; another group under identical shock conditions performs like controls in the shock escape test. The selection from two very different groups of animals, those that show no deficit in subsequent shock escape test and those that show a profound escape learning deficit, has provided a better medium for behavioral and pharmacological manipulations. The role of serotonin in the mediation of the learned helplessness effect is evaluated in these behaviorally distinct groups of rats.

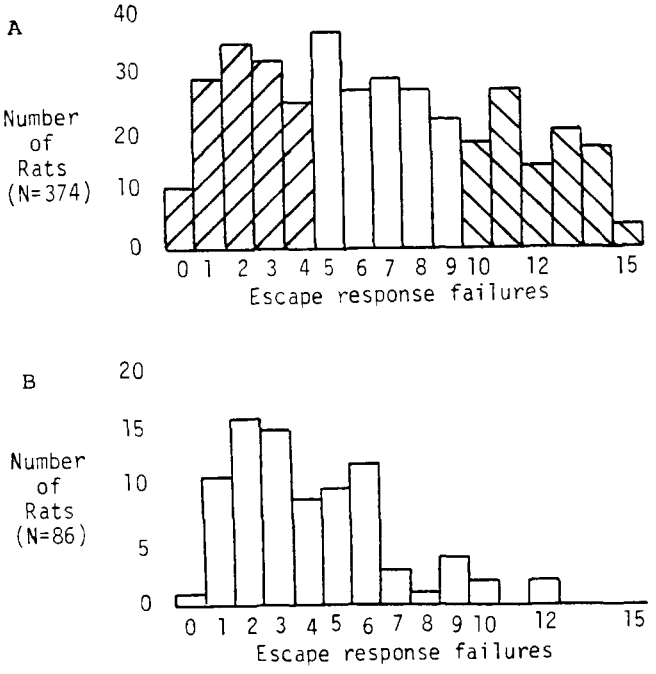

FIG. 1. Features of escape responding after exposure to inescapable shock. (A) Escape response in rats trained 24 hours previously. Hatching indicates response proficient (0–4 failures, 132 rats) and response deficient (10–15 failures, 107) animals. (B) Scoring of naive animals exposed to escape task, no prior training.

METHOD

Sprague-Dawley rats (150–200 g) obtained from Charles River Breeding Laboratories (Wilmington, MA) were housed in a temperature/humidity-controlled facility and a twelve hour dark-light cycle was maintained.

Animals were allowed a week of habituation in their new environment before use in an experiment. They were kept on an ad lib food and water schedule. The experiments were conducted between 8.00 and 14.00 hr.

Experiment 1: Establishment of the Model

Before proceeding with any pharmacological manipulations of the serotonin system in rats exposed to uncontrollable shock, we first determined the spontaneous response of this system following the course of treatment being studied. The critical element of this behavioral-biochemical study is the consistency and accuracy with which the behavior can be quantified. In this experimental set-up each of the 374 rats were placed in an experimental chamber with an electrified grid floor. Each chamber is 12 cm long × 18 cm high × 12 cm wide. Sides and ceilings are constructed of aluminum and Plexiglas. The floor is constructed of stainless steel rods spaced 1.9 cm apart. During the shock escape test a lever is mounted 7 cm off the grid floor on one wall. A yellow cue light is placed 5 cm above the lever. Shock is delivered to each chamber by a Coulbourn solid state shock source (Model E13-16).

Shock training. Training consisted of placing the rats in the experimental chamber where they receive intermittent inescapable 0.8 mA footshock. Each training session lasted forty minutes. The onset and offset of the shock being established by a probability generator resulted in an average schedule of 20 minutes of shock with a minimum time of 1.5 seconds between on and off events.

Shock escape testing. Twenty-four hours after training, each rat is individually tested in an escape situation where footshock can be eliminated by a single bar press. Shock is delivered at the intensity of 0.8 mA in a pulsating schedule of 35 msec on/35 msec off with the yellow cue light being on during the shock period. Shock onset begins one trial and pressing the lever or the end of 60 seconds shuts off the shock. Intertrial intervals of 24 seconds begin with the yellow cue light out. Fifteen trials are given in each testing session. Latencies up to 20 seconds before the lever is pressed and the shock terminated are considered a suc-

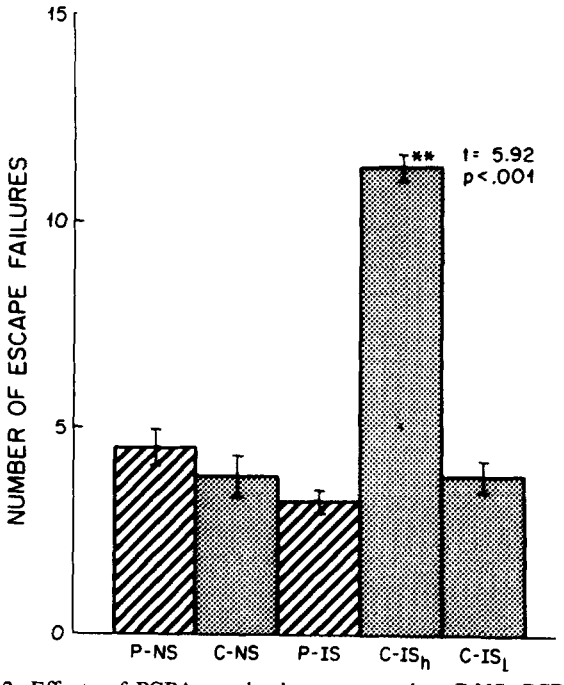

FIG. 2. Effects of PCPA on shock escape testing. P-NS: PCPA-treated, no shock pretreatment, N=20. C-NS: Control saline, no shock pretreatment, N=20. P-IS: PCPA-treated, inescapable shock, N=20. C-IS: Control saline, inescapable shock, N=20. After shock escape testing rats scoring 11–15 failures are represented as C-IS$_H$ (n=7). Rats scoring 0–4 failures are represented as C-IS$_L$ (n=8). Behavioral deficit is expressed as number of failures±SD.

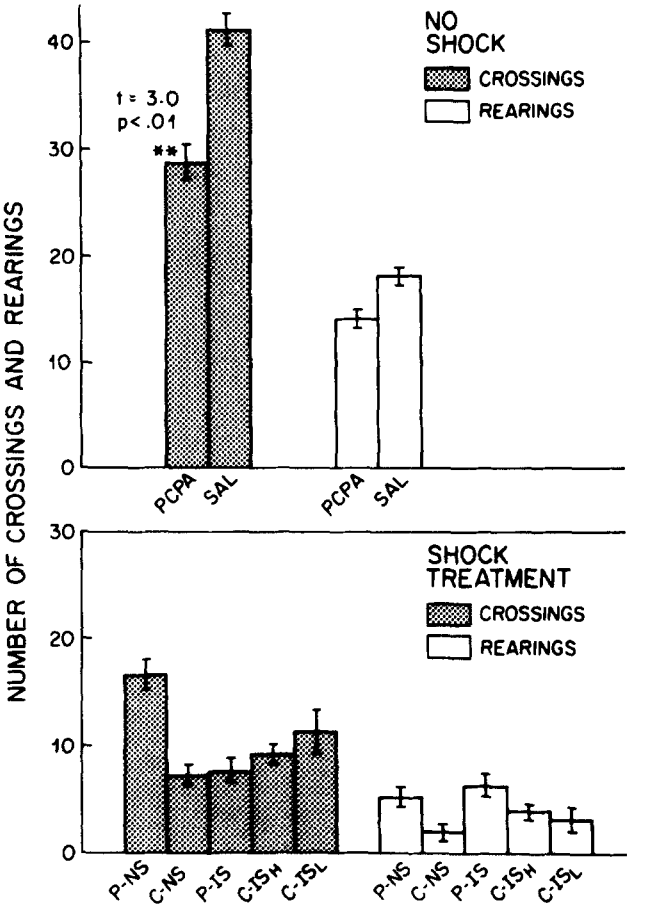

FIG. 3. Motor activity measurements in PCPA-treated rats and saline controls. P-NS: PCPA-treated, no shock pretreatment, N=20. C-NS: Control saline, no shock pretreatment, N=20. P-IS: PCPA-treated, inescapable shock, N=20. C-IS: Control saline inescapable shock, N=20. After shock escape testing rats scoring 11–15 failures are represented as C-IS$_H$. Rats scoring 0–5 failures are represented as C-IS$_L$. The upper panel shows the number of crossings and rearings prior to any shock training. The lower panel shows the activity measurements obtained after the shock escape test. All measurements are expressed as means±SD.

cessful escape to shock. Twenty to sixty second latencies are recorded as failures. Scores are recorded automatically. Nonspecific effects of footshock are eliminated by including internal controls in the testing paradigm. Behavioral, pharmacological and biochemical determinations are also carried out for these internal controls. These rats are only subjected to the shock escape test, enabling us to determine the effects of shock per se. From past experiments carried out in our laboratory, these controls routinely do not show any significant changes both behaviorally and biochemically from the non-deficit or naive rats. Behavioral deficits are measured as the number of failures, using the following criteria: rats scoring 0–4 failures in a 15-trial testing session are not "helpless" and learn this shock escape test equally as quickly as controls. Rats scoring 10–15 failures are considered deficient in the escape response while animals falling in the range of 6–9 failures are not considered in this analysis.

Experiment 2: P-Chlorophenylalanine Pre-Treatment

Experiment 2 consists of the following eight steps: drug treatment, 96 hr recovery, activity monitoring I, IS/NS training, 24 hr recovery, shock escape test, 2 hr recovery, activity monitoring II.

PCPA treatment: Drug schedule. Animals were randomly assigned to the four experimental conditions, each with an N of 20. PCPA-treated rats received 1 ml, IP injection of PCPA methyl ester (100 mg/kg of body weight) dissolved in physiological saline for three days. Control rats received 1 ml, IP injections of saline for three days. All rats were allowed a 96 hour period of recovery before the subsequent training and testing shock procedure.

Following PCPA treatment, the subsequent procedure of uncontrollable shock training and shock escape testing remained as previously described in Experiment 1. However, in the present experiment, the design was a 2×2 factorial design: inescapable shock versus no shock pretreatment and drug pretreatment versus no drug. Hence, the following groups were generated: PCPA—no inescapable shock (P-NS); PCPA—inescapable shock (P-IS); Saline control—no inescapable shock (C-NS); Saline control—inescapable shock (C-IS).

Behavioral activity. After exposure to the shock (testing session), both PCPA treated and saline treated rats (N=20/group) were returned to their home cages for a two-hour recovery period; they were then placed in the middle of an open field apparatus (kept in a separate room) similar to that described by Broadhurst [13]. The apparatus consisted of a circular plywood floor 70 cm in diameter. A circular wall 30 cm high constructed from Plexiglas and painted flat white served to enclose the test arena. To provide a basis for scor-

TABLE 1

REGIONAL LEVELS OF 5-HT, 5-HIAA, NE AND DA IN SALINE AND PCPA RATS

	P-NS	P-IS	C-NS	C-IS
ANC				
5-HT	0.9 ± 0.23	0.90 ± 0.3	4.5 ± 0.5[a]	5.4 ± 1.1[a, b]
5-HIAA	0.25 ± 0.08	0.20 ± 0.1	1.6 ± 0.3[a]	3.0 ± 1.0[a, b]
NE	3.2 ± 0.5	2.7 ± 0.4	3.3 ± 0.3	3.1 ± 1.1
DA	0.29 ± 0.05	0.15 ± 0.06	2.3 ± 0.1	0.2 ± 0.1
HC				
5-HT	0.84 ± 0.2	1.4 ± 0.5	4.2 ± 0.8[a]	5.5 ± 1.4[a, b]
5-HIAA	0.30 ± 0.07	0.50 ± 0.12	2.3 ± 0.15[a]	4.1 ± 1.4[a, b]
NE	3.9 ± 0.7	3.7 ± 0.4	4.3 ± 0.8	3.4 ± 0.4
DA	0.2 ± 0.08	0.25 ± 0.08	0.28 ± 0.07	0.2 ± 0.14
Septum				
5-HT	2.01 ± 0.25	2.0 ± 0.5	6.7 ± 0.7[a]	6.0 ± 2.0[a]
5-HIAA	0.90 ± 0.11	0.90 ± 0.2	3.01 ± 0.3[a, b]	4.9 ± 1.0[a, b]
NE	7.0 ± 1.2	5.8 ± 0.8	7.9 ± 1.3	7.4 ± 1.9
DA	5.8 ± 0.8	5.1 ± 1.6	5.8 ± 0.6	6.65 ± 0.82
Raphe				
5-HT	11.8 ± 0.94	10.8 ± 0.52	17.45 ± 0.70[a]	20.0 ± 0.51[a]
5-HIAA	6.8 ± 1.2	6.92 ± 0.33	12.6 ± 0.95[a]	13.2 ± 1.2[a]
NE	17.01 ± 0.6	17.68 ± 0.4	18.0 ± 1.26	17.91 ± 0.34
DA	2.3 ± 0.26	2.0 ± 0.19	2.28 ± 0.28	2.72 ± 0.24

5-HT, 5-HIAA, NE and DA levels in PCPA-treated rats and saline controls. Rats were sacrificed 2 hours after the shock escape test. Brains were rapidly removed and dissected on ice. ANC, HC, septum and raphe samples were sonicated in 0.2 M perchloric acid and aliquots were taken for the analysis of 5-HT, 5-HIAA, NE and DA levels by HPLC with electrochemical detection. Levels are expressed in ng/mg protein ± S.D.

[a] $p<0.001$ vs. P-NS and P-IS.

[b] $p<0.001$ vs. C-NS.

ing the ambulation of the animals, black markings were made from the center of the enclosed floor, dividing the total surface area into eight 74 sq. in. units.

Active motor behavior was measured in 5 minute sessions. Two behavioral parameters were measured: locomotion (the positioning of four paws within a floor space was defined as a unit of ambulation) and standing on the hind legs (rearing). These parameters were recorded with a video system (Esterline Angus event recorder). The video tapes were later displayed to two trained observers blind to the experimental condition, yielding independent rating scores for each animal. Baseline activity measurements were also recorded prior to training (inescapable shock exposure) and shock escape test.

Neurochemical Analysis

Brain dissection. Immediately after behavioral testing, animals were decapitated, their brains were removed and rapidly dissected on ice. Brain samples were taken, including five regions: hypothalamus, anterior neocortex, hippocampus, septum and raphe nuclei. The dissection of hypothalamus, hippocampus and anterior neocortex was done as described by Reinstein *et al.* [34] where anterior neocortex is taken as the cortical gray matter gently scraped from the frontal surface of the cerebrum. The "raphe nuclei" was taken as the entire region starting from the colliculi and extending to the pons and medulla. This region included clusters of 5HT-cells lying in or near the midline of the pons and upper brainstem. It also included more rostral 5HT cell groups (raphe dorsalis, raphe medianus and centralis superior, B_7–B_9) [9].

HPLC determinations. Brain regions were analyzed for their content of DA, NE, 5-HT and 5-HIAA. After homogenizing the brain regions in 0.2 M perchloric acid, aliquots were taken for analysis of DA, NE, 5-HT and 5-HIAA. Simultaneous assays were performed by reverse phase high performance liquid chromatography (HPLC) with electrochemical detection; separation was achieved at 37° using an Applied Science C_{18} column (150×4.6 mM, 3 μ absorbo sphere). The mobile phase consisted of 0.15 M monochloroacetic acid, 2.0 mM disodium-EDTA brought to pH 3.0 with NAOH, 270 mg/liter sodium octyl sulfate and 4.5% acetonitrile. The flow rate was set at 2 ml/minute and dihydroxybenzylamine (DHBA) served as an internal standard. We used the Beckman model 345 fitted with a Bioanalytical System (BAS) LC 4B detector. The glassy carbon electrode was set at a potential of 650 mV against Ag/Agcl/3M NaCl reference electrode. The sample injector (Altex 210) was fitted with a 20 μl loop. Peak height ratios were recorded and related to the internal standard.

Statistical Analysis

Individually scored behaviors were treated as independent measures. A two-way analysis of variance (ANOVA) was performed followed by Newman-Keuls' multiple range test for differences between means. The neurochemical

means were compared using a two-tailed Student's t-test. For all comparisons, the level of statistical significance was set at $p<0.05$.

RESULTS

Shock Escape Behavior

After exposure to uncontrollable shock, the response of Sprague-Dawley rats in a subsequent shock escape test was evaluated. The features of escape responding after prior exposure to shock and control responding are detailed in Fig. 1. In panel A, sub-populations of Sprague-Dawley rats are classified as: response deficients when they score 10–15 failures in a 15-trial shock escape test; non-deficient when they score 0–4 failures in a 15-trial shock escape test after undergoing identical treatment. The total number of Sprague-Dawley rats included in this analysis was 374 with 107 rats falling in the range of 10–15 failures, and 132 rats falling in the range of 0–4 failures. Panel B illustrates the shock escape scores of control rats (N=86). These controls are naive rats which are exposed to the escape paradigm with no prior training (inescapable shock exposure). Sixty-four percent of these rats (N=55) score 0–4 failures in the 15-trial shock escape paradigm.

In Experiment 2, (PCPA pre-treatment), the scores recorded in the shock escape test for the C-NS, C-IS, P-NS, P-IS groups are detailed in Fig. 2. Scores are expressed as number of failures±S.D. The C-IS group exhibited scores which averaged 7.6±3.3 failures. However, after exposure to a mild course of inescapable shock, two distinct groups of rats emerged within the saline controls (C-IS group, N=20). A significant percentage (N=7) of these rats exhibited a deficit in their shock escape behavior as compared to the control group (C-NS, N=20). These animals, C-IS_H group, scored an average of 11.3±0.6 failures while control animals scored an average of 3.8±1.04 failures. Conversely, some of the saline controls (C-IS group), although subjected to the same shock regimen, did not show any deficit in their shock escape behavior. These animals, C-IS_L group (N=8), scored an average 3.8±0.68 failures and their behavior closely paralleled that of the control group (Fig. 2).

Pretreatment with PCPA significantly decreased the adverse effect of exposure to inescapable shock. As seen in Fig. 2, the P-IS group (N=20) exhibited a minimal number of escape failures when tested in a shock escape paradigm (3.2±0.51). None of the rats from the P-IS group were "helpless." The average score for that group, 3.2±0.51 failures, was significantly different from the average score of the entire C-IS group (7.6±3.3: C-IS vs. 3.2±0.51: P-IS, $t=4.3$, $p<0.001$). A two-way analysis of variance examined the performance of the four groups: (N=20 per group) P-NS, P-IS, C-NS, C-IS and revealed a reliable shock pretreatment effect, $F(1,34)=35.6$, $p<0.001$, indicating that shock-pretreated animals were reliably slower in escaping from shock, and a reliable drug effect, $F(1,34)=30.06$, $p<0.001$, indicating that rats treated with PCPA escaped faster from shock. No other effects were significant. In the analysis of variance the entire C-IS group (not the pre-selected C-IS_H and C-IS_L groups) were used.

Behavioral Activity

A 96-hour recovery period was observed after the last PCPA injection. Drug effect (PCPA) on behavioral activity is shown in Fig. 3, top panel. Baseline activity measurements (before training with inescapable shock) revealed significant differences in units of ambulation between PCPA-treated and saline control rats (28.5±2.6:PCPA vs. 41.0±2.9: control, $t=3.0$, $p<0.01$, N=20/group). Rearing was not significantly different between PCPA-treated rats and saline controls (14.08±1.7:PCPA vs. 17.85±1.7:control, N=20/group). The same activity parameters were measured two hours after the shock escape test. The P-NS group compared individually to the C-NS, P-IS and C-IS displayed more crossings and rearings, $t=1.9$, $p<0.05$. Two sample t-test statistical comparison of the groups: P-IS vs. C-IS; C-NS vs. C-IS; P-IS vs. C-NS did not reach significance level, $t=0.20$, n.s. With a two-way analysis of variance, the differences in the number of crossings and rearings between the P-NS, C-NS, P-IS and C-IS group did not reach the significance level, $F(1,48)=1.1$ and $F(1,48)=0.02$ revealing no shock treatment effect and no overall drug treatment effect (Fig. 3, lower panel).

However, the differences in the number of crossings and rearings were striking when comparing baseline measurements (Fig. 3, top panel) and measurements taken after the shock escape test (P-NS, C-NS, P-IS, C-IS groups). A three-fold decrease ($p<0.001$) in the activity parameters was observed in the P-NS, C-NS, C-IS and P-IS groups. However, none of the rats in the P-NS, C-NS, P-IS groups were response deficient.

Regional Brain Levels of 5-HT, 5-HIAA, NE and DA

Previously, our laboratory has published data which indicate that exposure to uncontrollable shock within the time frame of our protocol, did not affect the levels of NE, EPI, DA in any of the brain regions examined [3].

PCPA pretreatment caused no change in NE levels in unstressed animals (P-NS group) as well as in animals exposed to shock (P-IS group) (see Table 1).

Preliminary experiments in our laboratory have shown that norepinephrine levels were decreased after the PCPA treatment (about 30%) while 5-HT and 5-HIAA levels were depleted up to 90% when compared to saline controls. However, at the time of the behavioral shock escape test, (96 hours after PCPA injection) catecholamine levels routinely returned to control levels [17]. Therefore, the time span between PCPA injections and the testing sessions is of primary importance in order to ensure the specificity of the administration of PCPA. Catecholamine levels in PCPA-treated rats returned to saline control levels within 48 hours while 5-HT levels are still depleted by 50–60% eight days after the last PCPA injection.

Exposure to uncontrollable shock resulted in an increase of 5-HT and 5-HIAA levels in all of the brain areas examined (Table 1). These changes were more evident in the anterior cortex and hippocampus. 5-HT levels were increased by 24% (C-IS group vs. C-NS group, $p<0.001$). 5-HIAA levels were increased by 48% (C-IS group vs. C-NS group, $p<0.001$).

Pretreatment with PCPA produced a highly significant decrease in 5-HT and 5-HIAA levels regardless of shock treatment. 5-HT and 5-HIAA levels were decreased by 80% in the anterior cortex and the hippocampus. The decreased levels in septum (68%) and raphe region (40%) also reached statistical significance ($p<0.001$, when P-NS and P-IS groups were compared to C-NS and C-IS groups).

DISCUSSION

The data from these experiments show that exposing rats

to a mild course of inescapable shock results in the development of a subsequent shock deficit in a distinct population of rats. The ability to subsequently escape exposure to electric shock was significantly decreased in that group. However, pretreatment with para-chlorophenylalanine (PCPA) blocks the shock-induced decreases in escape behavior. This decrease in behavioral deficit is concomitant with a substantial decrease in 5-HT levels in the brain while other monoamine levels shown no change within the same subjects.

This study complements the work of Brown and her group [15] where it was determined that manipulations known to increase levels of serotonin mimic the effect of inescapable shock. Treatment with L-tryptophan and 5-HTP produced a deficit in the acquisition of shuttle escape behavior which was prevented by treatment with the serotonin antagonist methysergide.

Support for serotonin (5-HT) involvement in the mediation of the effects of inescapable shock also stems from experiments where a specific behavior is equally modulated both by 5-HT and exposure to inescapable shock. Shock aggression decreases after treatment with 5-HT precursors [35] and similarly after exposure to inescapable shock [29,47]. Elevated 5-HT levels result in a decrease of affectively motivated behaviors [19, 31, 32] and inescapable shock also interferes with the subsequent acquisition of affectively motivated behaviors [36,37]. In studies employing parameters similar to ours, 5-HT turnover was enhanced after exposure to electric shock [10, 16, 42] and increased 5-HT levels produced deficits in the performance of a conditioned avoidance task [18]. Thus, our procedure yielded data consistent with those from other studies describing effects of stress on regional brain 5-HT status.

We cannot discount the possibility that PCPA-treated animals regardless of the shock pretreatment condition produced shorter response latencies in the shock escape testing because of a heightened reactivity to shock. This would lend support to the findings of Harvey and Lints [21] who report that reduction in brain 5-HT levels resulted in increases in the animal's sensitivity to electric shock. However, in our study, drug controls with no prior exposure to inescapable shock (C-NS group) were not statistically different from PCPA-treated rats with no prior exposure to inescapable shock (P-NS group) and PCPA treated rats exposed to inescapable shock (P-IS group) in their shock escape behavior (Fig. 2).

Some could argue that the enhanced shock escape response demonstrated in PCPA-treated rats could result from a general increased hyperactivity in these rats. The behavioral concomitants of daily injections of PCPA have been demonstrated to produce a transitory enhancement of daytime motor activity [12,49]. However, in our study, a four day recovery period was observed prior to the shock escape test and our data from the open field experiment refute the contention of hyperactivity in the PCPA-treated rats (Fig. 3).

Shock escape testing did produce a decrease in motor activity in the groups examined. Activity has always been found to be a confounding variable in the measure of a learning deficit. Several reports have claimed decreased motor activity in animals which have an associated learning deficit [2, 28, 45]. Weiss *et al.* [45] even suggested that the learned helplessness phenomenon could be explained by learned inactivity. Our data is at odds with this theory. Motor activity was decreased after the shock escape test in all the groups examined: P-NS, P-IS, C-NS and C-IS but simultaneously the PCPA-treated rats did not show any behavioral deficit in their shock escape response. Intensity of the shock treatment is an important determinant of the degree to which interference with ambulation will occur. A strong shock treatment intensity reduced open field movement [1,28] while a relatively weaker shock treatment intensity, similar to the shock levels we have used, only had a modest influence on subsequent activity [14]. Our data is in agreement with Jackson's studies where a number of learning tasks were developed in which activity and learning were either uncorrelated or negatively correlated [25].

The animal model we have presented has been considered a reasonable model of some types of human depression. Our data raises the possibility that a mechanism which decreases serotonergic processes in the brain may be beneficial in some human subjects exposed to severe stressors. At present, the status of the post-synaptic serotonin receptors, S_1 and S_2 is being investigated in our laboratory. Preliminary data indicate some supersensitivity of the S_1 serotonin receptor after PCPA treatment (also see [19]). Modifications at the receptor level in response to chronic PCPA action may provide a promising avenue in the search of a neurochemical basis of the behavioral deficit that we described.

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