Pharmacology Biochemistry & Behavior, Vol. 4, pp. 343–346.

BRIEF COMMUNICATION

Exaggerated Avoidance of Novel Stimulation in Rats Partially Recovered from Central Norepinephrine Damage

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(Received 2 June 1975)

BRESLER, D. E., J. DIAZ AND G. ELLISON. *Exaggerated avoidance of novel stimulation in rats partially recovered from central norepinephrine damage.* PHARMAC. BIOCHEM. BEHAV. 4(3) 343–346, 1976. — Rats in which central norepinephrine lesions are made with 6-OHDA and a recovery period allowed show a behavioral syndrome distinct from controls, from rats with more extensive norepinephrine damage (lesioned with 6-OHDA, allowed to recover, and relesioned), and from rats with general depletion of serotonin (chronic PCPA). These rats administered central 6-OHDA injections and then allowed a recovery period rear less in open field than all other groups and are characterized by an exaggerated avoidance of novel visual stimulation in light onset and light escape tests. It is proposed that this reflects the hyperresponsivity of partially repaired, supersensitive norepinephrine circuitry to novel or intense stimulation.

6-OHDA NE lesions Recovery and supersensitivity Attentional deficits Hyperkinesis

FOLLOWING central lesions of NE terminals made with intraventricular injections of 6-hydroxydopamine (6-OHDA), NE cells gradually develop an increased turnover of their remaining brainstem NE [10,17]. Other evidence of recovery is the sprouting and regrowth by damaged NE axons observed following mechanical lesions of the brain [5], 6-OHDA lesions in the spinal cord [11], and central injections of small amounts of 6-hydroxydopa [12]. Parallel evidence of compensatory recovery responses by damaged central NE neurons has been reported from behavioral studies. Rats given small amounts of 6-OHDA (three 25 μg injections, given over 3 days) and allowed to recover develop behaviors indicative of exaggerated NE activity (i.e., heightened aggressivity, hyperactivity in running wheels, increased emotional reactivity, and exaggerated responsivitiy to d-amphetamine); these supersensitive NE behaviors in these 6-OHDA × 3 rats are not present in rats who are lesioned, allowed to recover, relesioned, and then behaviorally tested (6-OHDA × 6; [15]). The purpose of the present study was to examine further the behavioral syndrome of these 6-OHDA × 3 rats, and to compare their behavior with nonlesioned controls, with animals with more extensive NE damage (6-OHDA × 6), and with animals treated with the 5HT synthesis inhibitor parachlorophenalanine (PCPA).

METHOD

The animals were 47 male Sprague-Dawley rats weighing 370–395 g. A 23 ga cannula was implanted in the right lateral ventricle of each animal, the rats were matched for body weight, divided into 4 groups, and injected under light ether.

6-OHDA × 6 (N = 12). These animals received 6 intraventricular 25 μg injections of 6-OHDA HCl (calculated as free base) in 10 μl of artificial CSF [9] with 5 μg/μl ascorbic acid added to prevent auto-oxidation. Three daily injections were given, a two week recovery period allowed, and three additional daily 6-OHDA injections given.

6-OHDA × 3 (N = 11). These animals received 3 daily 25 μg injections of 6-OHDA at the same time as the initial injections to the 6-OHDA × 6 group. At the time when the 6-OHDA × 6 group was given the second set of 6-OHDA injections these animals were given three daily 10 μl injections of the vehicle solution alone.

PCPA (N = 12). Three weeks after the second series of 6-OHDA injections to the 6-OHDA × 6 group, the PCPA group received 100 mg/kg subcutaneous injections of PCPA dissolved in 1N NaOH, neutralized with 1N HCl, and brought up to concentration (150 mg/ml) by adding McIlvain's citric acid/phosphate buffer solution at pH = 7. These injections were repeated every fourth day throughout the experiment.

Control (N = 12). This group received neither 6-OHDA nor PCPA but intraventricular control injections of artificial CSF and subcutaneous injections of buffer vehicle.

Behavioral Testing

Open field test. This test was carried out in a circular enclosure (130 cm across) with the floor divided into 22 cm squares. The animal was placed into the field's center, and

covered with a 22 x 22 x 46 box. After 30 sec this box was lifted, and the number of squares crossed and the number of rearings were recorded by 3 trained observers. Testing was conducted under dim room illumination with a masking white noise present (approximately 1 ft-candle and 54 db respectively at the center of the field). After 5 min intermittent flashing lights (60 ft-candles) and tones (78 db) were presented and the observers continued scoring behavior.

Light onset test. The animals were placed in 23 x 20 x 27 cm cages inside soundproofed, dark chambers. Protruding out 1 cm from the back of the cage and 9.5 cm above the floor were 2 wires side by side and bent into the shape of handles. When touched these wires operated contact circuits. One loop, when touched, activated a dim overhead light (12 W, incremented from 10 V ambient level to 18 V) as long as contact was maintained. Nothing happened when the other loop was touched. Each animal was tested for a half-hour session.

Aversion to bright light. The animals were placed in a 27 x 55 x 30 cm shuttle box with a 4 cm hurdle in the middle. Light bulbs (150 W) were mounted on each end of the shuttle box behind translucent panels. After 20 min of habituation to the box testing began (32 trials, average intertrial interval 1 min). A trial consisted of turning on either a dim (70 V) or a bright (110 V) flashing light in the end of the shuttle box nearest the rat. When the rat moved away from the light and crossed the hurdle, the light was automatically extinguished. Response speeds to turn off the light (reciprocal of latency in sec) served as the measure of aversiveness of the light. On every fifth trial no light was presented but latency was recorded.

Shock-elicited aggression. Pairs of rats from the same group were presented with a series of 30 shocks (1 mA rms, 0.5 sec duration) each separated by a 20 sec intershock interval. During shocks the presence or absence on each trial of the following behaviors was noted by two observers who were unaware of the animal's group: fights (aggressive contact made including pushing, biting, scratching, and nipping), stand and box (stereotyped aggressive response when the animals rear and face each other but no contact is made) and vocalizations (hissing or squealing).

Biochemical analyses. At the conclusion of testing (70 days after the first 6-OHDA injections), animals were decapitated and brains dissected into three pieces [3]: forebrain (all tissue anterior to a plane bisecting the superior colliculus and the optic chiasm, excluding the pineal gland), hypothalamus, and brainstem (medulla and pons, excluding the cerebellum). Brain pieces were homogenized in 5 volumes 0.02 N HCl saturated with KCl. A 2 ml aliquot was removed and added to 5 ml of butanol for determination of 5HT levels [19]. A 0.3 aliquot was removed for other analyses and 4 ml of 0.4 perchloric acid was added to the remaining homogenate for assays of NE and DA levels [14].

RESULTS

Assays

The results of the assays are shown in Table 1. Both 6-OHDA treated groups showed lowered forebrain NE levels, $F(2,65) = 120$, $p<0.001$; forebrain DA levels were much less affected. Animals chronically treated with PCPA had lowered brain 5HT, $F(1,65) = 1888$, $p<0.001$ but there was a lowering of both NE and DA in these animals as well.

Open Field

During the initial 5 min of the open field test the PCPA and 6-OHDA x 3 animals locomoted less than did the Controls ($p<0.05$, Dunnett's [1] tests); the 6-OHDA x 6 animals ambulated slightly more than the Controls. Both groups treated with 6-OHDA showed fewer rearing responses than Controls ($p<0.05$, Dunnett's tests) whereas the PCPA animals did not. The 6-OHDA x 3 group made the fewest number of rearing responses ($t = 3.1$, $p<0.01$ when compared with all other groups combined). Thus, the 6-OHDA x 3 animals showed a suppression of locomotion in open field like PCPA animals but a suppression of rearing more like 6-OHDA x 6 animals.

After 5 min in open field, stimulation was introduced (flashing lights and intermittent noise). The 6-OHDA x 3 group showed the largest increases in behavior when stimulated: they showed larger increases in locomotion ($t = 2.23$, 43 *df*, $p<0.05$) and in rearing ($t = 2.89$, $p<0.01$) than all other groups combined.

Shock Elicited Aggression

Table 1 also shows the fighting among pairs from the same group. The PCPA animals fought most when shocked. The only other group which showed significant differences from Controls was the 6-OHDA x 3 group, who showed increased fighting postures.

Light Aversion

Figure 1 shows mean speeds to escape (cross a hurdle) from a flashing light. Across all trials, all three drug-treated groups showed faster escape speeds when the bright light was presented than did Controls ($p<0.05$, Dunnett's tests), but there was a different pattern among these groups. As testing continued, the 6-OHDA x 6 animals showed progressively longer crossing latencies and engaged in fewer spontaneous crossings (they became more inactive) whereas PCPA animals showed progressive increases in activity, producing a significant interaction between groups and trials: $F(3,33) = 7.16$, $p<0.01$. The 6-OHDA x 3 animals showed decreasing spontaneous crossings but progressively shorter latencies to cross the Bright light. They learned to escape from bright light stimulation better than any of the other groups. In an analysis of discriminated crossing speeds (Bright minus Spontaneous) at the end of testing, the 6-OHDA x 3 group had faster discriminated crossing speeds than any of the other three groups ($p<0.05$, Dunnett's tests).

Light Onset

Preference for rearing and touching the bar which turned on the dim light was computed across blocks of trials by computing *t* tests, comparing each animal's responses to the neutral bar. The Controls had the greatest initial preference for the light, but this declined with time. The PCPA and 6-OHDA x 6 animals showed significant, although smaller initial preferences for the light, but learned and habituated at different rates (interaction between drug treatments and blocks of time was significant: $F(4,132) = 4.11$, $p<0.005$). Only the 6-OHDA x 3 group showed an initial avoidance of visual stimulation, making significantly more responses to the neutral bar than to the positive bar during the initial block of time ($t = 2.16$, $p<0.05$).

TABLE 1

	Control	Experimental Group 6-OHDA x 3	6-OHDA x 6	PCPA
Forebrain levels (μg/g tissue)				
Mean forebrain monoamine levels at sacrifice				
NE	.346	.210*	.182*	.290*
DA	.889	.884	.825	.754
5HT	.516	.497	.504	.042*
Open field				
Mean number of squares entered (Locomote) and rearing responses during the initial 5 min in open field				
Locomote	39.3	29.3*	41.2	29.0*
Rear	6.5	4.3*	4.9*	6.3
Shock-elicited aggression				
Mean number of occurrences of fighting and vocalizing during 20 shock trials. PCPA animals are the most aggressive, followed by 6-OHDA x 3 rats				
Fight	5.42	9.09	8.25	13.75*
Stand and box	8.42	15.36*	12.75	17.67*
Vocalize	1.83	3.91	2.17	9.17*

*Means significantly different from Controls, $p<0.05$, Dunnett's tests.

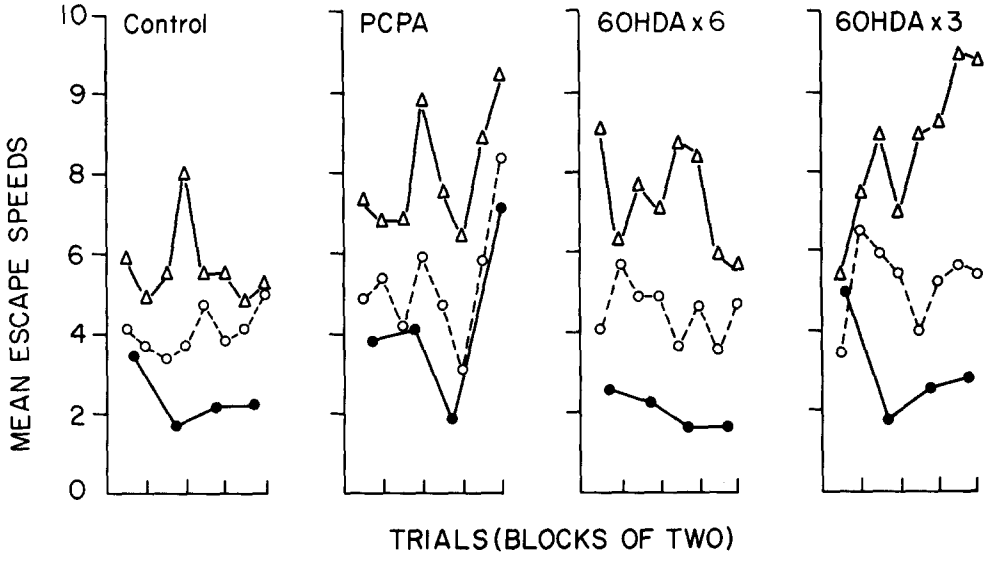

FIG. 1. Mean escape speed (reciprocal of latency in seconds X 100) to cross a hurdle in order to escape from photic stimulation upon presentation of △——△ Bright light; ○- - -○ Dim light; ●——● Spontaneous crossings (no light). "PCPA" animals show increasing activity as the stress of testing continues; "6-OHDA X 6" animals become progressively less active. "6-OHDA X 3" animals show progressively faster escape speeds to the Bright light but progressively decreasing spontaneous crossings, and learn to escape from strong visual stimulation most discriminantly.

DISCUSSION

Although there were relatively small differences in amount of central NE depletion observed in the two 6-OHDA groups, these two groups behaved distinctively differently on virtually every behavioral test. The major procedural difference between the two 6-OHDA injected groups was in the way they were lesioned and tested, with the 6-OHDA X 3 animals being given an extensive (6 week) recovery period before testing began, but the 6-OHDA X 6 animals being relesioned before testing. The present results provide evidence for recovery after 6-OHDA, for the 6-OHDA X 3 animals showed two behaviors indicative of increased arousal: decreased locomotion accompanied by freezing in a novel environment and increased shock-elicited aggression. These two high arousal behaviors were also present in PCPA animals, but not in the animals with more extensive NE damage (6-OHDA X 6). This finding is con-

sistent with other evidence of balanced and opposed actions of NE and 5HT on the nervous system [2,4], for it implies that low 5HT (PCPA) and supersensitive NE (6-OHDA × 3) animals are in a similar state of biochemical imbalance in stressful testing situations.

But there was a highly distinctive characteristic of the 6-OHDA × 3 rats in this study: they actively avoided visual stimulation in a way not present in any of the other groups. These 6-OHDA × 3 rats initially avoided novel visual stimulation in light onset when all other groups were working to produce it, and they learned to cross a hurdle faster than all other groups to escape from stronger visual stimulation in light escape. These characteristics of the 6-OHDA × 3 rat, together with other evidence of exaggerated arousal behaviors in these animals, imply that the exaggerated reactivity of partially recovered, supersensitive NE circuitry to novel stimulation produces an extreme of arousal that is aversive. The 6-OHDA × 3 rats of both this study and a previous one [15] also reared less in open field than any other groups tested.

The 6-OHDA × 3 rats are also highly hyperactive and self-stimulatory in activity wheels [15]. This complex behavioral syndrome suggests that these animals can serve as models for aspects of hyperkinesis or minimal brain dysfunction, for these animals are hyperactive, hyperemotional, actively avoid novel stimulation, and show a very low rate of rearing, or observing, responses. Electrophysiological evidence of lowered cortical arousal in hyperkinesis and data indicating lowered NE in depression [13] have led to the proposal of lowered NE in hyperkinesis [18]. Other studies of hyperkinesis have led to the description of intense electrical storms set off by novel stimulation and mediated via the reticular formation [6,8]. It is notable that both of these are precisely the mechanisms which appear to underlie the behavioral syndrome of the rat partially recovered from central NE disruptions: overall lowered NE levels accompanied by a supersensitive NE arousal system when stressed. Furthermore, studies of recovery following central NE lesions indicate that the earlier in life the lesion is made, including prenatally, the greater is there an exaggerated recovery of brainstem NE concomitant with lowered cortical NE [7, 16, 20].

Any therapeutic effects of d-amphetamine on the 6-OHDA × 3 rat would be clearly paradoxical, for these animals actually show a potentiated amphetamine activation effect [15]. But the chronic administration of such a stimulant might counteract the basic low NE depression in those brain areas which are still not innervated by NE circuitry and, by providing a steady, sustained background of NE release, counteract the extreme hyperreactivity and supersensitivity of NE circuitry which apparently develops in 6-OHDA × 3 rats.

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