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Effects of 6-Hydroxydopamine on Shock-Elicited Aggression, Emotionality and Maternal Behavior in Female Rats

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SORENSON, C. A. AND M. GORDON. *The effects of 6-hydroxydopamine on shock-elicited aggression, emotionality and maternal behavior in female rats.* PHARMAC. BIOCHEM. BEHAV. 3(3) 331–335, 1975. – Tests of emotionality and shock-elicited aggression, which have revealed consistent changes in behavior in male rats given 6-hydroxydopamine (6-OHDA), were administered to female rats intraventricularly injected with 250 μg of 6-OHDA to determine whether aspects of the 6-OHDA syndrome are shared by female and male rats. The results confirmed that female rats become hyperemotional as well as hyperaggressive after 6-OHDA, as do males. Using the hypothesis that the behavioral effects of 6-OHDA lesions of catecholamine neurons result from a general reduction in threshold to aversive stimuli, it was predicted that arbitrarily selected stimuli having aversive components should enhance the behavior normally elicited by these stimuli. Stimuli selected for testing were those that normally elicit maternal defensive aggression and maternal retrieval. Both of these behaviors were found to be enhanced in 6-OHDA treated female rats, thus supporting the prediction. These findings were interpreted as inconsistent with the hypothesis that norepinephrine chemically codes specific behaviors, particularly aggressive behavior.

6-Hydroxydopamine Open field test Shock-elicited aggression Maternal aggression Maternal retrieval Female rats

A NUMBER of studies have presented evidence that central catecholamines (CAs), particularly norepinephrine (NE), are involved in the manifestation of some forms of aggressive behaviors in animals, specifically those referred to by Moyer [12] as "irritable" or "spontaneous" aggression. For example, pharmacological treatments which are known to markedly increase the level and release of central NE elicit spontaneous fighting between rats. This has been reported following the combination of a monoamine oxidase inhibitor and a tricyclic antidepressant [7] or after a monoamine oxidase inhibitor plus L-DOPA or amphetamine [15]. Similarly, treatments known to elicit aggressive behaviors, such as decortication [16] or stimulation of the amygdaloid nuclei [17] or lateral hypothalamus [10] of cats, are associated with an enhanced turnover of central NE with no concomitant change in either dopamine [17] or serotonin [18].

Studies such as these have led to the hypothesis that central NE release is a causal factor in the types of aggressive behavior studied, i.e. that central NE somehow acts as a chemical code for these forms of aggression [4].

It would appear that such a hypothesis could not readily account for the chronic elevation in shock-elicited aggression (SEA) typically observed in the animal intraventricularly injected with 6-hydroxydopamine (6-OHDA) [4,25], since this treatment chronically depletes CAs [34] through selective terminal destruction [3]. However, it has been postulated that this elevation in aggressive behavior is based upon the development of a supersensitivity in CA neural circuits not destroyed by 6-OHDA [4,25]. This postulate is based partly on the observation that 6-OHDA lesions which produce enhanced SEA also increase behavioral activation to amphetamine, while more extensive lesions are followed by no increase in SEA and a subnormal response to amphetamine [25].

An alternative hypothesis which could account for the enhanced SEA seen following 6-OHDA treatment has been proposed [24]. It states that this increased aggressive behavior is due to a lowered threshold for responding to all aversive stimuli, so that the animal fights more vigorously in response to those stimuli which normally elicit aggression in the rat, e.g. the combined stimulus of pain and the presence of another animal. The important point is that this behavioral change is not viewed as a change specific to aggression. This hypothesis is based upon the observation that increased aggressive behavior in 6-OHDA treated animals is

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accompanied by a complex syndrome of hyperreactivity to aversive stimuli which include novel cages, especially those containing bright lights; handling; quinine; and loud tones [24, 25, 26], none of which involve aggression. The anatomical organization of the neural system damaged by 6-OHDA further supports a nonspecific interpretation. The system involved consists of neurons whose cell bodies are located within the reticular formation of the brain stem and whose processes extend to all levels of the neuraxis [33]. This configuration matches that of classic reticular formation neurons described by Scheibel and Scheibel [20], which are thought to be involved in the receipt and processing of incoming information [32].

The purpose of the present study was to test this reduced aversion threshold hypothesis. One specific prediction is that any aversive stimulus which elicits an innate response (or fixed action pattern) should produce an enhanced response in the appropriately lesioned animal. Therefore, two such behaviors were chosen for testing: maternal aggression, i.e. the protection of the young observed maximally for 24–48 hr post-parturition, and maternal retrieval, i.e. recovery of a pup removed from the nest – a behavior with no obvious aggression components. Both of these behaviors appear to be innate and to involve aversive stimuli [1].

Since the behavior of female rats treated with 6-OHDA has not been previously reported, a secondary purpose was to determine whether the 6-OHDA-treated female rat responds as the similarly-treated male does. Since 6-OHDA enhances emotionality [25] (as measured by a reduction in open field locomotor activity and rearing, and increased boli scores) as well as SEA in males when used in moderate doses [4,25], these measures were made on the 6-OHDA-treated female rats in the present study.

METHODS

Animals

Twenty-seven female Sprague-Dawley rats initially weighing 162–210 g were used in this study. They were housed individually and permitted free access to Purina rat chow and water.

Surgical Procedure

Twelve animals were stereotaxically injected in the right lateral ventricle with 250 μg of 6-OHDA HBr (calculated as the base) in 20 μl of saline solution (with 0.2 mg/ml of ascorbic acid added to retard autooxidation). The remaining 15 animals were injected with an equal volume of the saline solution alone. After a 2 week recovery period, the animals were administered the following behavioral tests.

Behavioral Tests

Open field test. This apparatus consisted of a 114 cm square field with 45 cm walls and a floor divided into 25 squares. Both walls and floor were painted a flat black. Two 150 W bulbs were suspended 45 cm above the center of the field and provided the only illumination. For testing, the animal was taken from her cage and placed in an 8 × 6 cm box, which was inverted over her in the start square of the field. After 30 sec the box was lifted, marking the beginning of the test trial. During the ensuing 3 min test period, the number of squares entered, the number of boli dropped, and the number of rearings were recorded. This test was run on 4 consecutive days. All animals were tested.

Shock-elicited aggression. Twenty-six animals (twelve 6-OHDA and 14 controls) were tested in a shock box with scrambled shocks (2 mA rms and 0.5 sec duration). Each animal was paired with another of its own group. Each pair was placed in the testing box, allowed a 2 min period of habituation, and then given a series of 100 shocks with a 10 sec inter-shock interval. Their behavior during each shock was scored according to the 4 categories established by Blanchard and Blanchard [2]: (A) both animals fighting, lunging, striking, or biting, (B) only one animal fighting, lunging, striking, or biting, (C) one animal or both standing and facing, (D) no response other than running, jumping or vocalization. This test was done blind.

Maternal retrieval. All animals were mated, resulting in pregnancies in eight 6-OHDA animals and 10 controls. Approximately 5 days prior to parturition, pregnant females were moved to special observation cages in a separate room. These cages (43 × 18 × 12 cm) were divided into 2 equal compartments by a wooden hatch which remained closed prior to testing. Each animal was placed in one of the compartments and provided with food, water, and nesting materials. Roughly 12 hr after the rat gave birth, testing was initiated. All of the pups were removed from the nesting side of the cage and 5 of them were placed on the floor of the adjoining compartment in a predetermined pattern corresponding to the points of an ×. The hatch was then lifted, and the time it took the mother to return all 5 pups to the nesting compartment was recorded.

Maternal aggression. Thirty-six hr following the birth of the pups, an unfamiliar male rat was introduced for 2 min into the nest of each of the 18 animals with pups, and the latency to an aggressive response by the female was recorded. An aggressive response was defined as the female fighting, lunging, striking or biting the male. The test was done blind.

Biochemical Assay

Upon the completion of the behavioral tests, eleven 6-OHDA and 11 control animals selected randomly from the larger groups were decapitated while unanesthetized and their brains quickly removed. NE and dopamine (DA) levels of each whole brain was determined using the fluorometric procedures of Walters and Roth [35].

RESULTS

The single 250 μg injection of 6-OHDA resulted in a depletion of both NE (73.6 percent) and DA (51.2 percent). These depletions were highly significantly reliable as compared to controls (see Table 1).

Under these conditions, 6-OHDA markedly and consistently altered open field behavior over the 4 days measured (Table 2). The 6-OHDA animals locomoted less, reared less, and dropped more fecal boli, thus displaying the full pattern of behaviors identified with heightened emotional reactivity.

When tested for shock-elicited aggression, 6-OHDA treatment again induced marked changes, characterized by increased aggressiveness as reflected primarily by increases in Categories B and C (one animal fighting, lunging, striking or biting, or one animal or both standing and facing) (Table 3).

TABLE 1

MEAN LEVELS OF WHOLE BRAIN NE AND DA

Group	NE	DA
6-OHDA 250 μg	68 ± 12	338 ± 43
Control	259 ± 18	693 ± 63
	(73.6%)	(51.2%)
	($p<0.0001$)	($p<0.0001$)

Values presented are means in ng/g ± s.e.m. In parentheses are shown percent depletion. Statistical comparisons were made between groups using two-tailed *t*-tests.

TABLE 2

EFFECTS OF 6-OHDA ON ACTIVITY, REARING AND BOLI SCORES IN THE OPEN FIELD OVER FOUR DAYS

	Day 1	Day 2	Day 3	Day 4	Overall Mean Score
Activity					
6-OHDA	10.8 ± 3.4	8.7 ± 2.6	4.3 ± 1.7	3.3 ± 1.9	6.8 ± 1.6
Control	29.9 ± 2.7	20.5 ± 5.7	22.4 ± 3.6	27.9 ± 3.6	25.3 ± 3.4
	($p<0.001$)	($p<0.1$)	($p<0.001$)	($p<0.001$)	($p<0.001$)
Boli					
6-OHDA	2.5 ± 0.8	2.3 ± 0.6	1.6 ± 0.6	1.4 ± 0.4	1.9 ± 0.4
Control	0.5 ± 0.2	0.6 ± 0.3	0.4 ± 0.1	0.1 ± 0.1	0.5 ± 0.2
	($p<0.02$)	($p<0.02$)	($p<0.02$)	($p<0.01$)	($p<0.01$)
Rearings					
6-OHDA	3.1 ± 0.9	2.4 ± 0.5	1.5 ± 0.4	1.7 ± 0.5	2.2 ± 0.4
Control	4.8 ± 0.7	4.9 ± 0.6	5.6 ± 0.6	6.0 ± 0.6	5.3 ± 0.4
	($p<0.2$)	($p<0.01$)	($p<0.001$)	($p<0.001$)	($p<0.001$)

Each score represents the mean ± s.e.m. of twelve 6-OHDA animals or 15 controls. Values were compared using two-tailed *t*-tests.

The effects of 6-OHDA on maternal retrieval are presented in Table 4. As can be seen, the 6-OHDA animals had a highly significant reduction in overall latency to retrieve the 5 pups. These results thus support the prediction that maternal retrieval would be facilitated in the 6-OHDA group.

Similarly, maternal aggression was facilitated in the 6-OHDA animals. None of the 10 control animals displayed any aggressive responses to the intruding male, but 4 of the eight 6-OHDA animals were aggressive. This difference is significant beyond the 0.025 level (using the Fisher Exact Probability test for small independent samples). The four 6-OHDA animals showing aggression had a mean latency to respond of 29.25 sec.

DISCUSSION

The results of the open field and shock-elicited aggression tests show that female rats injected intraventricularly with 250 μg of 6-OHDA display marked emotionality and

TABLE 3

EFFECT OF 6-HYDROXYDOPAMINE ON SHOCK-ELICITED AGGRESSION

Group	A	B	C	D
6-OHDA	1.3 ± 0.9	7.7 ± 1.9	42.8 ± 5.1	48.1 ± 7.1
Control	0.0	1.1 ± 1.0	18.3 ± 4.7	80.6 ± 5.4
	($p<0.2$)	($p<0.01$)	($p<0.01$)	($p<0.01$)

Statistical comparisons were made using two-tailed *t*-tests.

TABLE 4

EFFECT OF 6-OHDA ON MATERNAL RETRIEVAL

Group	N	Latency to Retrieve
6-OHDA	8	216 ± 18.1 sec
Control	10	372 ± 18.4 sec
		($p<0.0001$)

Statistical comparisons were made using two-tailed *t*-tests.

hyperaggressiveness, as defined by these tests. These results suggest, therefore, that there are no substantial differences between male and female rats on these measures and thus increases the generality of the syndrome which follows moderate doses of 6-OHDA.

The finding of an augmentation in maternal aggression and retrieval behaviors in these 6-OHDA treated females conforms precisely to the prediction that the 6-OHDA animal which shows enhanced SEA will hyperreact to arbitrarily chosen stimuli having aversive components. The list of stimuli to which 6-OHDA-treated animals have been found to hyperreact now includes: another animal plus footshock [4,25], novel cages plus bright light [25], quinine solutions [26], loud tones [24], an intruding male plus an appropriate hormonal condition and pups, and pups displaced from the nest. To view the elevation in aggressive behavior seen in 6-OHDA-treated animals in isolation from these other behaviors as evidence for a modification in aggressive circuitry *per se* would appear to be an unwarranted, or at least premature, conclusion. 6-OHDA-treated animals have been found to have a lowered threshold for each aversive stimulus which has been applied, suggesting that the NE system modified by the 6-OHDA lesion subserves a much more general function.

This interpretation can be applied to other preparations thought to support the hypothesis that NE chemically codes aggressive behavior. It may be that in each study reporting an increase in aggressive behavior associated with an increased turnover of NE that the aggressive behavior is due to the lowering of some general threshold for responding to aversive stimuli. Perhaps in situations where fighting is spontaneous the threshold is so low that only the presence of another animal is necessary, whereas when fighting is irritable, e.g. shock-elicited, the threshold is reduced less, so that a combination of painful stimulation and an appropriate releasing stimulus (the other animal) are necessary. Expanding testing procedures to include behaviors not obviously related to aggression might demonstrate whether or not manipulations producing aggression simultaneously enhance a variety of other behaviors elicitable by aversive stimulation.

It should be clear that the hypothesis of a reduced threshold for aversive stimuli possesses no mechanistic qualities. The question as to why this reduced threshold exists remains to be answered.

At least two possible mechanisms can be tentatively offered. First, it may be that a noradrenergic system involved in the control of both baseline arousal and in the receipt and processing of information is damaged by 6-OHDA. Recovery processes may compensate precisely for baseline arousal (baseline activity levels do recover to normal in 6-OHDA treated animals [25]), but the response to external inputs might be potentiated because of the additional demands these inputs place on the system, giving rise to a behavioral supersensitivity. Indirect evidence suggests that the development of a neuronal supersensitivity is involved in this recovery process. This is inferred from studies showing that animals pretreated with moderate doses of 6-OHDA display enhanced behavioral activation to amphetamine [6] even when lesions are restricted to NE terminals [25], while animals given larger doses of 6-OHDA, thus destroying more terminals, show subnormal behavioral activation to amphetamine [6,25]. This model would seem to implicate an altered function of the dorsal NE bundle as responsible for the observed behavioral changes, because it has an ideal distribution to function in the control of arousal and of information processing [8,33].

However, a second alternative (nor necessarily mutually exclusive) seems equally likely. It may be that the 6-OHDA-treated animal has deficient reward mechanisms. It has been proposed that reward-punishment is controlled by balanced neural systems [27,36]. If the balance is shifted by the lesion towards punishment, or negativity, the response to aversive stimuli would be expected to be enhanced as observed. In addition, the response to positive stimuli should be depressed, and this has been reported for consumption

of sucrose [26] as well as for brain stimulation [28]. This model would appear to implicate the ventral NE bundle, as its distribution is primarily to hypothalamus, amygdala, and other limbic system structures implicated in mechanisms of reward [33].

This model of the 6-OHDA-treated animal, which is hyperreactive to negative inputs, would view it as having essentially the converse syndrome as that of the animal treated with para-chlorophenylalanine (PCPA), which shows a hyperreactivity to positive stimuli. It is hypersexual [23,30], shows increased responding for rewarding brain stimulation [14], increases its consumption of sucrose solutions [5], and shows enhanced muricidal behavior [4], which may be viewed as appetitive in nature [9]. Consistent with a shift toward reward in the PCPA animal is a decreased responsiveness to the effects of punishment [11, 19, 29].

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