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Induction of Rage in Rats By Central Injection of 6-Hydroxydopamine[1]

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COSCINA, D. V., J. SEGGIE, D. D. GODSE AND H. C. STANCER. *Induction of rage in rats by central injection of 6-hydroxydopamine.* PHARMAC. BIOCHEM. BEHAV. 1(1) 1–6, 1973.– Intracisternal injection of 300 μg of 6-hydroxydopamine in male rats elicited a syndrome of hyperreactivity or hyperemotionality, i.e., rage, similar to that previously reported after septal or ventromedial hypothalamic (VMH) lesions. Specifically, rats showed increased resistance to capture as well as increased number and magnitude of startle responses compared to vehicle injected or normal controls. As with septal but not VMH lesions, this rage subsided with repeated testings (handling). These findings are discussed with regard to the possible importance of brain neurotransmitters in the expression of behaviors.

Rage Norepinephrine 6-hydroxydopamine Serotonin Intracisternal Dopamine

STIMULATION or destruction of certain structures along the limbic hypothalamic axis can produce profound effects on the expression of affective or emotional behaviors [12]. The importance of intact limbic circuitry for normal emotionality is suggested from reports of marked hyperemotionality and hyperreactivity after septal lesions. Among the many behavioral abberations associated with septal rage are a striking alertness to approaching movements, explosive startle reactions to auditory or tactile stimulation, and vicious attacks linked with loud vocalizations, urination and defecation when attempts are made to handle such animals [2]. Similar emotional responsivity has been reported after ventromedial hypothalamic (VMH) lesions [31]. This syndrome of rage behaviors appears immediately after lesions of either forebrain structure but, in the case of septal lesions, eventually subsides with repeated handling [2, 18, 24, 26]. Although anatomical, endocrinological and behavioral explanations have been advanced to account for certain aspects of these lesioned induced behaviors, a complete comprehension of the neural events associated with this syndrome is not yet available.

Injections of 6-hydroxydopamine (6-OHDA) directly into the ventricular system of mammals produce long lasting or permanent depletion of the brain catecholamine, norepinephrine (NE), but have little effect on the indoleamine, 5-hydroxytryptamine (5-HT or serotonin) [1, 3, 7, 10]. A second brain catecholamine, dopamine (DA), is usually depleted by such treatment as well, though often less severely so and not as permanently as with NE [3, 7, 10]. While conducting several neuropharmacological experiments with rats following intraventricular or intracisternal injections of 6-OHDA, the first author was struck by the irritable nature of these animals. Moreover, this irritability, which has been alluded to by others who have used this compound [1, 10], seemed qualitatively similar to that which occurs after septal or VMH injury. In the hopes of gaining further insight into neural mechanisms associated with aberrant emotional behaviors, we have begun to study systematically the neurochemical and behavioral time course of this drug induced rage. In this paper, we report the results of repeated observations on 6-OHDA treated rats using behavioral ratings previously employed to assess septal and VMH rage [2, 18, 24]. These data suggest that centrally administered 6-OHDA can mimic both qualitative and quantitative aspects of emotional behaviors which have, in the past, been associated with limbic diencephalic injury.

METHOD

Animals

Thirty-five male albino rats of the Wistar strain (High Oaks Ranch, Ontario) were used. Animals were housed in

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separate metal cages with wire mesh fronts and bottoms, and fed water and Rockland lab chow ad lib throughout the experiment. Cages were located in an animal colony with controlled temperature (24° ± 1° C) and lighting conditions (12-hr light-dark cycle; lights on at 0800 hr).

Injections

After two weeks adaptation to the controlled environment, rats weighed 300-320 g. At that time, 16 rats were anesthetized with 60 mg/kg of sodium pentobarbital (i.p.) after pretreatment with 0.1 mg atropine sulfate (i.p.) then placed in a David Kopf stereotaxic instrument. With the incisor bar adjusted to its lowest extreme, 300 μg of 6-OHDA (Calbiochem) was injected into the cisterna magna in 20 μl of vehicle (0.9% saline with 0.01% ascorbic acid added to retard oxidation). All injections were made through a 26 gauge needle attached to a 100 μl Hamilton Microsyringe. Rats were removed from the stereotaxic instrument and placed in a prone position with their feet elevated above their head to assist equal distribution of the injected material throughout the ventricular system [5]. An additional 14 rats were treated in exactly the same manner except that 20 μl of the vehicle without 6-OHDA was injected intracisternally. The remaining 5 rats were not injected nor behaviorally tested so as to provide normal brain tissue for chemical assays which were performed later.

Of the 16 rats receiving 6-OHDA, 3 became ill before the completion of the behavioral testing and were deleted from the experiment. Accordingly, the number of vehicle injected controls which were behaviorally tested for comparison with 6-OHDA rats was reduced from 14 to 10.

Behavioral Tests

Twenty-three rats were rated on 5 separate rating scales (see Table 1) on Days 3, 9, 17, 24, and 31 after injection. Four of these measures have been used before by one of us (J.S.) to rate septal rage [25]. In general, these tests represent similar assessments used by others for this same purpose [2, 18]. A fifth measure, the number of quadrants entered in three min (see Table 1), was included to provide some index of rats' activity.

The first measure, resistance to capture, was scored during capture in the homecage. The remaining four measures were scored while each rat was in a two-ft dia. drum marked off into equal quadrants. Table 1 describes the sequence of testing. All scores obtained were analyzed separately between groups for each test category rather than combining scores to give only one behavioral index as done previously [2, 18]. This avoided the necessity of considering the relative equality of any given measure to an overall summed index. All ratings were made by one observer who had much previous experience with such testing procedures.

Chemical Assays

Following the last rating session on Day 31, all animals were left undisturbed in their homecages for two weeks. Then, 5 rats which had received 6-OHDA and 4 rats which had received the vehicle were randomly selected from each of their respective groups and sacrificed by decapitation for determinations of forebrain NE and 5-HT levels. The 5 untreated and untested normals were also sacrificed at this time. All brains were rapidly removed from the calvarium, washed with cool 0.9% saline, and the forebrain dissected away from the hindbrain by a coronal section from the corpora quadrigemina to the mammillary bodies. Forebrains were blotted with filter paper, weighed, and then frozen in liquid nitrogen until assay the following day. Concentrations of both monoamines were determined by the method of Maickel and co-workers [22]. Fluorescence readings were made with an Aminco-Bowman spectrophotofluorometer. Separate determinations of NE and 5-HT recoveries, both from aqueous standards and from brain homogenates to which exogenous amines were added, produced reliable results across several independent runs (range = 75–85% for both amines). Therefore, the assay results obtained for brain extracts of test animals were not corrected for recovery.

Statistical Analyses

All behavioral data for each of the five test categories were analyzed by separate two-way analysis of variance (ANOVA), the two factors being groups (6-OHDA vs. vehicle treated) and days (five test sessions per measure). A correction for repeated measures on the second factor was employed. Fluorescence readings from brain extracts were analyzed between groups by *t*-tests for independent samples for each amine determination. All F-tests and *t*-tests were two-tailed.

RESULTS

Qualitative Observations

After recovery from anesthesia, rats which had received 6-OHDA appeared quiet, hypoactive, and, in some cases, mildly sedated in their homecages. Over the next week their general appearance returned to normal, although many animals were anorexic and hypodipsic and did not appear to groom normally during this time. It was felt that these anomalies, which have been observed before [1, 10, 28], were responsible in more severe form for the illness which developed in 3 of the 16 rats which received 6-OHDA.

By the second behavioral rating session (Day 9), the 13 rats which received 6-OHDA seemed virtually normal if left undisturbed. However, if aroused with a puff of air through the front of the cage, these animals typically exhibited an exaggerated startle reponse. In some cases, the response was so explosive that the rats would bounce around the cage for several seconds after such stimulation, then freeze on their haunches after the hyperreactivity subsided. In some of the animals, sliding the homecage open was sufficient to elicit such explosive responding that rats leaped out of their cages and hopped wildly around the floor. When attempts were made to seize the escaped rats, they offered extreme resistance to capture, i.e., struggled vigorously when contained with a gloved hand, biting the glove viciously while squealing, urinating and defecating. These behaviors abetted abruptly when rats were returned to their homecages. This general syndrome is strikingly similar to that described years ago for septal rats [2] and is qualitatively similar to that of VMH rats (Coscina, unpublished observations]. Such forms of hyperemotionality seemed maximal between one and two weeks after injection, then began to subside gradually over the next two weeks.

TABLE 1

FIVE CATEGORIES USED TO ASSESS SEPTAL-LIKE SYNDROME

Order of Testing	Category	Scoring Criteria
1	Resistance to capture (in homecage)	0 - rat remains calm when approached or grasped with gloved hand
		1 - rat avoids glove slightly and/or offers some resistance when grasped
		2 - rat clearly avoids glove by running around cage and/or struggles when captured
		3 - rat jumps out of cage and may struggle when captured
		4 - rat jumps out of cage and/or struggles vigorously and bites glove when captured
2 and 3	Number of Quadrants	Number of quadrants entered with three feet during 3 min inside cylinder
	Freezing	Number of seconds maintaining motionless posture (little or no vibrissa movements) during 3 min inside cylinder
4	Magnitude of first Startle Response (after tap on back with metal rod)	0 - no response
		1 - flinch or twist of whole body
		2 - flinch of body and rapid retreat
		3 - jump or hop (all feet leave floor)
		4 - high leap (at least 6 in. above floor) and rapid retreat
5	Number of Startles	Number of consecutive startle responses to tap on back before three consecutive nonresponses (maximum of 20 startles)

Vehicle treated rats showed none of these behavioral anomalies and, in all respects, appeared completely normal throughout the observation period.

Behavioral Testing

The qualitative findings reported above were largely reflected in the quantitative measures obtained with the five rating scales. As shown in Fig. 1, resistance to capture (top frame) for 6-OHDA rats was greater than for vehicle treated rats 3 days after injection, became maximal by Day 9, decreased on Day 17 and approached control levels by Days 24 and 31. The 2-way ANOVA results revealed that the differences between groups was statistically significant ($p<0.001$). In addition, test results were different across days of testing ($p<0.001$) and there was an interaction effect between groups and days ($p<0.001$).

Both the magnitude of the first startle response and the number of consecutive startles (Frames 2 and 3 from the top, Fig. 1) followed a time course similar to that seen for resistance to capture. Maximal responding occurred 9 days after injection with higher than control responding on Days 3 and 17. Although there was a gradual decline on both measures by Days 24 and 31, values for drug treated rats remained slightly higher than controls because the latter animals showed virtually no responding by this time. The 2-way ANOVA results for each measure showed that responses of drug treated rats were significantly higher on both measures than controls ($p<0.001$). In addition, scores varied significantly across days on both measures ($p<0.001$ and 0.005). Again, an interaction effect between groups and days was found ($p<0.001$ and 0.01).

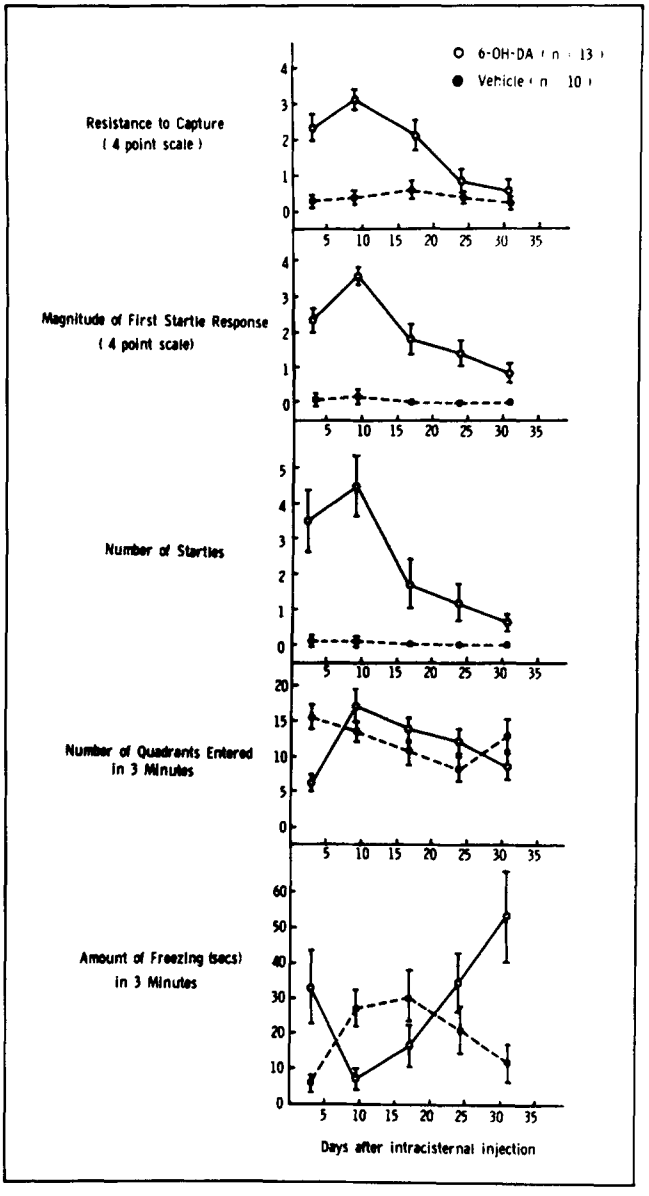

FIG. 1. Means and standard error of 6-OHDA group (○; n = 13) and vehicle control group (●; n = 10) on the five behavioral tests plotted across days after intracisternal injection. Description of tests and scoring criteria in Table 1.

In terms of number of quadrants entered in 3 min (Frame 2 from bottom, Fig. 1), 6-OHDA rats were slightly hypoactive on the first day, became more active on Day 9, then decreased activity along with controls over the remaining three test sessions. The 2-way ANOVA results revealed no significant differences between groups on this measure ($p>0.05$), a trend toward days effects ($0.10>p>0.05$) and a significant interaction effect between days and groups ($p<0.001$). Freezing behavior (bottom frame, Fig. 1) showed an inverse relationship, in general, to activity measures. Freezing was slightly greater than control values for 6-OHDA rats on the first test day, decreased on Day 9, and increased from then on to a maximum on Day 31. The 2-way ANOVA results revealed a trend toward group differences ($0.10>p>0.05$) but no differences across days regardless of group type ($p>0.05$). Again, there was a reliable interaction effect between groups and days of testing ($p<0.005$).

Chemical Assays

As shown in Fig. 2, mean levels of NE obtained from brain samples of selected 6-OHDA rats were 67% lower ($p<0.001$) than corresponding data from vehicle treated or normal untreated controls. On the other hand, means levels of 5-HT from these same brain samples were only 12% lower than controls after 6-OHDA. While this 5-HT depletion was considerably smaller ($p<0.01$) than that seen for NE concentrations, it was nonetheless statistically different from control values ($p<0.05$). There were no differences in NE or 5-HT concentrations between brain samples from vehicle treated or normal untreated animals.

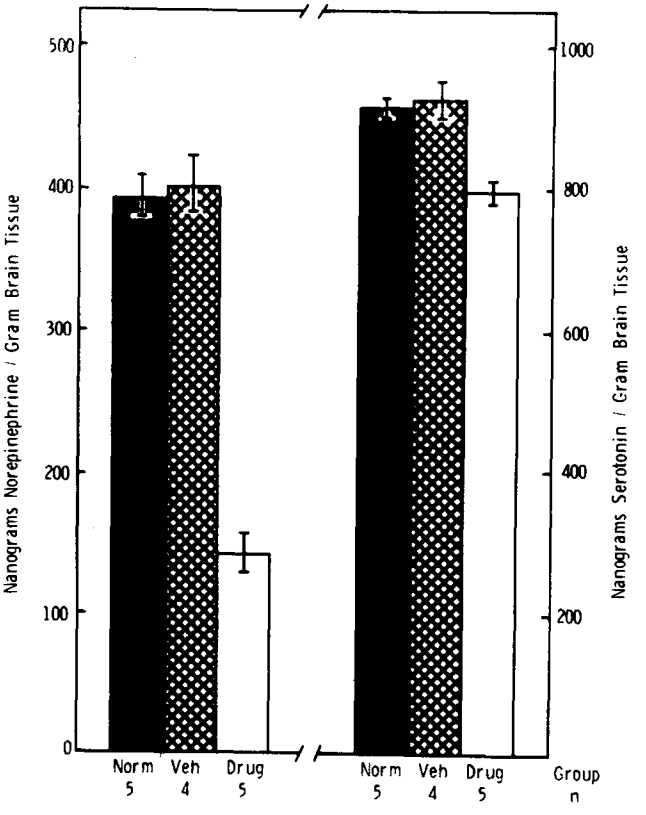

FIG. 2. Means and standard error of NE and 5-HT in brain samples from normal (solid bar; n = 5), vehicle treated (cross hatched bar; n = 4), and 6-OHDA treated (open bar; n = 5) rats.

DISCUSSION

From the data presented, it seems clear that centrally administered 6-OHDA is capable of producing several affective changes not unlike those seen after septal or VMH lesions [2, 26]. The gradual diminuition of this rage, as

assessed both qualitatively and by quantitative measures of resistance to capture, number of startles and magnitude of first startle, seems more like that reported after septal injury since VMH lesions produce permanent rage [26]. The mild hypoactivity seen after 6-OHDA on the first day of testing (see Fig. 1) might also be taken as a symptom of brain dysfunction common to septal or VMH rats since it compares favorably with reports of decreased locomotion in these animals [6, 17]. However, the lack of reliable statistical differences between groups across days of testing, linked with corroborating reports by others of no activity changes in rats after 6-OHDA [4, 10, 30], suggests that the initial decrement seen on Day 3 reflected the rats' illness proximal to drug treatment and not a true motor debilitation.

There are reasons to believe that behavioral changes other than those reported here after 6-OHDA treatment are common to septal or VMH rats. For example, previous work has shown that 6-OHDA increases aggression elicited by electric foot shock [9]. Similar increments in shock induced aggression have been seen after septal or VMH injury [8]. Of course, such parallels in behavior need not imply that 6-OHDA induces neural damage restricted to septal and/or VMH regions. In fact, the use of intracisternal or -ventricular routes to administer this compound assures widespread distrubition of the drug's effects thereby precluding any specific site of action per se. In addition, such drug treatment does not produce all of the behavioral anomalies known to occur after either type of forebrain lesion. For example, we found little consistency in freezing after 6-OHDA treatment in contrast to marked increments in such behavior after septal damage [24]. Also, our 6-OHDA rats were anorexic and hypodipsic during the first week after injection (c.f. [1,28] for similar observations). However, septal or VMH lesions induced increments rather than decrements in drinking or feeding, respectively [13, 17]. Finally, 6-OHDA produces little effect on jump flinch thresholds to foot shock [9] or on conditioned avoidance responding [29]. In contrast, septal lesions decrease jump flinch thresholds [21] and both types of brain lesions enhance avoidance responding [18, 19, 20]. Therefore, centrally administered 6-OHDA does not produce a spectrum of behavioral changes which mirror those produced by septal or VMH lesions. Nevertheless, such treatment does appear capable of mimicking portions of the syndromes associated with either type of forebrain insult. These behavioral similarities are perhaps best evidenced in emotionality changes as reported here.

It seems plausible to speculate that behavioral similarities and differences between drug treated and brain lesioned rats result from similarities and differences, respectively, in levels of brain monoamines. Such a notion presupposes that the behavioral effects which we were measuring and which occur in rats following discrete brain lesions are a consequence of neurochemical changes elsewhere in the C.N.S. [14]. So, for example, damage to the septum can produce decrements in forebrain levels of NE [15], 5-HT [15, 16] and acetylcholine [27]. As reported here and corroborated by many others [1, 3, 4, 7, 9, 10, 29, 30], 6-OHDA induces marked depletion of brain NE with little or no change in brain 5-HT. Additional work suggests that 6-OHDA does not alter levels of brain acetylcholine (no change in choline acetylase activity after 6-OHDA; G. R. Breese, personal communication). The possible behavioral consequences of brain lesions and resultant neurochemical changes is less clear regarding VMH injury. We could find no reports of marked changes in forebrain amine levels after discrete VMH damage.

The present data suggest that brain NE serves some functional, perhaps inhibitory, role in the expression of emotional responses to abrupt somatosensory stimulation. Very recent support for this suggestion is implied by the work of Nakamura and Thoenen [23] who observed long lasting irritability in rats after two 300 μg injections of 6-OHDA in the lateral ventricles. Unfortunately, it was not made clear whether animals were repeatedly tested over the 130 day observation period or whether different groups were tested only once at different times after the two injections. This is an important consideration since handling is known to diminish such irritability in the case of rage induced by septal lesions [2, 18, 24]. Alternatively, procedural differences such as dose of drug used, route of administration and specificity of amine depleted might explain the difference in duration of irritability observed by Nakamura and Thoenen and ourselves.

While 6-OHDA produced significant increments in several affective responses, the time course of these behavioral changes and their possible relationship to brain amine metabolism is not clear at this time. Our observations of decreased responsivity with repeated testings implies that drug induced rage was transient in spite of persisting NE depletion. This fact may mean that there is some type of functional reorganization of neurochemical mechanisms over time – a change which was not measureable by simple determinations of amine levels alone (e.g., altered turnover rates at remaining synaptic sites; receptor supersensitivity). It is also possible that the metabolism of other brain amines (e.g., DA), which were not measured, may have been affected by 6-OHDA. These additional changes, either alone or in some combination with altered NE metabolism, might play an important role in the magnitude and duration of the behavioral changes reported.

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