Pharmacology Biochemistry and Behavior, Vol. 3, pp. 525–528. ANKHO International Inc., 1975. Printed in the U.S.A.

BRIEF COMMUNICATION

Taming Effects of Handling on 6-Hydroxydopamine Induced Rage[1]

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(Received 2 August 1974)

COSCINA, D. V., J. GOODMAN, D. D. GODSE AND H. C. STANCER. *Taming effects of handling on 6-hydroxydopamine induced rage.* PHARMAC. BIOCHEM. BEHAV. 3(3) 525–528, 1975. – The purpose of this experiment was to determine the importance of handling to the expression of hyperemotional behaviors, i.e., rage, known to occur after chronic depletion of brain norepinephrine (NE) and dopamine (DA) following central injection of 6-hydroxydopamine (6-OHDA) in rats. Five min of handling per day for 6 consecutive days reduced resistance to capture as well as the magnitude and frequency of startle responding following one 300 μg injection of 6-OHDA intracisternally. Both 6-OHDA-handled and 6-OHDA-unhandled rats showed comparable levels of brain NE, DA, serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), comparable resting levels of plasma corticosterone, and comparable adrenal weights. These data demonstrate the importance of handling to the expression of 6-OHDA-induced rage and emphasize the importance of controlling for handling as a variable which can significantly affect the assessment of rage by behavioral criteria in this animal model of hyperemotionality.

6-Hydroxydopamine Hyperirritability Dopamine Hyperreactivity Serotonin Rage Taming 5-Hydroxyindoleacetic acid Hyperemotionality Handling Corticosterone Adrenal gland Norepinephrine

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COSCINA *et al.* have recently reported that one intracisternal injection of 300 μg 6-hydroxydopamine (6-OHDA) in rats is sufficient to chronically deplete brain norepinephrine (NE) and dopamine (DA) levels [2] and to produce a transient syndrome of hyperemotional behaviors operationally defined as rage [3]. The qualitative aspects of these behaviors as well as their transient time-course were similar to those known to occur after bilateral septal injury [1]. Only one other paper has appeared in which 6-OHDA-induced rage has been quantified using rating scales similar to ours. In that report, Nakamura and Thoenen [8] report permanent (130 days of testing) rage following two 300 μg injections of 6-OHDA intraventricularly spaced 24 hr apart. Since 6-OHDA-induced rage may represent a useful animal model for the study of certain biochemical abberations associated with affective disorders [8], we were interested in accounting for the time-course differences reported in these two studies.

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[1] This research was supported by funds from the Clarke Institute of Psychiatry. Portions of this work were presented at the 81st Annual Meeting of the American Psychological Association held in Montreal, Quebec, Canada in August, 1973. The behavioral data reported were obtained by the second author (J.G.) as part of a B.A. Honor's Thesis in Psychology at the University of Toronto (1973). We gratefully acknowledge the excellent technical assistance of Rick Johnston, Peter Chan, and David Wilson throughout various phases of this work. Appreciation is extended to Dr. G. M. Brown whose lab performed the plasma corticosterone assays.

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Several procedural aspects of 6-OHDA administration and/or testing of animals may account for Nakamura and Thoenen's [8] observation of permanent rage. These procedures include their use of: (1) higher total and/or spaced doses of 6-OHDA (2) different route of drug administration, and/or (3) slightly different rating procedures. Additional work by us (unpublished observations) suggests that neither the route of injection nor the rating scale differences can explain the time-course differences between these two studies. Nakamura (personal communication, 1973) feels that the higher total dose of 6-OHDA administered in a spaced fashion may be responsible. One additional variable which might have contributed to these differences was the amount and distribution of handling of animals during testing. Previous work shows that rage following septal ablation dissipates more quickly with repeated testing or handling [3, 4, 5, 10, 12]. In our previous paper [3], we tested our animals more frequently but for a shorter time duration than did Nakamura and Thoenen [8]. If handling contributed to the time-course differences between these two studies, we hypothesized that frequent handling would ameliorate the rage induced by central 6-OHDA administration.

METHOD

Animals

Forty-three male albino rats (Wistar strain, High Oaks Ranch, Ontario) were used. Rats were individually housed in a temperature (72°F ± 3°) and light (0800–2000 hr on) controlled colony with food (on the floor of cages) and water (in bottles with sipper tubes) available ad lib.

Injection and Testing Procedures

When rats weighed 320–350 g, 35 of them were pretreated with atropine methyl nitrate (2 mg, IP), anesthetized with sodium pentobarbital (Nembutal, 35–50 mg/kg, IP) and injected intracisternally with 300 μg (expressed as free base) 6-OHDA-HCl (Calbiochem) dissolved in 20 μl of 1 percent ascorbic acid – distilled water (see [3] for details of injection procedure). Another 8 rats served as uninjected, normal controls. Previous work has shown that vehicle injections produce neither biochemical nor behavioral effects [2,3].

Seven days after 6-OHDA treatment, all rats were rated for resistance to capture (0–4 point scale), the magnitude of startle response to the first tap on the hindquarters with a metal rod (0–4 point scale), and the number of consecutive startles (maximum of 20) before 3 consecutive nonresponses (see [3] for details of testing procedure and rating criteria). On the basis of these behavioral ratings, both 6-OHDA-treated and normal groups were divided into handled and unhandled subgroups with comparable main-group emotionality scores. Rats designated for handling were removed from cages and handled 5 min daily for the next 6 days. Handling consisted of placing rats on a table top, allowing 5–10 sec exploration, picking them up for 3–4 sec, and repeating this sequence. Unhandled rats were left undisturbed in homecages.

After 6 days of handling or no-handling, rats were rerated on the 3 emotionality scales (Day 14 of experiment). Since there were clear differences between 6-OHDA subgroups as a function of handling, one additional rating was performed on Day 21 after all rats were left undisturbed for 6 additional days.

All ratings and handling were performed by an experimenter well trained in the rating procedure. The experimenter was unaware of rats' group designations during testing.

Biochemical and Statistical Procedures

At the completion of behavioral testing, all rats were left undisturbed for one week, then sacrificed within 30 sec of removal from cages by decapitation between 1300 and 1500 hr. Brains were removed and prepared for fluorometric determinations of endogenous forebrain NE, DA, serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) levels [2]. In addition, blood was obtained from the cervical wound of each rat for determinations of plasma corticosterone levels (method of [7] as modified by [11]). Adrenal glands were removed and weighed on a Mettler Microgram Balance.

All between-groups comparisons were analyzed by *t*-tests for independent samples while within-groups comparisons were analyzed by *t*-tests for dependent samples [6]. Biochemical data derived from brain amine determinations were also subjected to cross-correlational analyses (Pearson product moment correlations). All tests were two-tailed.

RESULTS

General Effects of 6-OHDA Administration

Of the 35 rats receiving 6-OHDA, 14 died and 5 were too ill to complete the experiment. Mortalities reflected lack of recovery from anesthesia or frequent convulsive episodes. Sublethal side effects included cachexia due to hypophagia and hypodipsia. All of these side effects have been observed before in this dose range of 6-OHDA [2, 3, 13]. Because of these deletions, final group sizes were reduced to 8 handled and 8 unhandled 6-OHDA-treated rats.

Behavioral Testing

Seven days after 6-OHDA treatment, all injected rats showed exaggerated responses ($ps<0.001$) on all 3 rating scales ($\bar{X}$s of 3–4) compared to controls ($\bar{X}$s of 0–0.5). However, after 6 days of handling (Fig. 1), 6-OHDA rats showed significant diminuitions on all rating scales compared to 6-OHDA-unhandled rats (all $ps<0.01$). Handling produced no significant changes in behaviors of normals. An additional 6 days without handling produced no statistical changes from the profiles obtained on Day 14. Scores of 6-OHDA-handled rats were still lower than scores of 6-OHDA-unhandled rats (all $ps<0.01$) while within-group comparisons between Day 14 and 21 produced no differences. Again, normals were never distinguishable as a function of handling.

Biochemical Analyses

Forebrain tissues from both 6-OHDA-treated groups contained less ($ps<0.001$) NE and DA than samples from either normal group (see Table 1). Concentrations of NE and DA were positively correlated in tissues from all 6-OHDA-treated rats ($r = +0.574$, $df = 14$, $p<0.05$) but not from all normals ($r = +0.476$, $df = 6$, $p>0.10$). 5-HT levels were slightly lower ($p<0.05$) in 6-OHDA-treated rats

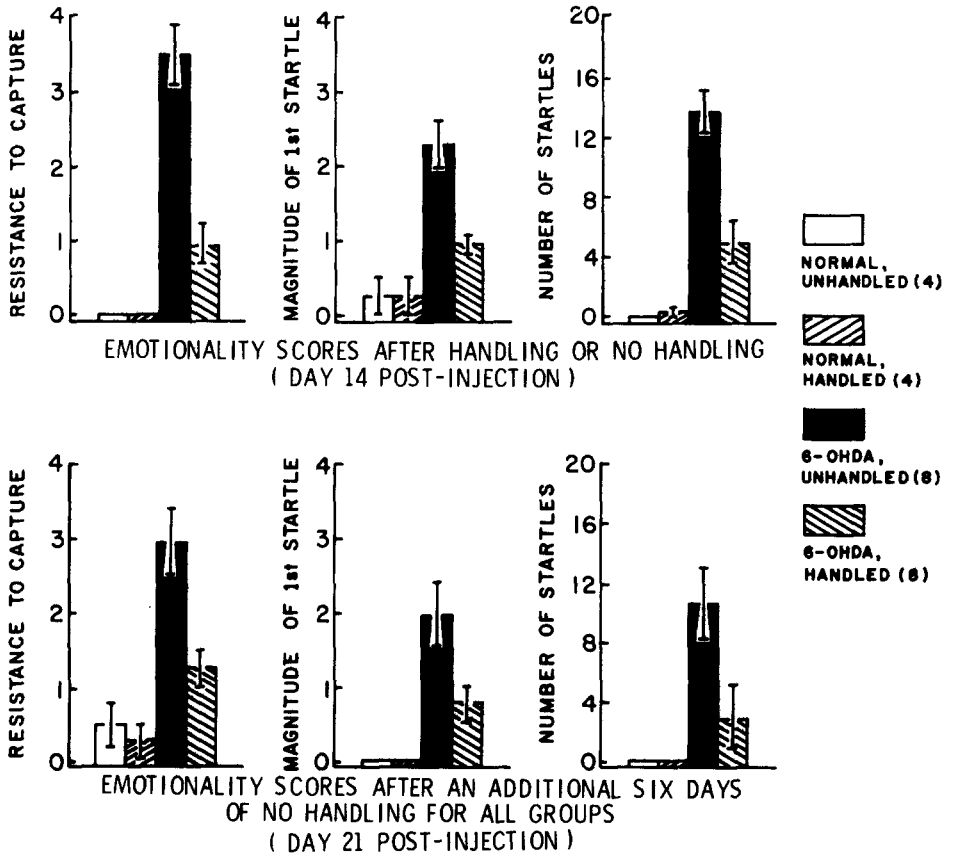

FIG. 1. Means and standard errors of mean emotionality scores, as described in the text for each rating scale, for normal or 6-OHDA-treated rats receiving handling or no-handling after intracisternal injection. Group designations and Ns are depicted in the figure for each group. The top 3 histograms represent individual emotionality scores of these groups after 6 days of handling or no handling (Day 14 post-injection). The bottom 3 histograms represent retests of these same groups after 6 additional days of no handling for all groups (Day 21 post-injection).

compared to controls (13 percent depletion). Levels of 5-HT were not correlated with NE or DA concentrations in either main group. No differences were found across groups for levels of 5-HIAA or for the ratios of 5-HT/5-HIAA. No differences were found among brain amine levels as a function of handling.

Concentrations of plasma corticosterone did not differ between 6-OHDA-handled and 6-OHDA-unhandled rats ($\bar{X}$s ± SEMs = 7.78 ± 0.95 μg/100 ml plasma vs. 7.72 ± 0.70, respectively). Plasma from unhandled normals contained slightly more ($p<0.02$) corticosterone than did plasma from handled normals (8.95 ± 0.17 vs. 7.45 ± 0.41, respectively). No differences were found between 6-OHDA-treated and normal controls as a function of handling. No differences were found among groups for total adrenal weights.

DISCUSSION

As hypothesized, brief (5 min) handling every day for 6 days produced marked reductions in rage, which lasted at least one week, after 6-OHDA treatment. A similar form of handling has been found effective in reducing septal rage [10]. In contrast to 6-OHDA-treated rats, normal rats could not be distinguished behaviorally as a function of handling.

Biochemical measurements of endogenous brain amines did not provide an explanation for the behavioral differences between 6-OHDA-handled and 6-OHDA-unhandled rats. Forebrain NE and DA levels in these groups were markedly depressed compared to controls while levels of 5-HT were marginally affected. In all cases, however, such depletions were unrelated to post-injection handling treatments. The observation of correlated NE and DA depletion in 6-OHDA-treated rats confirms our previous data [2] which implies a similar mode of action by this drug on both catecholaminergic systems in the dose range used.

Determinations of resting plasma corticosterone levels were also unable to explain the behavioral differences between 6-OHDA subgroups. If 6-OHDA-induced rage were related to enhanced adrenocortical activity, we expected to see higher corticosterone values in 6-OHDA vs. normal rats or between 6-OHDA-unhandled vs. 6-OHDA-handled rats. No such differences were observed. These findings, linked with the lack of difference in adrenal weights across groups, suggest that resting adrenocortical activity plays no clear role in the causation of 6-OHDA-induced rage. In keeping

TABLE 1

MEAN ENDOGENOUS LEVELS OF FOREBRAIN NE, DA, 5-HT AND 5-HIAA*

Group	N		NE	DA	5-HT	5-HIAA
6OHDA-handled	8	$\bar{X}$	118†	351†	763‡	550
		SEM	6.3	19.2	24.5	13.5
6OHDA-unhandled	8	$\bar{X}$	113†	378†	784‡	572
		SEM	11.1	18.1	18.1	11.7
Normal-handled	4	$\bar{X}$	417	1290	845	570
		SEM	6.3	38.1	42.5	39.8
Normal-unhandled	4	$\bar{X}$	385	1264	928	595
		SEM	16.4	13.8	31.3	20.5

*Data expressed as ngm/gm brain tissue, wet weight
†$p<0.001$ compared to appropriate normal control
‡$p<0.05$ compared to appropriate normal control

with this suggestion, rats with bilateral septal injury have been shown to have higher corticosterone levels than controls after stress, but are indistinguishable from them on resting measures [14]. Therefore, elevations in plasma corticosterone associated with septal-rage seem a consequence of over responsiveness after stimulation but not a cause of such responsiveness. Our preliminary data here do not refute a similar possibility in 6-OHDA treated rats.

In conclusion, the present data support the possibility that Nakamura and Thoenen's [8] observation of permanent rage after 6-OHDA treatment may in part reflect the less frequent testing, hence handling, of experimental animals than that which occurred in our previous study [3]. Additional support for the suggestion that multiple testings affect the time-course of rage is implied from a recent report [9] in which centrally injected 6-hydroxydopa produced hyperemotionality which diminished over three days of daily ratings using scales much like our own. Taken together, these and other [3, 4, 5, 10, 12] findings point out the need for careful control of handling in experimental models of hyperemotionality. Clearly, variations in handling can significantly alter the magnitude and time-course of various dependent variables used to assess these models.

REFERENCES

1. Brady, J. V. and W. J. H. Nauta. Subcortical mechanisms in emotional behavior: affective changes following septal forebrain lesions in the albino rat. *J. comp. physiol. Psychol.* **46**: 339–346, 1953.
2. Coscina, D. V., C. Rosenblum-Blinick, D. D. Godse and H. C. Stancer. Consummatory behaviors of hypothalamic hyperphagic rats after central injection of 6-hydroxydopamine. *Pharmac. Biochem. Behav.* **1**: 629–642, 1973.
3. 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**: 1–6, 1973.
4. Glass, J. D. and G. J. Thomas. Effects of cortical ablations upon recovery from the septal syndrome in hooded rats. *Physiol. Behav.* **5**: 879–882, 1970.
5. Gotsick, J. E. and R. C. Marshall. Time course of the septal rage syndrome. *Physiol. Behav.* **9**: 685–687, 1972.
6. Hays, W. L. *Statistics for Psychologists.* New York: Holt, Rinehart and Winston, 1963.
7. Murphy, B. E. P. Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurements of various steroids in body fluids by competitive protein-binding radioassay. *J. clin. Endocr.* **27**: 973–990, 1967.
8. Nakamura, K. and H. Thoenen. Increased irritability: a permanent behavior change induced in the rat by intraventricular administration of 6-hydroxydopamine. *Psychopharmacologia* **24**: 359–372, 1972.
9. Richardson, J. S., N. Cowan, R. Hartman and D. M. Jacobowitz. On the behavioral and neurochemical actions of 6-hydroxydopa and 5,6-dihydroxytryptamine in rats. *Res. Communs. chem. path. Pharmac.* **8**: 29–45, 1974.
10. Seggie, J. Effect of somatosensory stimulation on affective behavior of septal rats. *J. comp. physiol. Psychol.* **66**: 820–822, 1968.
11. Seggie, J. and G. M. Brown. Septal lesions and resting adrenal function. A possible explanation of conflicting findings. *Neuroendocrinology* **8**: 367–374, 1971.
12. Singh, D. Comparison of hyperemotionality caused by lesions in the septal and ventromedial hypothalamic areas in the rat. *Psychon. Sci.* **16**: 3–4, 1969.
13. Stricker, E. M. and M. J. Zigmond. Effects on homeostasis of intraventricular injections of 6-hydroxydopamine in rats. *J. comp. physiol. Psychol.* **86**: 973–994, 1974.
14. Uhlir, I., J. Seggie and G. M. Brown. The effect of septal lesions on the threshold of adrenal stress response. *Neuroendocrinology* **14**: 351–355, 1974.