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Impairment of Avoidance Performance by Intrastriatal Administration of 6-Hydroxydopamine

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NEILL, D. B., W. O. BOGGAN AND S. P. GROSSMAN. *Impairment of avoidance performance by intrastriatal administration of 6-hydroxydopamine.* PHARMAC. BIOCHEM. BEHAV. 2(1) 97–103, 1974. – Bilateral administrations of crystalline 6-hydroxydopamine to the ventral anterior striatum of rats reliably impaired the performance of avoidance responses. The 6-OHDA treatments depleted the forebrain of dopamine but did not reliably alter forebrain levels of norepinephrine. A significant correlation was found between the extent of the depletion of forebrain dopamine and the magnitude of the avoidance deficit. These results support the hypothesis that dopaminergic components of the striatum may be involved in avoidance behavior.

Corpus striatum Dopamine Norepinephrine Avoidance 6-hydroxydopamine

SEVERAL investigators have reported that lesions in the corpus striatum of rats impair the acquisition of both one-way [10,11] and two-way (shuttlebox) [7,13] avoidance responses. Neill and Grossman [13] have reported that lesions in the ventral portion of the anterior striatum of rats impaired shuttlebox avoidance learning, whereas the local administration of the anticholinergic agent scopolamine to the same site facilitated the acquisition of the same behavior. This pattern of effects suggested that the ventral striatum of the rat might contain either fibers of passage or non-cholinergic synapses which are destroyed by a lesion but are not affected by a drug that may preferentially interfere with cholinergic mechanisms. The first of these interpretations is supported by Runnels and Thompson [17], who have suggested that the effects of striatal damage on avoidance behavior may be due to the disruption of a descending fiber system which originates in the frontal cortex. That non-cholinergic mechanisms in the striatum may play a role in avoidance behavior is suggested by the results of experiments [12] which have demonstrated that ventral striatal lesions impair the performance of pre-operatively trained avoidance responses more effectively than comparably sized lesions in the dorsal striatum and that the ventral lesions also deplete greater quantities of forebrain dopamine than dorsal lesions.

The following experiment was designed to further investigate these alternatives. Recent experiments [5,9] have suggested that 6-hydroxydopamine (6-OHDA) may selectively damage central catecholaminergic nerve terminals. Administered intraventricularly, 6-OHDA depletes dopamine and norepinephrine from the brain but spares serotonin and acetylcholine [9]. Ungerstedt [19] has recently reported that microinjections of this compound along the nigrostriatal dopaminergic path deplete dopamine from the striatum without producing significant non-specific damage. This suggests that the local administration of 6-OHDA may selectively destroy the catecholaminergic components of the striatum while sparing non-catecholaminergic cells and fibers of passage. Such a preparation permits a direct test of the alternative hypotheses discussed above.

METHOD

Animals

Twenty male albino rats, obtained from Holtzman (Madison, Wisc.), were used. All weighed 350–380 g at surgery. They were singly housed in a continuously lighted colony with food and water available ad lib.

Apparatus

Two identical shuttleboxes, each measuring 55 × 22 × 32 cm, were used. Each box was constructed of Plexiglas with a floor of 4 mm dia. stainless steel rods, placed 1 cm

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apart. The CS was supplied by a 7 W bulb placed in a recess in the center of the top cover of each side. Constant-current shock was obtained from a high voltage source [2] and was pulsed (0.3 sec on/0.3 sec off). Movement of the rat from one side to the other was detected by photocells located 7 cm on each side of the center of the box. Each crossing was recorded by a high speed timer/printer. Each box was housed in a sound-attenuating chamber with an exhaust fan.

Procedure

Training. The animals were trained before surgery as follows: Sixty-three seconds after the animal was placed into the shuttlebox, an overhead light (CS) which illuminated only the half of the apparatus in which the animal happened to be was turned on. If the rat ran into the dark half of the apparatus within 5 sec after the CS onset, an avoidance response was recorded. If the animal remained in the illuminated half of the apparatus longer than 5 sec, the current (0.1 ma, RMS) was applied to the grid floor in the illuminated half of the apparatus. The CS and US were terminated as soon as the animal had broken an infrared light beam 7 cm inside the dark half of the apparatus. Sixty-three sec separated the onset of successive CS presentations. During the intertrial interval (ITI) the animal could cross between the two sides of the apparatus without being shocked. Twenty such trials were given each day until each animal made at least 12 avoidances in 20 trials on each of three successive days. After this criterion was attained, the animals underwent surgery.

Surgery. The animals were placed in a stereotaxic apparatus under Nembutal anesthesia (50 mg/kg), supplemented with atropine methyl nitrate (10 mg/kg) to reduce respiratory problems. Double-walled stainless steel cannulas (outer: 23G; inner: 30G) were implanted bilaterally in 15 animals such that the tips of the cannulas terminated in the striatum. The upper portion of these cannulas was cemented to the skull. The tips of the cannulas were aimed at AP = 8.6, L = 2.7, H = 1.5 using the Pellegrino and Cushman [15] atlas of the rat brain. Five animals served as unimplanted but operated controls (the scalp was opened and burr holes drilled, but the cannulas were not lowered).

Drug administration. One week after surgery, the animals were tested for retention of the avoidance task and were given additional training until they again performed at least 12/20 avoidances on each of 3 consecutive days. After this criterion was regained, crystalline 6–hydroxydopamine hydrobromide (Regis Chem. Co., Chicago, Ill.) was administered to the striatum as follows: The inner cannulas were removed and cleaned. 6–OHDA was spread thinly on a glass plate and tamped into the tip of the inner cannulas. The inner cannulas were then returned to the animals' brains. Ten of the 15 implanted animals received cannulas containing the drug; the remaining 5 received empty cannulas (sham injections).

Total dosage of 6–OHDA administered over the five-day testing period was at two levels. An estimate of the amount of drug administered was made by weighing similarly loaded cannulas on a microbalance. Six animals received approximately 15 μg/cannula 2 hr before the first test session and an equal amount 20 hr before the fourth test session. The remaining 4 experimental animals received 15 μg/cannula 20 min before the first test session and 45 μg/cannula 20 hr before Test Sessions 2, 3, and 5. Thus, 6 animals received a total of 30 μg/cannula and 4 received 150 μg/cannula over the five-day test session. The injection interval before the first test session was varied to determine if any effects were observable within 2 hr of the application.

The effect of the drug treatments on avoidance performance was analyzed by comparing the number of avoidance responses on the fifth day after the first drug injection with the animals' average scores for the two days before the drug administration began. Student's *t*-tests for correlated means [18] were performed to evaluate statistical reliability.

In order to examine the possibility [16] that the behavioral effects of intrastriatal applications of crystalline 6–OHDA might be due to nonspecific neural damage, six rats not used in the behavioral experiments were sacrificed 30 days after intrastriatal 6–OHDA. Three received the high and 3 the low doses of 6–OHDA given to animals in the avoidance experiment. Two additional rats receiving the low dose of 6–OHDA were sacrificed 3 days after the last drug application. Fifty-micron frozen sections were taken through the region of the cannula tips of these animals and stained with cresyl violet for microscopic examination.

Biochemistry. Six days after the first 6–OHDA application, forebrain and hindbrain levels of dopamine (DA) and norepinephrine (NE) were determined in all experimental and control animals by a modification of the method of Anton and Sayre [1]. Forebrain included all tissue anterior to the superior colliculi; hindbrain included all tissue between the superior colliculi and the upper end of the spinal cord. Statistical comparisons were performed using *t*-tests for independent means [18].

RESULTS

Application of crystalline 6–hydroxydopamine to the ventral anterior striatum reliably impaired the performance of two-way avoidance (see Fig. 1). The magnitude of this impairment was related to the total amount of drug applied. A total dose of 30 μg/cannula lowered avoidance responding to 64% of the pre-drug level on the average, while a total dose of 150 μg/cannula lowered avoidance to 39% of the pre-drug level (see Table 1). Both of these impairments were statistically significant (low dose: $t = 3.29$, $df = 5$, $p<0.05$; high dose: $t = 3.40$, $df = 3$, $p<0.05$).

Biochemical assays revealed that the implantation of cannulas into the striatum did not reliably ($p>0.20$) alter either dopamine or norepinephrine in fore- or hindbrain when compared to unimplanted controls (see Table 2). The low dose of 6–OHDA depleted forebrain DA to 55% of the unimplanted control level, while the high dose depleted forebrain dopamine to 37% (see Table 2). These depletions were statistically reliable (low dose: $t = 3.31$, $df = 9$, $p<0.01$; high dose: $t = 3.31$, $df = 7$, $p<0.02$). Forebrain levels of norepinephrine were not significantly affected in the low dose group ($t = 0.50$, $df = 9$, $p>0.20$). A statistically unreliable tendency for NE depletion was observed in the high dose group ($t = 2.23$, $df = 7$, $0.05<p<0.10$). Hindbrain levels of NE were similar in all groups ($p>0.10$). Hindbrain levels of dopamine were reliably depressed ($t = 2.35$, $df = 9$, $p<0.05$) only in the group receiving a total of 30 μg/cannula of 6–OHDA.

These results indicate that reliable impairments in avoidance behavior can be produced by intrastriatal applications of 6–OHDA which deplete forebrain dopamine and spare norepinephrine. Further support for a specific relationship

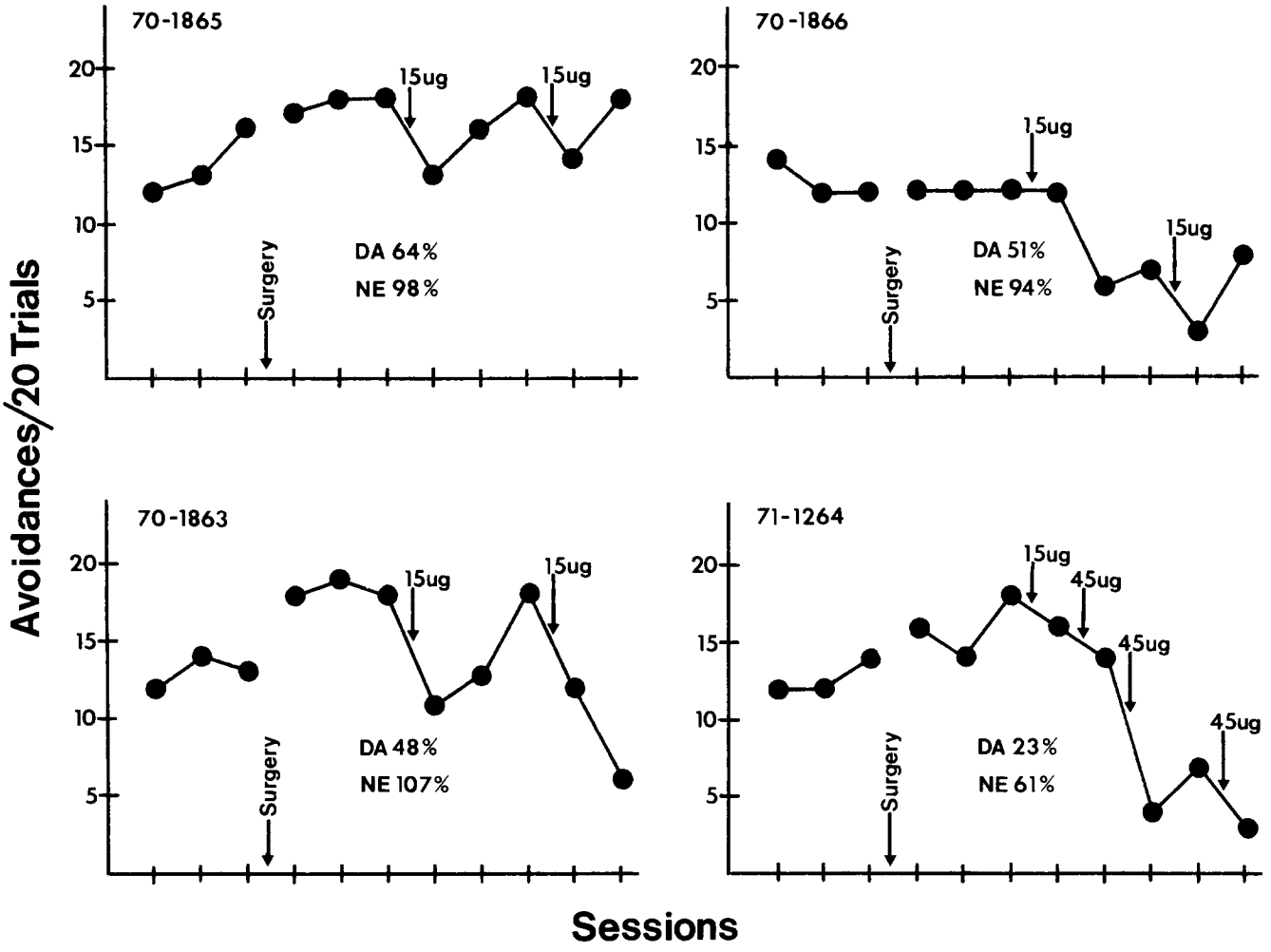

FIG. 1. Data from representative animals demonstrating the effects of intrastriatal applications of 6–OHDA on avoidance behavior. Forebrain levels of DA and NE are expressed as percentages of unimplanted control levels.

between forebrain dopamine and avoidance behavior was obtained by correlating the DA loss and avoidance decrement for individual animals receiving 6–OHDA. DA was calculated as a percentage of the average DA level of the non-cannulated control animals, and avoidance performance was calculated in terms of a percentage of each animal's average performance the last 2 days prior to the first drug treatment. A statistically significant ($r = 0.67$, $p<0.05$) product-moment correlation [18] was found in this comparison, suggesting that the severity of the avoidance impairment was related to the degree of DA depletion. Using the same procedure, there was no reliable correlation between avoidance behavior and NE levels ($r = 0.28$, $p>0.10$).

The drug required more than 20 min but less than 2 hrs to produce a reliable avoidance impairment. Fifteen μg/cannula applied 2 hrs before the first test session lowered avoidance responding to 80% of pre-drug levels ($p<0.05$), while application of the same amount 20 min before the first test session was completely ineffective (109% of pre-drug levels).

Examination of the areas around the cannula tips of the animals sacrificed 3 or 30 days after receiving intrastriatal 6–OHDA revealed small black deposits in the immediate vicinity of the implant tip. The remainder of the striatum did not show grossly abnormal tissue reactions (see Fig. 2).

TABLE 1

AVERAGE AVOIDANCES/20 TRIALS (± S.E.M.) BEFORE AND FIVE DAYS AFTER BEGINNING OF INTRASTRIATAL APPLICATIONS OF 6-OHDA

	Pre-drug Avoidances	Post-drug Avoidances	Percent Pre-drug Avoidance Responding	Percent Unimplanted Control Forebrain Dopamine
Unimplanted Controls (5)	14.5 ± 1.0	13.4 ± 0.8	92.4	–
Cannulated Controls (5)	16.3 ± 1.6	15.0 ± 1.1	92.0	95.0
Total 30 μg/cannula 6-OHDA (6)	16.3 ± 1.2	10.5 ± 2.3*	64.4	55.4
Total 150 μg/cannula 6-OHDA (4)	14.8 ± 0.9	5.8 ± 2.8*	39.2	37.5

*$p<0.05$, compared to pre-drug level

TABLE 2

CONCENTRATION (ng/g ± S.E.M.) OF CATECHOLAMINES IN FORE- AND HINDBRAIN AFTER INTRASTRIATAL APPLICATION OF 6-OHDA

	Forebrain		Hindbrain	
	Norepinephrine	Dopamine	Norepinephrine	Dopamine
Unimplanted Controls (5)	506 ± 75	1483 ± 212	424 ± 37	478 ± 76
Cannulated Controls (5)	511 ± 47	1410 ± 174	509 ± 62	466 ± 34
Total 30 μg/cannula 6-OHDA (6)	543 ± 27	822 ± 50†	493 ± 15	311 ± 14*
Total 150 μ/cannula 6-OHDA (4)	310 ± 24	556 ± 164*	423 ± 18	461 ± 36

*$p<0.05$, †$p<0.01$, compared to unimplanted controls

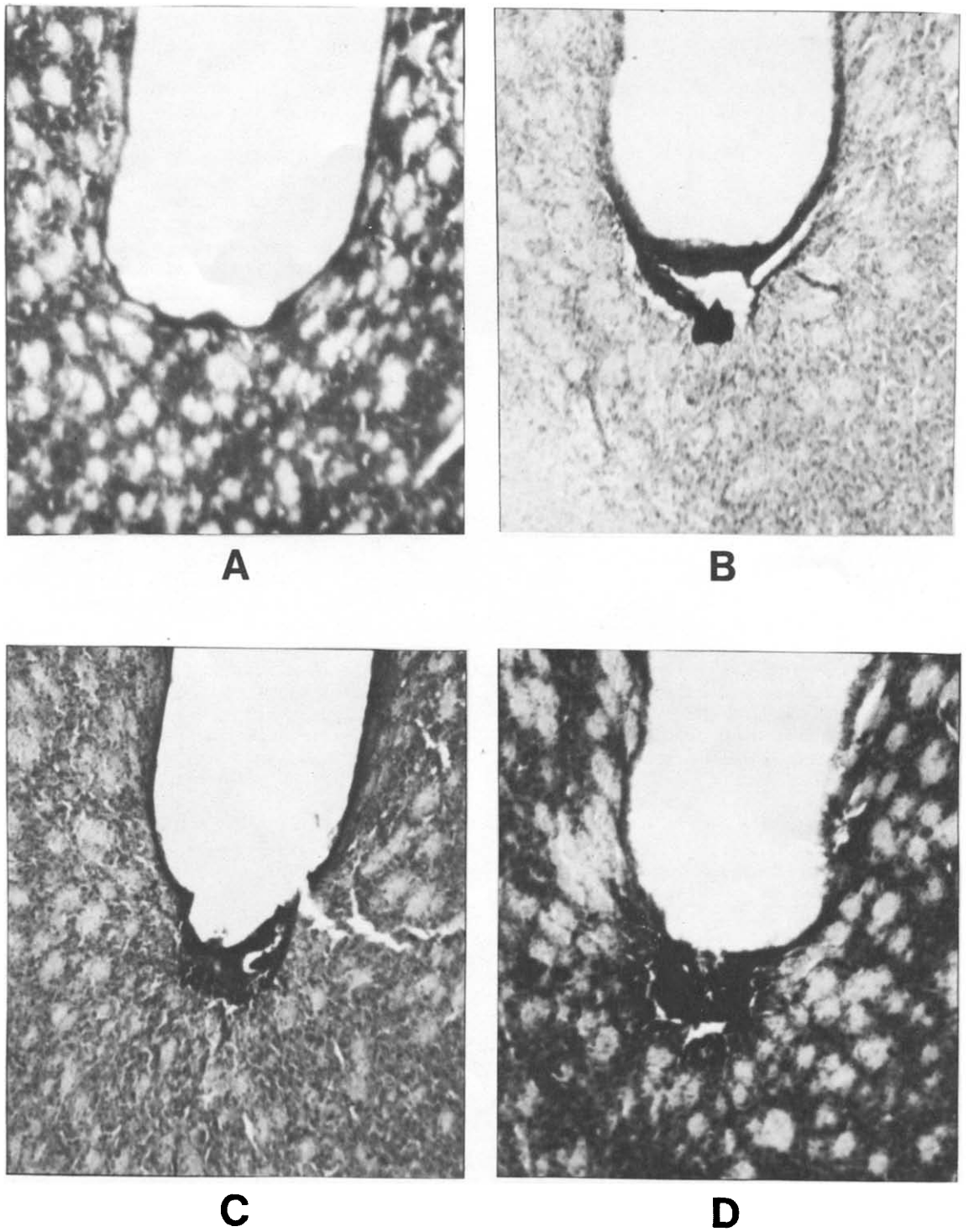

FIG. 2. Photomicrographs of 50 micron thick sections of corpus striatum showing the region of the cannula tips of rats: (a) after sham applications (b) 3 days after receiving the second of two 15 μg applications of 6–OHDA (c) 30 days after receiving the second of two 15 μg applications of 6–OHDA, and (d) 30 days after receiving the last of multiple applications of 6–OHDA totaling 150 μg.

DISCUSSION

The results of the present experiment demonstrate that intrastriatal applications of crystalline 6–hydroxydopamine impair the performance of shuttlebox avoidance and selectively deplete forebrain dopamine. Since the vast majority of forebrain DA is in the striatum [3], striatal depletion of this amine was probably responsible for most, if not all, of the loss reflected in our assay. Our biochemical results agree with data reported by Ungerstedt [19], and contrast with the effects of intraventricular injections of 6–OHDA, which are more effective in depleting forebrain NE than DA [5].

A statistically significant correlation was found between forebrain dopamine loss and the magnitude of the avoidance impairment. This pattern of effects supports previous suggestions [12,13] that the deficits in avoidance behavior seen after electrolytic lesions in the striatum may be due to an interruption of non-cholinergic components of the region. The results of our biochemical assays suggest that the avoidance impairments observed may be specifically related to a depletion of striatal dopamine. Recent results using intracisternal injections of 6–OHDA [6] have also suggested a critical role of dopamine in avoidance responding.

The principal issue which must be raised before we can attempt to interpret these interesting findings concerns the similarity between the behavioral and biochemical effects of 6–OHDA injections into the striatum and lesions in the same region. We [12,13] have previously shown that lesions in the ventral as well as dorsal striatum impair the acquisition and performance of avoidance responses and that the magnitude of this effect of the lesions correlates well with their effects on forebrain dopamine. Whenever a compound such as 6–OHDA is injected intracerebrally and is found to mimic the effect of a lesion, one must ask whether the effects of the drug might not be due to the non-specific blockade or destruction of tissue rather than a selective effect on catecholaminergic terminals. We would like to offer the following evidence in support of the tentative conclusion that the behavioral effects of our injections may indeed be specific to 6–OHDA: (a) microscopic inspection of the area surrounding the tips of cannulas used to repeatedly administer 6–OHDA in doses comparable to those applied in our behavioral experiments did not uncover evidence of abnormal tissue reactions outside a narrow band of tissue immediately surrounding the tip of the implant. This should be viewed in the context of the observation that the relatively large lesions produced by the implant itself did not reliably affect avoidance behavior or forebrain DA content (see Fig. 1 and Table 1). It appears unlikely that the addition of relatively little additional damage due to the drug could be responsible for the marked behavioral effects observed in the present study. (b) The application of various other compounds in crystalline form to the same area has been shown to either have no effect on avoidance performance (notably dopamine and norepinephrine; unpublished observations) or, in the case of scopolamine, to enhance avoidance learning [13] while not affecting performance [12]. These DA and NE applications which do not alter avoidance performance also leave deposits at the cannula tips similar to those of 6–OHDA, which does impair performance. (c) Finally, although the behavioral measure is different, intrastriatal applications of 6–OHDA depress responding for stimulation of the lateral hypothalamus, and this depression is reversed by subsequent application of dopamine through the same cannula [14].

If it is assumed that the effects of intrastriatal 6–OHDA on avoidance behavior and forebrain DA may, indeed, be due to a selective destruction of dopaminergic nerve terminals in the striatum, an interesting picture of the possible role of the striatum in avoidance learning emerges. We have previously shown that the administration of the anticholinergic agent scopolamine to the same region enhances shuttlebox avoidance learning [13]. The contrast of the effects of scopolamine and 6–OHDA suggests an interaction between cholinergic and dopaminergic components of the area for which there is some neurophysiological support. Investigations of the effects of iontophoretic applications of drugs to single striatal neurons have shown that single cells in that region are typically inhibited by dopamine but excited by acetylcholine [4,8]. One can relate these electrophysiological observations to our behavioral data to suggest that the efferent influences of the striatum on avoidance behavior may be inhibitory. A blockade of the cholinergic components of the system may reduce this inhibitory influence and thereby facilitate avoidance learning. A decrease in the activity of the dopaminergic component of the system, on the other hand, might facilitate the activity of this behaviorally inhibitory system and thus interfere with avoidance behavior.

REFERENCES

1. Anton, A. H. and D. A. Sayre. The distribution of dopamine and dopa in various animals and a method for their determination in diverse biological materials. *J. Pharmac. exp. Ther.* **145:** 326–336, 1964.
2. Belluzzi, J. D. and S. P. Grossman. A source of "scrambled" constant current with solid state control. *Physiol. Behav.* **10:** 133–135, 1973.
3. Bertler, A. and E. Rosengren. Occurrence and distribution of catecholamines in the brain. *Acta physiol. scand.* **47:** 350–361, 1959.
4. Bloom, F. E., E. Costa and G. C. Salmoiraghi. Anesthesia and the responsiveness of individual neurons of the caudate nucleus of the cat to acetylcholine, norepinephrine and dopamine administered by microelectrophoresis. *J. Pharmac. exp. Ther.* **150:** 244–252, 1965.
5. Breese, G. R. and T. D. Traylor. Effect of 6–hydroxydopamine on brain norepinephrine and dopamine: evidence for selective degeneration of catecholaminergic neurons. *J. Pharmac. exp. Ther.* **174:** 413–420, 1970.
6. Cooper, B. R., G. R. Breese, L. D. Grant and J. L. Howard. Effects of 6–hydroxydopamine treatments on active avoidance responding: evidence for involvement of brain dopamine. *J. Pharmac. exp. Ther.* **185:** 358–370, 1973.
7. Green, R. H., W. W. Beatty and J. S. Schwartzbaum. Comparative effects of septo-hippocampal and caudate lesions on avoidance behavior. *J. comp. physiol. Psychol.* **64:** 444–452, 1967.
8. Herz, A. and W. Zieglgansberger. The influence of microelectrophoretically applied biogenic amines, cholinomimetics and procaine on synaptic excitation in the corpus striatum. *Int. J. Neuropharm.* **7:** 221–230, 1968.

9. Jacks, B. R., J. DeChamplain and J. P. Cordeau. Effects of 6–hydroxydopamine on putative transmitter substances in the central nervous system. *Eur. J. Pharmac.* **18:** 353–360, 1972.
10. Kirkby, R. J. and D. P. Kimble. Avoidance and escape behavior following striatal lesions in the rat. *Expl. Neurol.* **20:** 215–227, 1968.
11. Mitcham, J. C. and R. K. Thomas Jr. Effects of substantia nigra and caudate nucleus lesions on avoidance learning in rats. *J. comp. physiol. Psychol.* **81:** 101–107, 1972.
12. Neill, D. B., W. O. Boggan and S. P. Grossman. Behavioral effects of amphetamine in rats with lesions in the corpus striatum. *J. comp. physiol. Psychol.*, in press.
13. Neill, D. B. and S. P. Grossman. Behavioral effects of lesions or cholinergic blockade of the dorsal and ventral caudate of rats. *J. comp. physiol. Psychol.* **71:** 311–317, 1970.
14. Neill, D. B. and S. D. Parker. Hypothalamic self-stimulation: Long-lasting impairment by intrastriatal applications of 6–hydroxydopamine. Submitted to *Science*.
15. Pellegrino, L. J. and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain.* New York: Appleton-Century-Crofts, 1967.
16. Poirier, L. J., P. Langelier, A. Roberge, R. Boucher and A. Kitsikis. Non-specific histopathological changes induced by the intracerebral injection of 6–hydroxydopamine (6–OH–DA). *J. neurol. Sci.* **16:** 401–416, 1972.
17. Runnels, P. and R. Thompson. Hypothalamic structures critical for the performance of a locomotor escape response in the rat. *Brain Res.* **13:** 328–337, 1969.
18. Runyon, R. P. and A. Haber. *Fundamentals of Behavioral Statistics.* Reading: Addison–Wesley, 1967.
19. Ungerstedt, U. 6–Hydroxydopamine induced degeneration of central monoamine neurons. *Eur. J. Pharmac.* **5:** 107–110, 1968.
20. Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta physiol. scand. Suppl.* **367:** 1–48, 1971.