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Deficits in Instrumental Responding after 6-Hydroxydopamine Lesions of the Nigro-Neostriatal Dopaminergic Projection

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FIBIGER, H. C., A. G. PHILLIPS AND A. P. ZIS. *Deficits in instrumental responding after 6-hydroxydopamine lesions of the nigro-neostriatal dopaminergic projection.* PHARMAC. BIOCHEM. BEHAV. **2**(1) 87–96, 1974. – Rats subjected to bilateral injections of 6-hydroxydopamine (8 μg) into the zona compacta of the substantia nigra completely failed to learn either a one-way active avoidance response or a simple approach response for food reinforcement. The neurotoxic lesions reduced striatal dopamine and tyrosine hydroxylase activity to less than 10 percent of control levels. A significant loss of hypothalamic norepinephrine was also produced by these lesions suggesting that this procedure also destroyed part of the ventral noradrenergic bundle. When bilateral lesions of the latter pathway were made caudal to the substantia nigra, so that similar losses were produced in hypothalamic norepinephrine levels without reducing striatal tyrosine hydroxylase activity, normal acquisition of both avoidance and appetitive responses were observed. In another experiment, almost complete retention of avoidance responding was obtained if the animals were overtrained on this response prior to the bilateral nigral lesions. These results suggest that the nigro-neostriatal dopaminergic projection may play an important role in the acquisition of learned instrumental responses.

6-Hydroxydopamine Nigro-neostriatal projection Catecholamines Instrumental responding Learning

DESPITE the fact that there exists a substantial pharmacological literature suggesting that central catecholamines play an important role in the acquisition and retention of a variety of learned behaviours, the relative roles of noradrenaline (NA) and dopamine (DA) remain unclear. A major difficulty in differentiating between the relative importance of pathways utilizing these two putative neurotransmitters is that peripherally administered drugs which influence the storage, synthesis or metabolism of one of these amines almost inevitably also have profound effects on the other. Recently Breese and coworkers have taken advantage of the fact that 6-hydroxydopamine (6-OHDA) can, when injected intraventricularly under the appropriate pharmacological conditions, produce variable degrees of destruction of either dopaminergic or noradrenergic neurons [7,9]. Based on this approach these workers have concluded that DA neurons are important substrates in the acquisition and maintenance of conditioned avoidance responding [9,41]. Additional support for this hypothesis can be found in a recent report by Mitchum and Thomas [28] who demonstrated that lesions of the substantia nigra, the source of a major DA projection in the brain [47], produce substantial deficits in conditioned avoidance responding (CAR). Numerous other workers have also found that lesions which encroach upon the nigro-striatal projection disrupt avoidance responding (e.g. [21,38]). Furthermore, drugs such as pimozide and haloperidol which in small doses may be relatively specific in blocking DA receptor sites produce deficits in avoidance but not escape behaviour [31].

While data accumulated from these diverse procedures point to a role for dopamine in CAR, each one of these approaches has certain methodological limitations. Intraventricular application of 6-OHDA can produce only a relative decrease in dopamine over norepinephrine so that some effects on brain NE levels are always observed when brain DA levels are substantially reduced. Electrolytic lesions and knife cuts produce, of course, completely nonspecific damage making it difficult to ascribe behavioural effects to any particular system. Electrolytic lesions of the substantia nigra are also rather ineffective in producing substantial and reproducible decreases in neostriatal dopamine levels suggesting that this technique results in partial and variable destruction of the nigro-neostriatal projection [15,19]. Finally, some questions remain regarding the specificity of neuroleptic drugs even when given at relatively low doses [2, 6, 10, 20].

With Ungerstedt's [47] discovery that it was feasible to selectively lesion specific catecholaminergic pathways by stereotaxic injection of 6-OHDA into the cell bodies or axons of these projections, new possibilities for the investigation of the functional roles of these systems have

emerged. The present experiments were designed to further evaluate the role of the ascending dopaminergic projections in conditioned avoidance responding. Furthermore, insofar as 6-OHDA has been used almost exclusively in investigations of avoidance responding the role of these systems in a non-aversive learning task was also examined.

EXPERIMENT 1

The role of the nigro-striatal dopaminergic projection in conditioned avoidance learning was examined following localized bilateral injections of 6-OHDA into the substantia nigra.

METHOD

Animals

Twenty-two naive male Wistar rats were maintained in individual stainless steel cages, with Purina rat chow and water available ad lib, prior to surgery. Throughout the experiment, colony lighting was on between 7 a.m. and 7 p.m.

Surgery

Twenty-two animals were anesthetized with sodium pentobarbitol (50 mg/kg) and prepared for stereotaxic surgery in a Kopf stereotaxic instrument. Twelve animals received 8 μg of 6-hydroxydopamine hydrobromide (dosage expressed as the free base) injected bilaterally into the substantia nigra in a volume of 2 μl by the method of Fibiger *et al.* [16]. The 6-OHDA was dissolved in a solution of 0.9% NaCl containing ascorbic acid (1 mg/ml). The injection coordinates according to König and Klippel [22] were A + 3.2 mm; L ± 2.1 mm; and DV − 2.1 mm. The 10 control animals received identical treatment with an empty injection needle lowered to within 1.5 mm of the substantia nigra. Previous results have shown that hypothalamic norepinephrine levels and tyrosine hydroxylase activity are significantly reduced after bilateral injections of 6-OHDA into the substantia nigra ([17], Fibiger and Phillips, unpublished observations). This probably reflects damage to the ventral noradrenergic projection [47]. To control for the possible effects of damage to this system an additional group of five animals received bilateral injections of 6-OHDA (8 μg) into this bundle caudal to the substantia nigra so as not to damage the nigro-striatal projection. The coordinates according to König and Klippel [22] were A + 1.0 mm; L ± 1.3 mm; and DV − 2.1 mm.

Apparatus

The test chamber was a wooden shuttlebox measuring 90 × 28 × 45 cm, divided into two equal compartments by a manually operated guillotine door. The floor was constructed from 0.6 mm diameter copper rods mounted 12 mm apart and the grid floor of one compartment could be wired for the delivery of foot shock. In the avoidance tests, a 1.5 mA shock was supplied by a LaFayette shocker (Model 5226) to the grid floor.

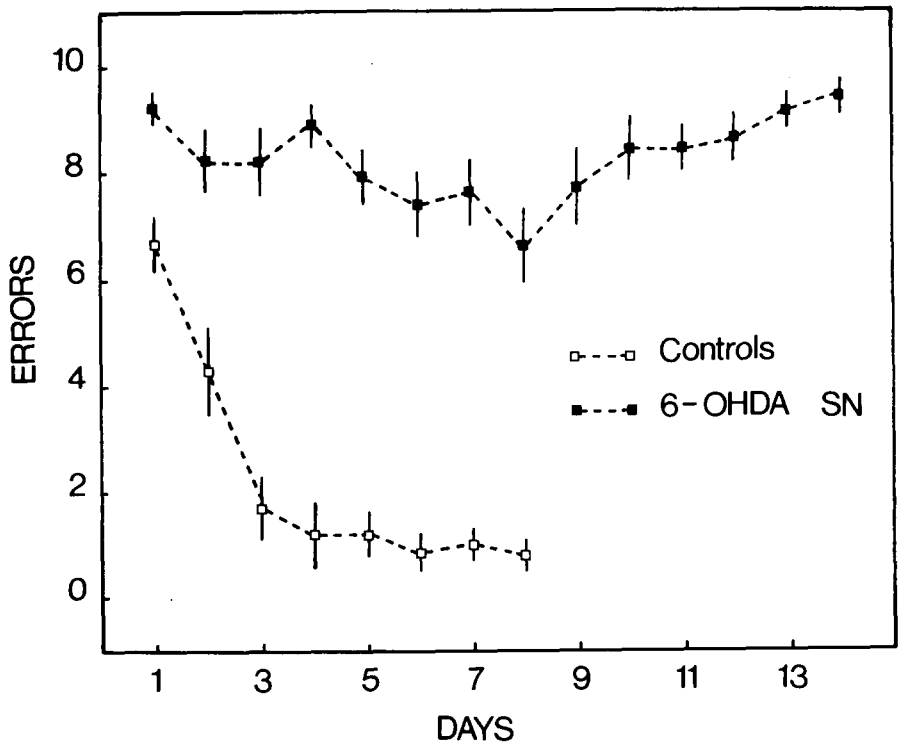

FIG. 1. Effect of bilateral 6-OHDA lesions of the substantia nigra on the acquisition of a one-way active avoidance response. Animals were given 10 trials per day and the number of errors each day (lack of avoidance response) are given on the abscissa. Data represent means (±1 S.E.M.) of 9 animals in each group.

Procedure

Convalescence. All animals receiving 6-OHDA lesions of the substantia nigra became aphagic and adipsic and required tube feeding until they recovered the ability to feed and drink. Each animal was intubated with 12 cc of artificial mother's milk (Lactol 25% by volume, 75% tap water), twice daily. Three daily feedings were given when necessary to maintain body weight. A daily record of each animal's body weight was kept and intubation was continued until the animal displayed a sustained increase in body weight and could maintain itself. Our past experience has shown 6-OHDA treated animals to have a preference for canned dog food and consequently this diet was made available after the operation and for the remainder of the experiment. Despite these precautions, three animals died prior to testing.

One-way avoidance learning. Nine experimental animals which had received bilateral 6-OHDA lesions of the substantia nigra, and had regained feeding and drinking behaviour, and nine control animals were trained for avoidance behaviour in the shuttlebox described above.

On Day 1 each animal was placed in the apparatus and permitted to explore for 15 min with the guillotine door raised. Testing began the next day at which time the rat was placed on the right side of the chamber with the door closed. After 5 sec the door was raised, the concomitant noise serving as a CS and the rat allowed 10 sec to reach the left side before the 1.5 mA footshock was delivered. When the rat reached the left side, the door was lowered and the rat allowed 30 sec on the safe side before the next trial began. Each animal was given 10 trials per day until a criterion of 9 of ten correct responses on two consecutive days was reached. The results were analyzed by analysis of variance and Student's t test.

Biochemistry

After the behavioural tests were completed the animals were killed by cervical fracture and the striatum and hypothalamus were dissected out from each brain. Tyrosine hydroxylase activity was measured in the striatum by the method of McGeer, Gibson and McGeer [25]. Hypothalamic norepinephrine and striatal dopamine were measured according to McGeer and McGeer [26].

Histology

The caudal portion of the brains of all experimental animals were fixed in buffered Formalin prior to embedding in paraffin wax. Serial sections (20 μ) were taken through the substantia nigra and every fourth section mounted, stained with luxol fast blue and counterstained with thionin.

RESULTS

The results of this experiment are seen in Fig. 1. Highly significant differences were observed between the controls and the 6-OHDA substantia nigra lesioned group on CAR ($p<0.001$). All of the control animals reached criterion (9 out of 10 avoidance responses on 2 consecutive days) by the seventh experimental day. The average was 4.70 ± 0.47 days. None of the substantia nigra lesioned animals reached criterion during the 14 day test period. In fact, 9 out of 10 avoidance responses were never obtained in any of the animals in this group on any of the 14 test days. The animals sustaining bilateral 6-OHDA lesions of the ventral bundle reached criterion in 5.00 ± 0.36 days which was not significantly different from controls. While the substantia nigra lesioned group never learned to avoid the shock, they had no difficulty in performing the escape response after shock onset. The escape latencies were not systematically recorded but they typically fell within 2–3 seconds after shock onset.

The biochemical results are seen in Table 1. The data in this table represent the combined results from Experiments 1 and 2 because animals in these experiments received identical lesions. 6-OHDA lesions of the substantia nigra reduced striatal tyrosine hydroxylase activity to about 4 percent of control values. Hypothalamic norepinephrine was reduced by 63 percent. Striatal tyrosine hydroxylase was not significantly altered in the animals with ventral noradrenergic bundle lesions, but hypothalamic norepinephrine was reduced by 65 percent.

Histological analysis revealed that the damage produced

TABLE 1

EFFECT OF 6-OHDA LESIONS ON STRIATAL TYROSINE HYDROXYLASE ACTIVITY AND HYPOTHALAMIC NOREPINEPHRINE LEVELS

	Striatal Tyrosine Hydroxylase (n moles Dopa/gm/hr)	% Control	Hypothalamic Norepinephrine (μg/gm)	% Control
Controls	104.5 ± 3.5	100	2.53 ± 0.10	100
6-OHDA SN	4.1 ± 1.2*	3.9	0.94 ± 0.11*	37.1
6-OHDA Ventral Bundle	103.5 ± 3.3	99.0	0.89 ± 0.06*	35.2

Data represent means (± S.E.M.) of 17 (controls and 6-OHDA SN) or 8 (6-OHDA ventral bundle) animals. Tyrosine hydroxylase was measured by this method of McGeer, Gibson and McGeer (1967) and norepinephrine was measured according to McGeer and McGeer (1961).

*Significantly different from controls, $p<0.01$.

by 6-OHDA was confined primarily to the zona compacta of the substantia nigra. Considerable gliosis was observed in this region. Cells in the zona reticulata appeared normal. Small amounts of gliosis were observed in cell group A10 near the interpeduncular nucleus [47]. In some animals a few necrotic cells were observed in the ventral part of the red nucleus, but the vast majority of cells in this region appeared normal.

DISCUSSION

Bilateral injections of 6-OHDA into the substantia nigra produced profound deficits in oneway active avoidance learning similar to those reported after bilateral electrolytic lesions of this nucleus or the caudate [28]. Immediately after the neurotoxic lesions, all of the animals became aphagic and adipsic, confirming previous observations [17]. The animals were initially maintained by intragastric intubation of milk and after one to three weeks began eating canned dog food. This period of aphagia and adipsia was marked by a pronounced lethargy and a degree of ataxia, but these symptoms disappeared when the animals regained the ability to feed themselves. At the time of testing which was 35 days after the lesion, the experimental subjects appeared as active as controls and displayed no marked motor disabilities making it unlikely that these factors could account for the results.

Although no formal assessment of shock threshold was made, the experimental animals would react vigorously to the presentation of the footshock by squealing and escaping into the safe compartment. This reaction to the shock was in contrast to the response elicited by raising the guillotine door at which time the animals would remain relatively quiet until the shock was initiated. Although the CS only consisted of the sight and sound of the door being raised, the control animals reacted to it immediately as they acquired the avoidance response, but the experimental subjects did not appear to pair these cues with the ensuing foot shock.

EXPERIMENT 2

The preceding experiment provides strong evidence for the role of the nigro-striatal dopaminergic system in the acquisition of shock-motivated behaviour, but the question still remains as to whether this role is limited to avoidance behaviour or may be generalized to learning similar responses under different states of motivation. One difficulty which often arises in studies of this nature is the use of different test apparati for the avoidance and appetitive tasks. In the following experiment, animals which had recovered from aphagia and adipsia produced by bilateral 6-OHDA injections into the substantia nigra were food deprived and tested for the acquisition of a simple approach response in the same shuttlebox as was used in Experiment 1.

METHOD

Animals and Surgery

Twenty-six additional male Wistar rats were housed in conditions identical to Experiment 1. Twelve animals received bilateral injections of 6-OHDA into the substantia nigra as described above, and ten animals served as operated controls. As a control for possible damage to the ventral NA bundle, four animals had 6-OHDA injected bilaterally into this projection as described in Experiment 1.

Apparatus and Procedure

The wooden shuttlebox employed in Experiment 1 was again utilized in the appetitive testing. As in the previous experiment all animals were placed on a diet of dog food after the 6-OHDA substantia nigra lesioned animals no longer required intragastric feeding. The animals, including controls, remained on the canned dog food for the remainder of the experiment. Thirty-five days after surgery, eight surviving rats with 6-OHDA lesions, and eight controls were placed on a 21-hr food deprivation schedule on which they were maintained until the end of testing. Water was available ad lib.

Prior to the learning trials, each animal was accustomed to the apparatus by first placing it next to the food dish, then moving it slowly to the opposite compartment of the test chamber, a procedure which took approximately 15 min per animal. On the first day of testing, each rat was placed in the right side of the chamber facing away from the lowered guillotine door. The door was raised after 5 sec. Each rat was given 10 sec to move out of the start compartment. On reaching the food the animal was given 10 sec to eat the dog food before being put into a separate cage during the 30 sec inter-trial interval. Ten trials were conducted per day until a criterion of 9 of 10 approach responses were displayed within the initial 10 sec after the door was raised on two consecutive days. A trial was not considered correct unless the rat also ate within 30 sec on the food side. In the event the rat did not leave the start side within 30 sec on the first trial, he was placed on the goal side by the experimenter and left near the food dish for 10 sec. After the learning trials the animals were returned to their home cages and allowed food and water for 3 hr. The amount of food consumed during the 3-hr eating period was measured on four days. This measure was used to determine whether the experimental animals differed from control animals in terms of food motivation. Following completion of the experiment, all animals were sacrificed and prepared for biochemical measures and histology as described in Experiment 1.

RESULTS

The results of this experiment are seen in Fig. 2. The performance of the 6-OHDA substantia nigra lesioned group differed significantly from the controls ($p<0.001$). All control animals reached criterion within 10 days, the mean being 8.50 ± 0.29 days. As in the first experiment, none of the substantia nigra lesioned animals attained criterion performance during the 14 days. The group receiving lesions of the ventral noradrenergic bundle reached criterion in 5.00 ± 0.72 days which tended to be shorter than controls, but because of the small number of animals in this group, this observation requires further validation. It is obvious however, that none of the profound deficits found in the substantia nigra lesioned animals were present in the animals receiving bilateral ventral bundle lesions. The biochemical results are as for Experiment 1 (Table 1). The controls and substantia nigra lesioned animals did not differ significantly in the amount of food eaten during the three hour feeding period each day (Controls: 38.6 ± 1.94 gms; 6-OHDA SN: 39.1 ± 1.03).

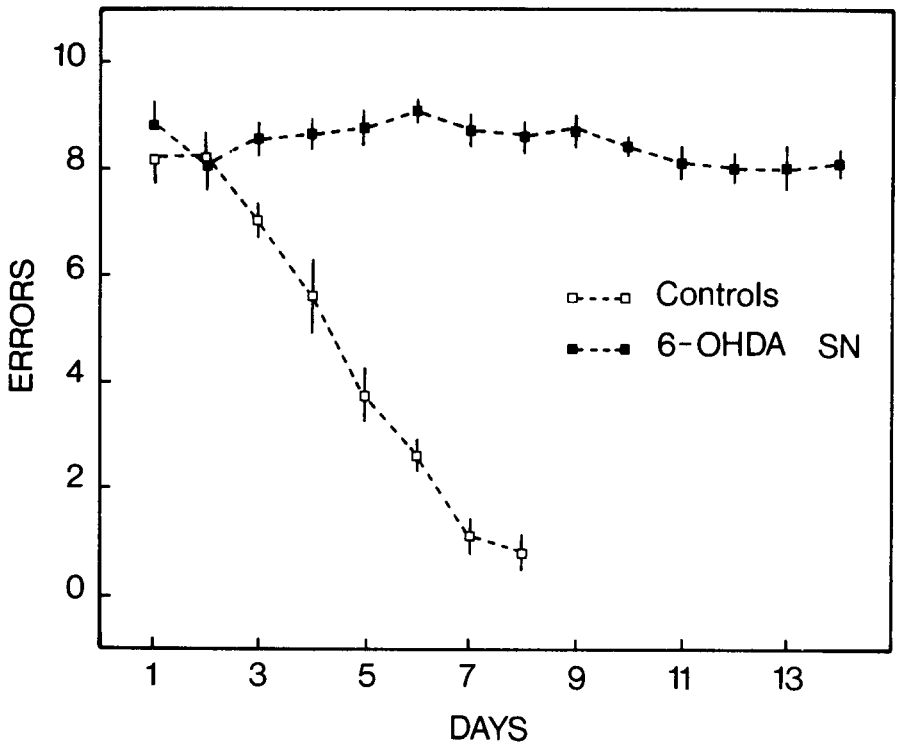

FIG. 2. Effect of bilateral 6-OHDA lesions of the substantia nigra on the acquisition of an approach response for food reinforcement. Animals were given 10 trials per day and the number of errors on each day (failure to approach the food within 10 seconds) are given on the abscissa. Data represent means (±1 S.E.M.) of 8 animals in each group.

DISCUSSION

The results of this experiment are directly comparable to those obtained in the avoidance task employed in Experiment 1, in that animals sustaining bilateral destruction of the substantia nigra never learned a simple approach response for food. In spite of the fact that the same apparatus was employed, this task appeared more difficult to learn than the avoidance task as evidenced by the greater number of trials required by the controls to reach criterion. Despite the apparent increase in difficulty, both of the control and the ventral noreadrenergic bundle lesioned groups attained criteria whereas none of the experimental animals acquired the response. This failure cannot be attributed to the unfamiliarity with the type of reinforcement employed, as all groups had been eating the canned dog food in their home cages for over a month prior to the start of the experiment, and consumed equal quantities when returned to the home cage for the 3 hr daily feeding period which occurred after each test session. Furthermore, the nigral lesioned animals would eat the food when placed next to the dish, but would not learn to run from one compartment to the goal compartment within a specified period of time for the food reward. The observed impairment therefore, seems best attributed to deficits other than decreased food motivation. These results suggest that 6-OHDA lesions of the substantia nigra produce a learning deficit which may be of a generalized nature rather than a specific impairment of conditioned avoidance responding.

EXPERIMENT 3

Many researchers engaged in the study of shock-motivated active avoidance responding have emphasized the importance of providing measures in addition to the avoidance response, as these other variables may aid in the interpretation of any observed deficits [3, 5, 33]. Activity levels represent a particularly important variable in these studies, and they become even more critical when the effects of damage to a part of the extrapyramidal system is under investigation. Consequently, measures of activity were taken before and after a foot shock. This test was conducted prior to the learning tests in Experiments 1 and 2.

METHOD

Animals

All animals receiving substantia nigra lesions (N = 17) and their controls (N = 17) in Experiments 1 and 2 were tested.

Apparatus and Procedure

A Plexiglas chamber measuring 45 x 45 x 45 cm with a grid floor was employed in measuring activity levels. The floor area was divided into 16 equal squares to facilitate the recording of activity and the shock source employed in Experiment 1 was connected to the grid floor and calibrated to deliver a 1.5 mA current. Each animal was placed in the bottom left hand corner of the chamber

and allowed to move freely. The number of squares traversed was recorded over a 3 min pre-shock period after which a shock was delivered for 5 sec. The animals were immediately returned to their home cages for 1 min after which they were returned to the test apparatus for an additional 3 min period during which post-shock activity was measured.

RESULTS

No significant difference was observed between 6-OHDA lesioned animals and controls in pre-shock activity levels. The lesioned subjects crossed over an average of 45.2 ± 3.5 squares in the 3 min pre-shock period as compared to a control mean of 46.6 ± 2.9. The groups differed significantly however, in activity levels after the 5 sec shock. The post shock activity of the lesioned animals was significantly higher than the controls ($p<0.01$). Virtually all of the control animals froze for the entire 3 min period ($\bar{X}$ = 1.1 ± 0.4) whereas the experimental animals were active although at a significantly reduced level ($p<0.01$) relative to their pre-shock scores ($\bar{X}$ = 15.3 ± 2.6).

DISCUSSION

These data are important in that they reduce the possibility that differences in general activity were the major factor responsible for the observed decrements in both approach and avoidance behaviour. Furthermore, these findings suggest that the inability of the 6-OHDA group to acquire the avoidance response cannot be attributed to freezing in this situation. In the present experiment, the lesioned animals in fact showed less post-shock freezing than controls. It may be argued from these results that the experimental animals were less reactive to footshock. While this point remains to be determined empirically, the fact that a significant decrease in activity was observed after the shock serves to emphasize again the experimental animals' ability to react to the shock. On the other hand, the reduced freezing could be taken as evidence of impaired learning of a conditioned emotional response by the 6-OHDA lesioned group. Verification of this possibility in a formal conditioned response suppression task is in progress and should help elucidate whether the disruptive effects of nigral damage are limited to instrumental learning.

EXPERIMENT 4

Insofar as Experiment 1 demonstrated that destruction of the nigrostriatal dopaminergic bundle abolished the acquisition of a conditioned avoidance response, the following experiment was undertaken to determine the effects of bilateral 6-OHDA lesions of the substantia nigra on the retention of a well-established conditioned avoidance response.

METHOD

Animals and Surgery

Eighteen male Wistar rats were assigned either to a group receiving 6-OHDA lesions of the substantia nigra or to a shamoperated control condition based on preoperative performance in a shuttlebox. The groups were matched in terms of days to reach criterion (9 out of 10 avoidance responses on 2 consecutive days). The stereotaxic microinjection procedure was the same as described in Experiment 1, as were the biochemical and histological analyses. Post-operative convalescences were as described above.

Apparatus and Procedure

The animals were trained in a shuttlebox (75 × 25 × 30 cm), divided into two equal compartments by a wooden door extending from the top of the apparatus to the grid floor. Raising the door triggered the presentation of a descrete tone for 10 sec, which served as the CS. Termination of the CS coincided with the delivery of a 0.7 mA shock to the grid floor which was composed of 0.6 mm diameter copper rods mounted 12 mm apart. Training took place over a 13-day period and the animals received 10 trials per daily session. In this manner, the animals were assured at least 7 days of overtraining after having reached the criterion of 9 out of 10 correct responses on two consecutive days. After a 15-day post-surgery recovery period, both groups were retested on post-operative Days 15 and 21, and the number of errors in each 10 trial session were recorded.

RESULTS

Both groups learned the avoidance response preoperatively at very similar rates (because of matching) and to an equal level of performance (Fig. 3). Both the control and the experimental groups showed substantial retention on the retest days. In Fig. 3 it is evident that the controls in fact showed complete retention of the avoidance response. Although on both retest days the performance of nigral lesioned group was significantly poorer than controls ($p<0.05$), this group also clearly showed considerable savings, making a mean of approximately 8 out of 10 avoidance responses (Fig. 3). This differs dramatically from the animals in Experiment 1 which were lesioned before avoidance training. These animals were making an average of only one or two out of ten avoidance responses per session after 14 days of training (Fig. 1).

The biochemical results are seen in Table 2. Bilateral injections of 6-OHDA into the substantia nigra reduced striatal dopamine to 5.5 percent of controls, a value comparable to the 96 percent decrease in striatal tyrosine hydroxylase obtained in Experiments 1 and 2. Hypothalamic norepinephrine was also reduced in the lesioned animals by 67 percent which is also comparable to the first two experiments.

DISCUSSION

In marked contrast to the disruptive effects of substantia nigra lesions on the acquisition of an active avoidance response, similar lesions produced only a slight deficit in retention of this task after overtraining. The overtraining may be a critical variable, as Cooper *et al.* [9] have reported poor retention of a similar response acquired in a non-overtrained massed trial paradigm, following intraventricular injections of 6-OHDA in rats pretreated with a monoamine oxidase inhibitor. In the present experiment the response pattern appeared to be sufficiently well established prior to surgery as to ensure its immunity from the lesion effects. This observation leads to the conclusion

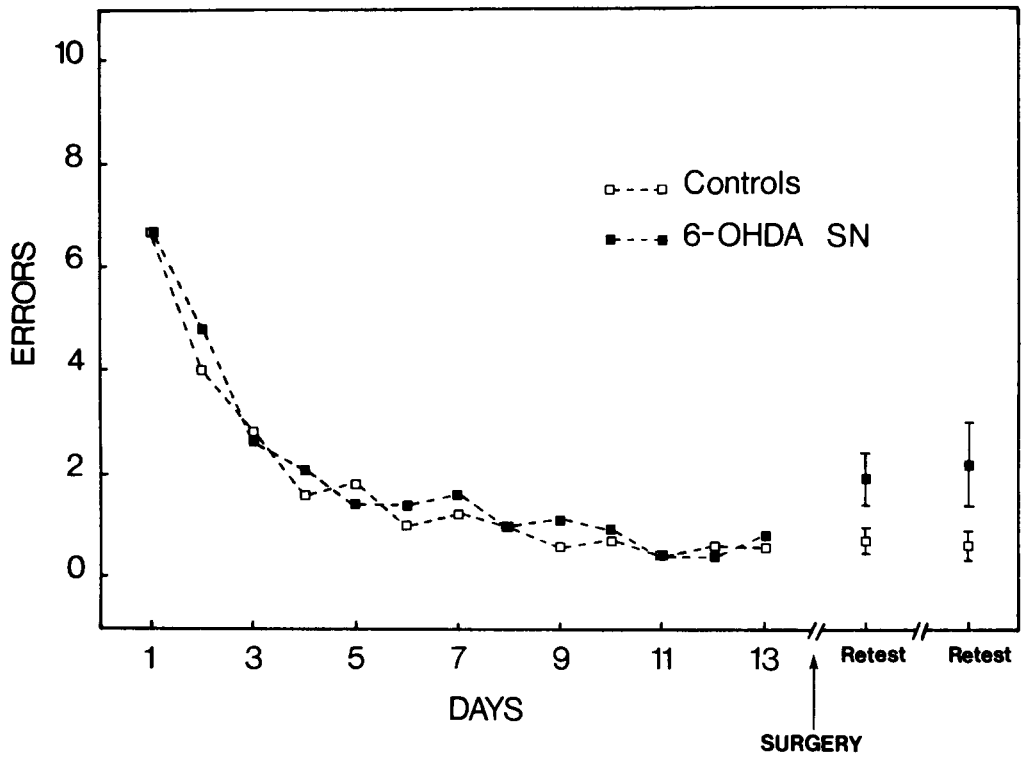

FIG. 3. Effect of bilateral 6-OHDA lesions of the substantia nigra on the retention of an overtrained conditioned avoidance response. Animals were trained on 10 trials per day for 13 days, and then received lesions or sham operations. The animals were tested for retention 15 and 21 days after surgery. Data represent means (±1 S.E.M.) of 9 animals in each group.

TABLE 2

EFFECT OF 6-OHDA LESIONS OF THE SUBSTANTIA NIGRA ON STRIATAL DOPAMINE AND HYPOTHALAMIC NOREPINEPHRINE LEVELS

	Striatal Dopamine (μg/gm)	% Control	Hypothalamic Norepinephrine (μg/gm)	% Control
Controls	8.74 ± 0.44	100	2.76 ± 0.12	100
6-OHDA SN	0.48 ± 0.12*	5.5	0.91 ± 0.10*	33.0

Data represent means (± S.E.M.) of 9 animals in each group. Norepinephrine and dopamine were measured according to McGeer and McGeer (1961).

*Significantly different from controls, $p<0.01$.

that 6-OHDA lesions of the substantia nigra may impair the acquisition of learned instrumental responses rather than resulting in non-specific performance decrements. It should be pointed out that for technical reasons a different shuttle-box was used in this experiment than in Experiments 1 and 2. We have observed however, that animals given bilateral 6-OHDA lesions of the substantia nigra prior to CAR training in this apparatus, show the identical deficits as reported in Experiment 1 (Zis, Fibiger and Phillips, in preparation), so that this variable cannot account for the substantial savings observed in the lesioned animals.

GENERAL DISCUSSION

Several laboratories have now reported that intraventricular injections of 6-OHDA can produce deficits in active avoidance responding [9, 23, 43, 45]. Because 6-OHDA appears to be a relatively specific agent for the destruction

of CA neurons, these reports have confirmed earlier suggestion that central CA mechanisms play an important role in this behaviour [34, 35, 39, 40]. Due to the fact that there are a number of well-defined CA projections in brain [13,47], it next becomes important to attempt to determine which of these systems are important in CAR. Using intraventricular injections of 6-OHDA several laboratories have concluded, on the basis of preferential reduction of either norepinephrine or dopamine, that dopamine is the more important amine for the maintenance of CAR [9, 41, 45]. These conclusions are consistent with the fact that drugs such as chlorpromazine, haloperidol and pimozide, which at low doses are thought to be relatively specific blockers of dopamine receptor sites, produce disruption of CAR in rats [14,31]. While highly suggestive, these conclusions are weakened by the facts that (a) intraventricular 6-OHDA inevitably produces destruction of NE neurons when major destruction of DA neurons is obtained [9] and (b) that neuroleptic drugs may also block NE receptor sites albeit less effectively [2, 6, 10, 20].

In the present experiments, massive destruction of the ascending nigro-neostriatal projection was obtained by stereotaxic injection of 6-OHDA into the substantia nigra. Thus, neostriatal tyrosine hydroxylase activity and dopamine levels were reduced by more than 90 percent (Tables 1 and 2). These lesions produced substantial learning deficits in both approach and avoidance tasks. Hypothalamic norepinephrine levels were also reduced by this procedure and this was probably due to partial lesioning of the ventral noradrenergic bundle which ascends just dorsal to the substantia nigra [47]. That the behavioural deficits in the nigral lesioned group were not due to destruction of the ventral NE bundle is indicated by the fact that when hypothalamic NE levels were similarly reduced by lesions of the ventral NE bundle caudal to the substantia nigra, normal and perhaps enhanced acquisition was observed. While the lack of pronounced deficits in the animals with lesions in the ventral NE bundle is clear from the few animals tested, the possibility that these lesions may even enhance the acquisition of learned responses is the subject of on-going experiments.

The fact that neurotoxic lesions of the nigro-striatal dopaminergic projection produce an inability to learn a conditioned avoidance response may be related to the numerous reports which have found deficits in CAR after lesions of the caudate nucleus [30, 46, 48, 49]. The present data are also consistent with the results of experiments in which lesions which may have produced damage at various levels of the nigro-striatal projection have been observed to produce learning deficits. Electrocoagulation of the basal diencephalon in the region of the medial cerebral peduncle for example, produces severe deficits in avoidance behaviour [38,44]. Mitchum and Thomas [28] observed impaired avoidance after electrolytic lesions of the substantia nigra or ventral caudate nucleus. Kent and Grossman [21] have reported that knife cuts in the hypothalamus which would interrupt the nigro-striatal projection prevent the acquisition of conditioned avoidance responding. Some of the above authors have interpreted their results as reflecting the involvement of other central projections, but to hypothesize that damage to the nigro-striatal system plays an important part in these observations would appear to be more parsimonious.

Further evidence for the involvement of the nigro-striatal projection in learning and memory comes from findings observed after electrical stimulation of the zona compacta of the substantia nigra. Routtenberg and Holzman [37] observed that electrical stimulation of this nucleus at low currents disrupted retention of a simple passive avoidance response. Such low level stimulation may have blocked activity in the nigro-striatal system and in essence created a reversible functional lesion, the effects of which may be comparable to the deficits observed after the neurotoxic and electrolytic lesions referred to above. Electrical stimulation of the caudate nucleus also disrupts passive avoidance learning [50]. Furthermore, stimulation in the medial forebrain bundle in the lateral hypothalamus impairs two-way avoidance responding [8]. Carder[8] demonstrated that new learning was retarded whereas well-established responses were enhanced by comparable stimulation. This dichotomy between the effects of stimulation on new versus well-learned behaviour is comparable with the present results. All of these findings are consistent with a hypothesis of nigro-striatal involvement in the establishment of relatively permanent changes in behaviour.

The fact that bilateral 6-OHDA lesions of the substantia nigra produced profound deficits in both the acquisition of an operant response for food reinforcement as well as in conditioned avoidance responding indicates that the nigro-neostriatal projection may play an important general role in learning. Furthermore, the fact that subtantial savings are observed when the animals are overtrained prior to the nigral lesions indicates that this pathway may be of particular importance in the acquisition of learned responses. It remains to be determined whether the functional integrity of this pathway is a prerequisite for the development of the special significance of the conditioned stimulus (i.e. the acquisition of the classically conditioned emotional response to the CS) or whether the lesioned animal may indeed learn the significance of the CS but may not be able to generate the appropriate instrumental response to that stimulus [29].

While the discussion to this point has revolved around the importance of the nigro-striatal projection, it should be emphasized that a contribution of the meso-limbic dopaminergic projection to the results cannot at present be excluded. Using the Fink-Heimer stain we have found that when 8 μg of 6-OHDA are injected into the substantia nigra significant destruction of the meso-limbic DA projection also occurs (Maler and Fibiger, unpublished observations). Lorens, Sorensen and Harvey [24] found that electrolytic lesions of the nucleus accumbens, a site of extensive termination of mesolimbic DA axons, enhance the acquisition of CAR and this may indicate that this projection is not critically involved in the learning deficits observed after 6-OHDA lesions of the substantia nigra. More work will be required to deal with the possible involvement of the meso-limbic projection however. An additional note of caution stems from recent evidence suggesting that 6-OHDA, despite being highly specific at lower doses [27], may have nonspecific toxic actions when given in the amounts required to cause nearly complete destruction of the nigro-striatal DA projection [1,42]. Damage to a second nigro-striatal projection, which is not dopaminergic, could for example be responsible for some of the behavioural effects of the 6-OHDA injections [17,18]. Although we cannot at present be sure that some of the deficits produced by bilateral 6-OHDA injections into the substantia nigra are not due to nonspecific damage produced by the drug, our preliminary observation that

l-dopa can reverse these deficits argues that damage to central dopaminergic mechanisms are indeed the critical variable.

Recently Anlezark, Crow and Greenway [4] found that bilateral lesions of the locus coeruleus can impair the acquisition of a response for food reinforcement in an L-shaped runway. This observation, together with present findings, indicates that several central catecholaminergic systems may be important substrates of learning. It is interesting to note that both the dorsal noradrenergic bundle which originates from cells in the locus coeruleus, as well as nigro-striatal and meso-limbic dopaminergic projections will support high rates of intracranial self-stimulation [11, 12, 32, 36]. This suggests that these systems may play important roles in reinforcement and leads to the speculation that some of the learning deficits observed after lesions of these projections are due to an inability of these animals to be reinforced by correct responses. It will be necessary for future research to consider possible functional differences of these catecholaminergic pathways in learning.

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