

THE ROLE OF THE NIGRO-STRIATAL DOPAMINERGIC
SYSTEM IN LEARNING IN THE RAT

by

MAURICE FREDRICK SHAPIRO

B.A. University of British Columbia

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF ARTS

in the Department
of

Psychology

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA

September, 1974

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study.

I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of

Psychology

The University of British Columbia
Vancouver 8, Canada

Date

Sept. 19/74

Abstract

The possibility that the nigro-striatal dopaminergic system is involved in learning processes was tested by damaging the substantia nigra (SN) of the rat either electrolytically or neurochemically and testing the animals for deficits on a one-way active-avoidance task and a simple appetitive task. In Experiment 1 the animals were tested to determine whether the lesion resulted in increased freezing following footshock (FS) or in changes in general activity. No such differences were revealed. The deficit in active-avoidance learning observed in Experiment 2a could then be attributed to a disruption of the learning process following nigro-striatal system damage. However, these results must be qualified by the results of Experiment 2b in which it was observed that the experimental animals were less sensitive to FS than the controls. In experiment 3 an appetitive task was used to overcome the confounding effects of footshock and avoidance paradigms. Testing revealed a deficit in learning following damage to the nigro-striatal system. These combined results strongly suggest that the nigro-striatal dopamine system is an integral part of the neural substrate of learning.

Table of Contents	page
Abstract.	i
Table of Contents	ii
List of Figures	iii
List of Tables	iv
Acknowledgements	vi
Introduction	1
Experiment 1	12
Introduction	12
Method	12
Results	14
Experiment 2a	17
Introduction	17
Method	17
Results	18
Experiment 2b	25
Introduction	25
Method	25
Results	26
Experiment 3	29
Introduction	29
Method	29
Results	30
Discussion	37
Bibliography	43
Appendix A	51
Appendix B	55

List of Figures

	page
Figure 1: A graphic representation of the location and magnitude of the lesions found in each animal of Experiment 2a depicted at the same coronal section redrawn from Pelligrino and Cushman (1967).	24
Figure 2: A graphic representation of the location and magnitude of the lesions found in the animals of Experiment 3 depicted at various coronal sections which best illustrate this damage	36

Table I:	The results of Experiment 1 illustrating differences in activity before and after footshock for immediate and 24 hr. retest conditions of the electrolytic, biochemical and control groups	15
Table II:	Results of Experiment 2a showing number of trials and errors to criterion for the experimental and control groups	20
Table III:	Effects of 6-OHDA and SN lesions in Experiment 2a on caudate dopamine levels as indicated by tyrosine hydroxylase activity (N mole DOPA/gm Tissue/hour and hypothalamic noradrenaline levels (ug/gm) expressed as percentage of controls	21
Table IV:	Levels of caudate dopamine for control animals of Experiments 2a and 3	22
Table V:	Results of Experiment 2b showing differences in "jump" thresholds between experimental and control groups . .	27
Table VI:	Results of Experiment 3 indicating individual and group trials and errors to criterion scores for the experimental and control groups	31
Table VII:	Mean amount of food consumed by the experimental and control groups during the 3 hr. feeding period on four consecutive days of Experiment 3	33

Table VIII:	Effect of 6-OHDA and SN lesions on caudate dopamine levels in Experiment 3 as indicated by tyrosine hydroxylase activity and hypothalamic noradrenaline levels expressed as percentage of controls	34
-------------	--	----

Acknowledgements

I would like to express my sincerest gratitude to all those who have helped me to complete this manuscript. Special thanks are due to Tony Phillips without whose assistance this research could never have been conducted.

A great deal of research in biopsychology has been concerned with devising strategies for investigating the neural substrates of learning. Because of the obvious relationship between reward and learning, it has been argued that understanding of the neural reward processes could serve as an important beginning for further insight into the neural processes of learning (Olds, 1969). At present much is known about the neural substrates of reward. Early investigation revealed that brain stimulation reward (BSR) was comparable to natural rewards such as food and water. Furthermore, a great deal has been learned about the underlying neuroanatomy and biochemistry of reward systems. It remains for subsequent research to utilize this detailed information on the neural substrates of reward to determine the role of these systems in learning. The following pages review the literature concerning brain stimulation as a reward and the subsequent investigations of the anatomy and biochemistry of the reward systems. The literature pertaining to the role of these reward systems in learning is also reviewed.

Is brain stimulation reward comparable to natural rewards such as food, water, and sex?

In 1954 Olds and Milner discovered that electrical stimulation to specific areas within the rat brain produced a rewarding effect. Before neural reward systems could be seriously pursued as a key to understanding learning processes, it was necessary to show that such reward was comparable to natural reinforcers. Subsequently, Olds had attempted to prove that the rewarding effect was due to an artificial activation of the neural substrates mediating the effects of natural

rewards (Olds, 1955, 1956, 1967, 1969; Olds and Olds, 1962, 1963, 1964). The early data associated with the brain stimulation phenomenon indicated a large discrepancy between behaviour rewarded with brain stimulation and naturally rewarded behaviour. The greatest differences were found in the areas of schedules of reinforcement and extinction (Deutsch, 1963; Seward, Ueyda, and Olds, 1959; Brodie, Moreno, Malis, and Boren, 1969). For example, in bar press situation FR 200 schedules have been maintained for food reward, whereas FR 20 schedules are difficult to maintain when brain stimulation is the reward (Brodie, et al., 1960). Recently it has been demonstrated that discrepancies between brain stimulation rewarded behaviour and naturally rewarded behaviour can be accounted for largely by the experimental situation itself (Trowill, Panksepp, and Gandelman, 1969). For example, Pliskoff, Wright, and Hawkins (1965) found that ratio schedules, comparable to naturally rewarded schedules, could be maintained using brain stimulation reward if a consummatory bar was added to the apparatus. In this way the rat pressed on the instrumental bar until the consummatory bar was presented, the pressing of which resulted in brain stimulation. Several other investigators established schedules of reinforcement and extinction comparable to those maintained by natural rewards (Gibson, Reid, Sakai, and Porter, 1965; McIntyre and Wright, 1965; Terman and Kling, 1968). Such research has indicated that BSR is comparable to natural rewards such as food, water and sex. It would appear then, that in stimulating subcortical structures yielding BSR one is

"...stimulating genuine reward systems in the brain..."

that the effect derives from no mere compulsion or automatism, but from stimulation of the cells actually involved in the food, sexual, or other reward process."

(Olds, 1958c)

The anatomy of brain reward systems

Studies of the anatomical correlates of BSR paralleled attempts to establish the similarity between rewarding effects of BSR and natural rewards. By testing various brain sites for self-stimulation and then verifying the placement of the electrodes by histology, Olds and Olds (1963) mapped a medial forebrain bundle (MFB) reward system which included the MFB as an essential structure in BSR. However, Valenstein (1966) found that large ablations of the MFB failed to abolish self-stimulation of midbrain stimulation sites (Valenstein and Campbell, 1966; Lorens, 1965). Furthermore, studies by Routtenberg and Malsbury (1969) and Routtenberg (1971) indicated that the MFB was not the only structure which would mediate brain stimulation reward. From the brainstem reward sites mapped by Routtenberg and Malsbury (1969), high rates of brain stimulation were elicited from the region of the fibers and nucleus of the ventral tegmental decussation, ventral tegmental area of Tsai, and the substantia nigra (SN). Moreover, Routtenberg (1971) mapped reward sites in the rat forebrain implicating the medial and posterior regions of the anterior hippocampus, the area dorsal to the cingulum and the corpus callosum, and caudate nucleus. Posteriorly, he mapped the lateral septal region, septohypothalamic tract, MFB, stria medullaris

of the SN, and a dorsal system lateral to the central grey matter (an area which Arbuthnot, Crow, Fuxe, Olson, and Ungerstedt, (1970) had shown to be noradrenergic). The ventral sites corresponded to areas A9 and A10 which previously had been shown to be dopaminergic (Anden, et al., 1966; Fuxe, Hokfelt and Ungerstedt, 1970). Thus it appears that BSR can also be maintained by a DA system and that any theory involving only noradrenergic mechanism must be modified accordingly (Phillips and Fibiger, 1973).

The anatomical and biochemical complexity of the brain reward systems was further emphasized by the suggestion that the reward systems do not work autonomously but function reciprocally with punishment systems. Olds and Olds (1963) and others (Delgado, Roberts, and Miller, 1954; Hess, 1954) found areas of the brain which induced escape behaviour when stimulated and several investigators (Stein, 1964b; Olds and Olds, 1963; Poschel and Ninteman, 1971) have suggested that these two systems may be reciprocally related.

Poschel and Ninteman (1971) tested the possibility that a noradrenergic reward system and a serotonergic aversion system were reciprocally related as both of these monoaminergic systems have parallel projections from the midbrain to the limbic lobe (Anden et al., 1966). This relationship is further substantiated by the findings of Spector, Gjoerdsma, and Udenfriend (1965) who showed that self-stimulation was virtually abolished when alpha-methyl-p-tyrosine was used to deplete the brain of NA while leaving serotonin (5-HT) levels unaffected. Poschel and Ninteman (1971) demonstrated that NA and 5-HT act reciprocally on

reinforcement thresholds. They found that when 5-HT levels were reduced by the use of p-chlorophenylalanine, brain self-stimulation rates increased. Furthermore, when 5-HT levels were raised by the use of p-chloroamphetamine, self-stimulation was virtually eliminated. These results indicate that NA does not act alone in mediating reward but acts in proportion to levels of 5-HT.

Brain reward systems and learning

In spite of the important theoretical relationship between reward and learning processes, this relationship has not been thoroughly investigated at the neural level. However, a few studies can be cited. Kent and Grossman (1973) examined avoidance and appetitive learning in rats following knife cuts of the lateral border of the diencephalon. Such cuts essentially severed all fibers which cross the lateral border of the hypothalamus including the pallidofugal fiber system, nigro-striatal bundle, ventral amygdala hypothalamic pathway, and portions of the stria medullaris. Their animals were not able to learn to lever press for rewarding brain stimulation or to lever press to escape shock. As well their animals did not learn to run a straight alley for brain stimulation or food reward. Such deficits in avoidance and appetitive learning suggested to Kent and Grossman that connections between the brainstem and basal ganglia were essential for learning to occur.

Mitcham and Thomas (1972) investigated the effects of SN and caudate lesions on avoidance learning in rats. Compared to controls, animals with small bilateral lesions in the rostral SN were significantly impaired in the acquisition of one-way active avoidance, passive avoidance

task. Treatment of 6-OHDA plus pargyline resulted in a depletion of both brain NA and DA. Preferential depletion of brain NA resulted in facilitation of the avoidance task but this was confounded by an increase in locomotor activity during the inter-trial interval. Preferential depletion of brain DA resulted in no deficit in acquisition of the avoidance task. This result seems to contradict the hypothesis that reduction in brain DA (particularly of SN) is an important factor in avoidance acquisition (Mitcham and Thomas, 1972). It becomes even more difficult to interpret the role of DA in light of further findings by Smith, Cooper, and Breese (unpublished data) in which preferential depletion of DA during development blocked acquisition of the shuttle-box avoidance response when the rat reached 60 days of age. Cooper et al. (1972) attribute the lack of a deficit in avoidance acquisition to an inadequate depletion of brain DA or NA. An interpretation which is difficult to reconcile with their reported 63% reduction in brain DA and a 17% reduction in brain NA.

Earlier investigations of the SN has implicated this structure in position reversal learning (Thompson and Langer, 1963), in the integration of approach and avoidance behaviours (Thompson, Rich, and Langer, 1964), and in relearning brightness, visual pattern, and kinesthetic discriminations (Thompson, Kukaszewska, Schweigerdt, and McNew, 1967). Thompson (1959) and Breen, Beatty, and Schwartzbaum (1967) found deficits in active avoidance following lesions of the caudate nucleus. However, Winocur and Mills (1969) and Albert and Bignami did not find deficits following such lesions. The crucial

factor distinguishing these studies was the locus and extent of the lesions. In the studies reporting deficits the lesions were placed in a postero-ventral part of the caudate whereas in the no-deficit studies the lesions occurred in the more anterodorsal regions.

The results of previous studies (Mitcham & Thomas, 1972; Cooper et al., (1972) indicate that damage to the nigro-striatal system causes an active avoidance deficit. Studies involving lesions of the caudate nucleus (Thompson, 1959; Green et al., 1967) similarly support a SN or nigro-striatal pathway involvement in avoidance learning. One factor which complicates interpretation of avoidance data is that most active avoidance paradigms presently used do not provide measures in addition to the avoidance response which might be valuable in quantifying the extent to which motivational factors, associative factors, or an interaction between the two, were mediating the response. The results of experiments by Barrett, Leith, and Ray (1973) strongly indicate that many experimental manipulations which have been shown to affect avoidance behaviour may be simply altering the subject's normal reactivity to shock. Thus, the active-avoidance deficit need not be a learning deficit.

Purpose of this study

The present experiments were designed to examine the role of the DA nigro-striatal reward system in learning. In previous studies this system had been examined in only a general way, a major problem being the nonspecificity of the lesions used. Mitcham and Thomas (1972), for instance, used electrolytic lesions resulting in damage to structures

and fibers other than the SN. Cooper et al. (1972) used intracisternal injections of 6-OHDA which resulted in DA and NA depletions throughout the whole brain. In the present experiments 6-OHDA was injected directly into the SN in half of the animals in order to restrict damage to the nigro-striatal system and to permit direct comparison between these animals and animals sustaining more gross damage from electrolytic lesions of the SN.

A second problem with previous studies was the bioassay technique employed to assay DA and NA depletions following damage to the nigro-striatal system. For example, Cooper et al., used a whole brain assay technique which could not measure DA depletions specifically within the nigro-striatal system, and Mitcham and Thomas (1972) did not perform bioassays. In the present study bioassay was performed directly on the caudate putamen which indicated the amount of catecholamine depletion within the nigro-striatal system and on hypothalamic tissue which provides a measure of nonspecific CA damage.

A third problem inherent in previous experiments was the use of only a single behavioural test (usually avoidance learning) and the attribution of behavioural deficits to the disruption of learning processes. The danger of such assumptions has been recently discussed by a number of investigators. An experiment by Barrett et al. (1973) for example has demonstrated that many experimental manipulations which have been shown to affect avoidance behaviour may be simply altering the subject's normal reactivity to shock, indicating that the deficit is not a learning deficit per se. Any study of learning deficits

must include controls to rule out the possibility of deficits other than learning. In the present experiments performance was evaluated on a number of tasks so that the precise nature of any deficits could be specified.

Experiment 1

The purpose of Experiment 1 was to determine whether differences in avoidance could be attributed to differences in general activity or FS-produced freezing. Animals with electrolytic or biochemical lesions of the SN were compared with control animals on the learning of a one-trial conditioned emotional response (CER). Following a baseline test of open field activity, subjects were given a single foot-shock (FS) and retested either 1.5 min or 24 hr later.

Method

Subjects. Fifty-four male, adult, Wistar albino rats weighing 300-350 grams at the time of surgery served as subjects. They were randomly assigned to one of three groups, (1) electrolytic lesions of the SN (electrolytic), (2) 6-OHDA lesions of the SN (6-OHDA), (3) a sham operated group (control). Each group was further subdivided into a 1.5-min test group (IMM) and 24-hr test group (24 HR). All animals were housed in individual stainless steel cages and maintained on ad lib food and water.

Surgery. All animals were anesthetised using sodium pentobarbital (50 mg/kg, i.p.). The electrolytic lesions were aimed at the SN at coordinates AP 3.0, L $\pm$ 2.2, DV-8.0 (de Groot) by means of a Krieg stereotaxic instrument. A 1.5-ma current was passed for 20 sec. through a nichrome electrode insulated except for approximately .5 mm at the tip. The biochemical lesions were produced by injecting 2 microlitres of 6-OHDA dissolved in a solution of sodium chloride, ascorbic acid, and distilled water* bilaterally into the SN at coordinates AP 3.1, L2.1 DV 2.1 from

*5.8 mg of 6-OHDA were dissolved in 1 ml of solution consisting of 54 mg NaCl, 5 mg ascorbic acid, 5 ml distilled water.

stereotaxic zero. A Kopf stereotaxic instrument was used for placement of the injection needle into the SN. The control animals had the electrode or injection needle lowered to an area approximately .5 mm dorsal to the SN to avoid SN damage but no current was passed or chemical injected.

Immediately following the surgery each animal was injected with a solution of 5% dextrose in 0.9% saline (0.05 ml/gm b wt i.p.) to facilitate recovery of those animals which had become both adipsic and aphagic following SN damage.

During the recovery period which lasted from 10 to 40 days, all animals were intubated with 12 cc. of a 25% by volume, solution of lactol and tap water twice daily (in several instances three feedings were given). A record was kept of each animal's body weight and intubation was terminated when an animal's weight began to stabilize. (Stabilization defined as no weight loss over 3 consecutive days).

Apparatus. A Plexiglas chamber measuring 18 x 18 x 18 inches was used as the test chamber. The floor consisted of copper grids (0.25 in. in diameter) separated by 0.5 in. and wired so that a 1.50-ma, 75-V FS could be delivered through a Lafayette shocker (model #5226). The floor of the chamber was divided into 16 equal-sized squares so that the number of squares traversed during the test session could be recorded.

Procedure. Each animal was placed in the same corner of the test chamber and the number of squares traversed was recorded over a 3-min. period. FS was then delivered for 5 sec. and the animal was immediately returned to the home cage. The animals were retested either 1.5 min.

(IMM) or 24 hr (24HR) later depending upon the group to which they had been assigned.

Results

Behavioural. No differences in locomotion scores were found between any of the groups prior to FS presentation ($F(5,46) = 1.43, p > .05$). This finding suggested that any difference found in learning ability would not be attributable to simple locomotor deficits due to the lesions. Analysis of variance indicated that activity scores following FS were reduced ($F(1,92) = 113.6, p < .001$) but the reduction in activity scores for the control animals was greater than that for the experimentals ($F(2,92) = 2.5, p < .05$). The electrolytic group did not differ from the 6-OHDA group ($p > .05$). No differences were found between groups retested at 1.5 min as compared to those retested at 24hr. Table 1 shows the results of this study with the raw scores transformed by the formula

Raw Score

mean score of all controls on pre-FS test

The results of this experiment indicate that any deficit in active avoidance would not be the result of too much freezing because the lesioned animals showed more activity than controls following FS. This experiment also indicated that any differences in appetitive learning task would not be the result of difference in general activity between lesioned and control groups. It would further appear from the results that CER learning is impaired. It should also be pointed out that the task is not a

Table 1: The results of Experiment 1 illustrating differences in activity before and after footshock for immediate and 24 hour retest conditions of the electrolytic, biochemical, and control groups.

<u>Electrolytic 24 HR</u>			<u>Electrolytic Immediate</u>		
	pre	post		pre	post
1	.89	.14	47	1.44	.66
2	1.78	.38	48	.72	.47
3	1.07	.47	49	1.74	1.83
5	.36	.31	50	.41	.26
7	1.35	.59	51	1.06	.25
8	.86	0	52	.99	.31
11	1.01	.17	53	1.59	.20
12	1.40	.70	54	.96	.56
13	1.66	1.72	55	1.12	.35
$\bar{X}$ =	1.15	.49		1.11	.54
<u>6-OHDA 24 HR</u>			<u>6-OHDA Immediate</u>		
	pre	post		pre	post
1	.48	.07	23	.59	.33
3	1.84	1.09	25	.90	.17
5	1.09	.14	27	.63	.48
6	1.14	.07	30	1.08	.54
9	1.69	.39	31	1.12	.04
12	.82	.27	33	.73	.25
13	1.18	1.23	34	.83	.29
			36	.85	.40
			38	.63	.15
$\bar{X}$ =	1.18	.47		.82	.29
<u>Control 24 HR</u>			<u>Control Immediate</u>		
	pre	post		pre	post
16a	1.06	.09	41	1.12	.04
17a	1.07	.04	42	.81	0
18a	.98	.07	43	1.15	0
19a	1.12	.04	44	.90	.02
20a	.77	.15	45	1.00	0
16b	.75	.05	60	1.15	.15
17b	1.11	0	61	1.05	.22
18b	.89	.09	62	.78	0
19b	1.23	.05	63	1.02	.20
$\bar{X}$ =	.99	.06		.99	.07

Score is expressed by ratio formula: Raw score $\div$ Mean of all controls
on pre-shock test

a traditional CER in that only one trial was involved, and the box rather than a tone served as the CS. Nevertheless, the animals do appear to associate FS with movement in the open field as this response is inhibited following FS.

Experiment 2a

Experiment 2a was designed to compare animals with electrolytic or biochemical lesions of the SN with control animals on a one-way active avoidance learning task. Both electrolytic and biochemical lesions can result in a depletion of DA in the caudate-putamen and it was this depletion that Mitcham and Thomas (1972) considered integral in active avoidance deficits. Experiment 1 has indicated that deficits in active avoidance learning can not be the result of differences in either general activity or freezing. Therefore, positive results in the following experiment may be interpreted as a true learning deficit although the role of reactivity to FS (Barrett et al., 1973) cannot be ruled out until appropriate tests are made.

Method

Subjects. Nine animals from the electrolytic, 6-OHDA and control groups of Experiment 1 were randomly selected for Experiment 2a (see Appendix A). The animals were housed in individual cages and maintained on ad lib food and water.

Apparatus. A two-chamber wooden box measuring 36 x 11 x 18 in. separated by a Plexiglas guillotine door was used. The floor consisting of 0.25 inch diameter grids separated by 0.50 in was wired so a 1.5 ma, 75V FS could be delivered to either side of the chamber by a Lafayette model #5226 shocker.

Procedure. 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 and the rat was allowed 10 sec. to reach the left side before the onset of a 20-sec. FS. The latency to reach the left side (including shock time) was recorded. 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 was begun. Each animal was given 10 trials per day until a criterion of at least 9 of 10 correct responses on two consecutive days was reached.

At the completion of the test for reactivity to FS (after Experiment 2b) the animals were sacrificed by cervical fracture and the left and right striatum were dissected from each brain. The remainder of each brain was put into 10% formaline and saved for histology. Striatal tyrosine hydroxylase activity was assessed by the method of McGeer, Gibson and McGeer (1967) in order to determine the extent of damage to the DA neurons in the nigro-striatal bundle in each of the rats. This damage was indicated by the amount of tyrosine hydroxylase (TH) remaining in the caudate, tyrosine hydroxylase being the immediate precursor of DA and NA. As there is no NA in the caudate, reduction of TH may be interpreted as a reduction of DA in the caudate.

Results

Behavioural. The electrolytic lesioned animals required an average of 139 trials and 98 errors to criterion while the 6-OHDA did not reach criterion within 200 trials and averaged 171 errors during these trials. The control animals required a mean 31 trials to reach criterion and averaged 8 errors to criterion. An analysis of variance indicated that both the electrolytic and 6-OHDA groups were impaired on the active

avoidance task when either trials to criterion or errors to criterion were compared to those of the control group ($F(2,24) = 22.9, p < .01$ and $F(2,24) = 26.7, p < .01$). A Newman-Keuls test revealed that the 6-OHDA group was more impaired than the electrolytic group ($p < .05$) and that both experimental groups were more impaired than the controls, ($p < .05$). Table 2 shows the scores for all the animals as well as the group means. Although none of the 6-OHDA animals reached criterion within 200 trials, in all cases the animals did escape the shock within the 20-sec. maximum shock delivery time. The mean latency to escape for both the 6-OHDA group and the electrolytic group was approximately 5 sec. These observations suggest a deficit in avoidance learning.

Biochemical. Table 3 shows the effect of the surgical manipulations on catecholamine levels at the caudate nucleus and hypothalamus. The 6-OHDA group suffered a mean caudate DA depletion of 98.83% from control levels. Accompanying this depletion in DA content was a depletion of hypothalamic NA to 42.1% of control levels. (Control levels shown on Table 4).

The electrolytic group suffered a mean DA depletion of 30.6% with no consequent reduction of hypothalamic NA. A Spearman rank-order correlation between degree of DA depletion and score on active avoidance yielded a negative correlation of $-.64$ on trials to criterion and $-.54$ on errors to criterion. Such a correlation indicates that learning performance on the active avoidance task decreases with a reduction in DA.

Table II: Results of Experiment 2a showing number of trials and errors to criterion for the experimental and control groups.

<u>Electrolytic</u>		<u>6-OHDA</u>		<u>Control</u>	
<u>Trials</u>	<u>Errors</u>	<u>Trials</u>	<u>Errors</u>	<u>Trials</u>	<u>Errors</u>
80	55	200	173	30	11
120	77	200	171	30	9
200	197	200	174	30	11
200	151	200	171	40	12
100	60	200	167	30	7
50	24	200	168	30	7
50	22	200	174	30	5
190	113	200	172	30	4
90	65	200	169	30	6
$\bar{X} = 120$	62.7	200	171	31.1	8.0

Table III: Effects of 6-OHDA on SN lesions in Experiment 2a on caudate dopamine levels as indicated by tyrosine hydroxylase activity (n mole DOPA/gm tissue/hour) and hypothalamic noradrenaline levels (ug/gm) expressed as percentage of controls.

	<u>Caudate Tyrosine Hydroxylase</u>	<u>Hypothalamic Noradrenaline</u>
<u>Electrolytic</u>		
2	36.5	94.5
5	68.75	110.0
7	60.5	88.0
8	96.0	130.0
11	76.5	119.0
12	60.5	102.0
13	87.0	108.0
$\bar{X}$	<u>69.4</u>	<u>107.4</u>
<u>6-OHDA</u>		
1	1.2	--
9	2.3	--
12	2.0	--
13	0	--
27	0.47	22.4
30	0	69.9
33	0.7	57.0
34	1.18	79.0
36	2.7	57.0
$\bar{X}$	<u>1.17</u>	<u>56.9</u>

Table IV: Levels of caudate dopamine for control animals of
Experiments 2a and 3

	<u>Caudate Tyrosine Hydroxylase</u>	<u>Hypothalamic Noradrenaline</u>
16a	63	101
17a	98	95
18a	116.5	113
19a	85	92
43	113	126
44	114	102
45	76	102
46	97	69
60	103	113
61	109	90
62	91	94
63	97	104
$\bar{X}$	<u>96.9</u>	<u>100.1</u>

Scores expressed as percentage of control mean

Anatomical. Histological examination of the electrolytic lesioned animals revealed a great deal of variability with respect to locus. In three of the animals sustaining the most complete and symmetrical lesions damage was located primarily in the ventro-medial aspects of the SN, also extending dorsally into the reticular formation, and ventrally into the cerebral peduncles. In one animal, the lesions were restricted mainly to the cerebellar peduncles, with only slight damage to the SN. The lesions in two animals were asymmetrical resulting in unilateral damage to the SN. Histology from the remaining animal revealed a large unilateral lesion to the SN on the right side of the brain. Reproduction of the lesion at the same coronal section in each of the animals is depicted in Figure 1.

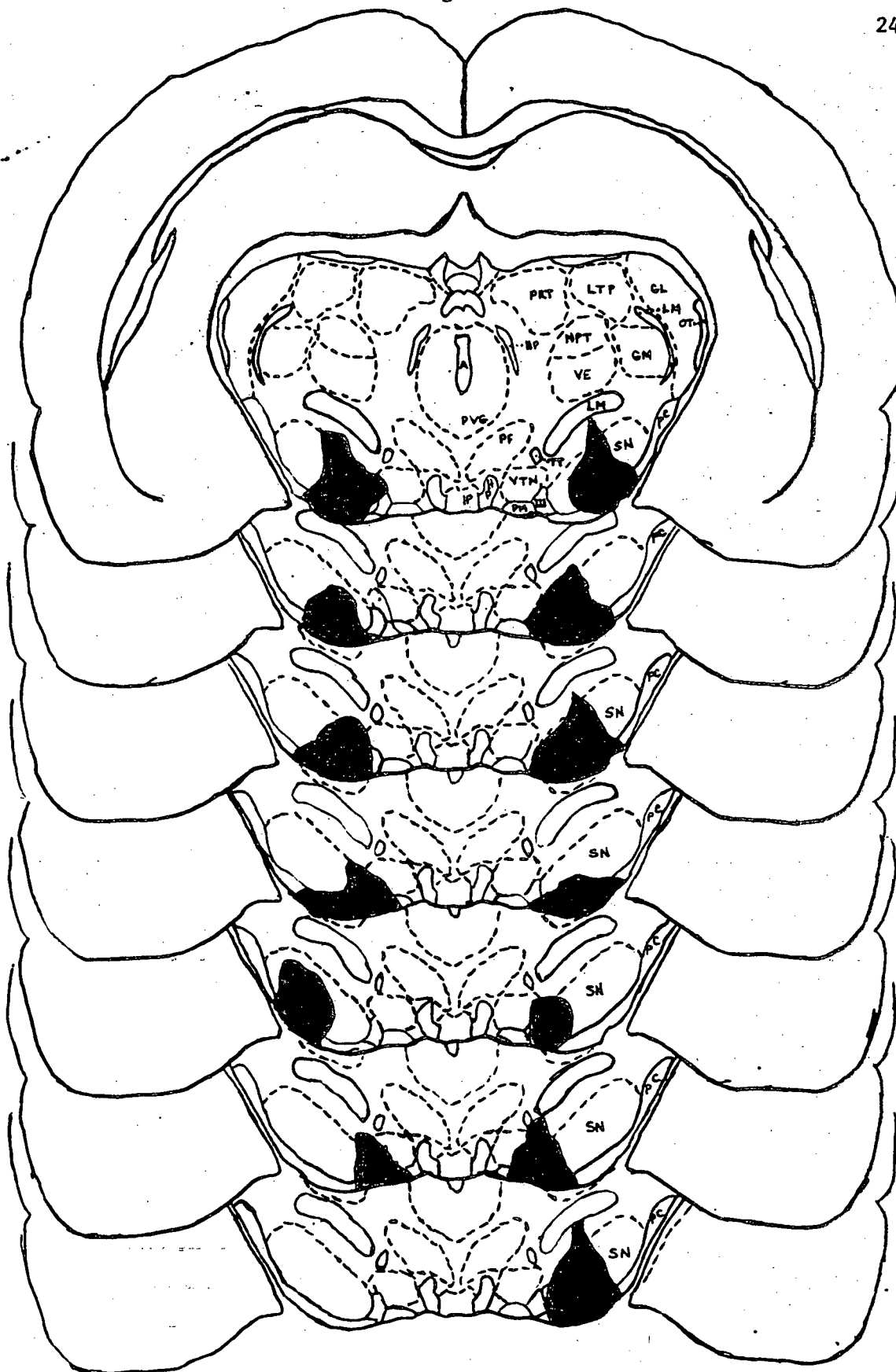
Figure 1

A graphic representation of the location and magnitude of the lesions found in each animal of Experiment 2a depicted at the same coronal section redrawn from Pelligrino and Cushman (1967).

Figure 1

24b

A 2.8



E 2

E 5

E 7

E 8

E 12

E 13

E 11

Experiment 2b

The results of Experiment 1 have shown that general activity deficits and freezing cannot explain the avoidance deficits observed in Experiment 2a. Therefore, the deficits may be true learning deficits. However, as mentioned previously, differences in reactivity to FS may confound the avoidance results as animals less sensitive to FS would be less motivated to avoid the shock. Experiment 2b was designed to test for differences in the unconditioned reactivity to footshock which may have resulted from the experimental manipulations.

Method

Subjects. Seven animals from the electrolytic, 6-OHDA, and control groups of Experiments 1 and 2a were randomly selected for Experiment 2b (see Appendix A). They were housed in individual stainless steel cages and maintained on ad lib food and water.

Apparatus. The animals were tested in a Plexiglas chamber measuring 10 x 10 x 10 in. Shock was delivered to the grid floor (0.25 in. in diameter, 0.50 in. separation) by a Lafayette shocker (model #5226). Shock delivery was timed by a Hunter timer.

Procedure. Each rat was placed in the testing chamber and subjected to 5 ascending and descending series of electric FSs. Each series consisted initially of 10 FS intensities: .6, .8, 1.0, 1.2, 1.5, 2.0, 2.8, 3.5, and 4.7 ma. The 10 shocks were presented in alternate ascending and descending order with the first series for each animal being ascending. Once the jump level for an animal was obvious, the level was never raised more than one intensity level above this value.

Each shock duration was 0.2 sec with a 30-sec. interval between each series.

Responses were rated as either: (a) no response, when the rat did not respond to the shock, (b) flinch, when the rat displayed a startle response but did not jump from the floor, (c) jump, when the rat jumped up from the floor (after Lints and Harvey, 1969).

Results

The average "jump level" for the 6-OHDA group was 2.54 ma while for the electrolytic group the average was 1.98 ma. The average jump level for the controls was 1.46 ma (see Table 5). An analysis of variance indicated the the "jump level" of the experimental groups was significantly higher than that of the controls ($F(2, 18) = p < .01$). A Newman-Keuls test indicated that the 6-OHDA group had a higher "jump level" than did the electrolytic group ($p < .05$) and both experimental groups showed a higher jump level than the controls ($p < .05$). Thus the 6-OHDA group showed the highest "jump levels" followed by the electrolytic group and the controls.

Seizures were observed in two rats of the electrolytic group during testing (E1 and E3). In both instances the rats died despite attempts by the experimenter to resperate them. The seizures could be attributed to the high shock levels used in this study but the fact that seizures were observed only in the electrolytic groups suggests strongly that some damage occurred to the brain which could have resulted in greater seizure susceptibility.

The results of this experiment suggest that the avoidance deficits observed in Experiment 2a may be attributable to a decreased reactivity

Table V: Results of Experiment 2b showing differences in "Jump" thresholds between experimental and control groups.

	<u>6-OHDA</u>		<u>Electrolytic</u>		<u>Control</u>
	jump		jump		jump
1	2.54	1	1.83	17a	1.04
9	2.46	2	1.89	16a	1.35
12	2.54	3	1.99	19a	1.50
13	2.54	5	1.98	16b	1.75
27	2.46	7	1.87	17b	1.69
30	2.62	11	2.14	18b	1.41
36	2.62	12	2.14	41	1.49
$\bar{X}$	<u>2.54</u>		<u>1.98</u>		<u>1.46</u>

Scores are expressed in milliamperes.

to FS by the lesioned animals. This would result in the FS being less motivating for the lesioned animals and may account for the apparent learning deficit. This is unlikely, however, because the shock level used in Experiment 2a (1.5 ma) resulted in a jump response in Experiment 2b and the animals did escape once the FS was presented.

Experiment 3

Because of the confounding effects of FS on active avoidance, it was considered necessary to replicate the learning paradigm using a different state of motivation. Experiment 3 was designed to test for learning deficits following electrolytic and neurotoxic lesions of the SN on a food-motivated task using the same runway as employed in Experiment 2a.

Method

Subjects. Twenty-four naive male, adult, Wistar albino rats weighing 300-350 grams were used. Eight rats were assigned to 3 groups, 6-OHDA, electrolytic, control, and housed in individual stainless steel cages.

Surgery. The surgery and postoperative care were the same for these rats as for previous rats.

Apparatus. The same test chamber as in Experiment 2a was used with the addition of a food dish on one side.

Procedure. All animals were placed on a diet of wet dog food (Husky brand, Vancouver, B.C.) immediately following surgery and maintained on this diet throughout the experiment. Five days prior to testing all animals were put on a 21-hr food deprivation schedule which was maintained throughout the experiment.

On day 1 each animal was accustomed to the apparatus by placing him first beside the food cup and then, in two stages, moved back to the starting side of the test chamber. This procedure took 15 min per animal.

On the first day of testing the rat was placed in the starting side of the chamber facing away from the lowered guillotine door. The door was raised after 5 sec. and two latencies were measured: (1) the time for the rat to pass through the doorway and (2) the time until the rat began to eat from the food cup. Each rat was allowed 10 sec to eat before the next trial was begun. Ten trials were conducted per day until a criterion of 9 of 10 correct responses was reached. A correct response was defined as leaving the start box within 10 sec and eating in the goal box within 30 sec. In the event the rat did not leave the start side within 30 sec, he was placed in the goal side by the experimenter for a maximum of 30 sec. The testing continued for 14 days allowing 140 trials for criterion to be reached.

During the experiment the amount of food consumed during the 3-hr eating period was measured on four days.

Following completion of the experiment all animals were sacrificed and prepared for bioassay and histology as in Experiment 2a.

Results

Behavioural. None of the animals in the electrolytic or 6-OHDA groups reached criterion within 140 trials (Table 6). The 6-OHDA group averaged 119 errors throughout the trials while the electrolytic group averaged 110 errors. The controls reached criterion within 101 trials with an average of 43 errors. An analysis of variance on the group data indicated that the difference in error scores was significant ($F(2,21) = 144.9$, $p < .001$). A Newman-Keuls test indicated that both experimental groups made more errors than did the controls ($p < .05$) but the 6-OHDA

Table VI: Results of Experiment 3 indicating individual and group trials errors to criterion scores for the experimental and control groups.

	<u>6-OHDA</u>			<u>Electrolytic</u>			<u>Control</u>	
	<u>Trials</u>	<u>Errors</u>		<u>Trials</u>	<u>Errors</u>		<u>Trials</u>	<u>Errors</u>
23	140	123	47	140	107	43	80	42
25	140	114	48	140	100	44	110	50
28	140	121	49	140	93	45	100	54
31	140	115	54	140	130	46	100	52
35	140	129	56	140	119	60	80	37
37	140	118	57	140	106	61	80	43
38	140	111	58	140	124	62	90	32
40	140	119	59	140	99	63	70	30
$\bar{X}$	140	119±2.1		140	110±4.6		101±6.8	42.5±2.5

group did not differ significantly from the electrolytic group ($p > .05$).

Table 7 shows the mean food consumption of the three groups for each of the four test days. Food consumption during the 3-hr eating period did not differ between the experimental and control groups ($p > .05$) suggesting that there were no large differences in motivation to eat during the testing period.

Biochemical. Table 8 shows the effects of the surgical manipulations on catecholamine levels at the caudate nucleus and hypothalamus for the animals used in Experiment 3. Measures were taken on the hypothalamus to monitor the degree of NA depletion. The 6-OHDA group suffered a caudate DA depletion of 99.12% from the control levels. The accompanying hypothalamic NA depletion averaged 45.9% from control levels. The magnitude of these depletions is very similar to that reported in Experiment 2a where DA depletion was 98.83%. The electrolytic group suffered a DA depletion of 33.0% from control levels. The animals in this group, however, suffered a 31.6% depletion of hypothalamic NA not incurred in the electrolytic group of Experiment 2a, due probably to greater tissue damage to the ventral NA bundle.

The results of this experiment indicated a deficit in appetitive learning by the lesioned animals. As the animals were unable to acquire an appetitive response, these results may be indicative of a true learning deficit following damage to the nigro-striatal DA system.

Anatomical. The electrolytic lesions for this group were in general larger than and more posterior to those found in the group of Experiment 2a. The lesions extended more dorsally and more laterally into the

Table VII: Mean amount of food consumed by the experimental and control groups during the 3 hr feeding period on four consecutive days of Experiment 3.

<u>Day</u>	<u>6-OHDA</u>	<u>Electrolytic</u>	<u>Controls</u>
1	39.6	41.9	46.1
2	36.7	34.8	33.8
3	42.2	36.2	37.5
4	37.9	35.0	38.6
$\bar{X}$	39.1	37.0	37.5

Scores expressed in grams of food consumed during 3 hr feeding period on four days of the experiment.

Table VIII: Effect of 6-OHDA and SN lesions on caudate dopamine levels in Experiment 3 as indicated by tyrosine hydroxylase activity and hypothalamic noradrenaline levels expressed as percentage of controls.

	<u>Caudate Tyrosine Hydroxylase</u>	<u>Hypothalamic Noradrenaline</u>
<u>Electrolytic</u>		
47	56	59
48	34	90
49	76	107
50	77	69
56	101	85
57	88	69
58	47	79
59	57	69
$\bar{x}$	<u>67.0</u>	<u>78.4</u>
<u>6-OHDA</u>		
23	1.85	57
23	0.59	45
28	0.18	69
31	0.95	63
35	0.03	57
37	0	0
38	0.33	69
40	3.1	73
$\bar{x}$	<u>0.88</u>	<u>54.1</u>

reticular formation. In four of the animals the lesion was found to be symmetrical and fairly complete but extended dorsally into the reticular formation. In two animals damage was restricted almost entirely to the reticular formation with only the very dorso-medial aspects of the SN sustaining damage. Two of the histologies are not depicted in Figure 2. One histology was poorly performed and the results uninterpretable. The other animal received asymmetrical lesions which were too dorsal on the left side of the brain and too medial on the right side. Figure 2 depicts reproduction of the lesion at various coronal sections.

Figure 2

A graphic representation of the location and magnitude of the lesions found in the animals of Experiment 3 depicted at various coronal sections which best illustrate this damage.

Figure 2

36 b

2.4

2.4

2.4

2.6

2.0

2.4

E 47

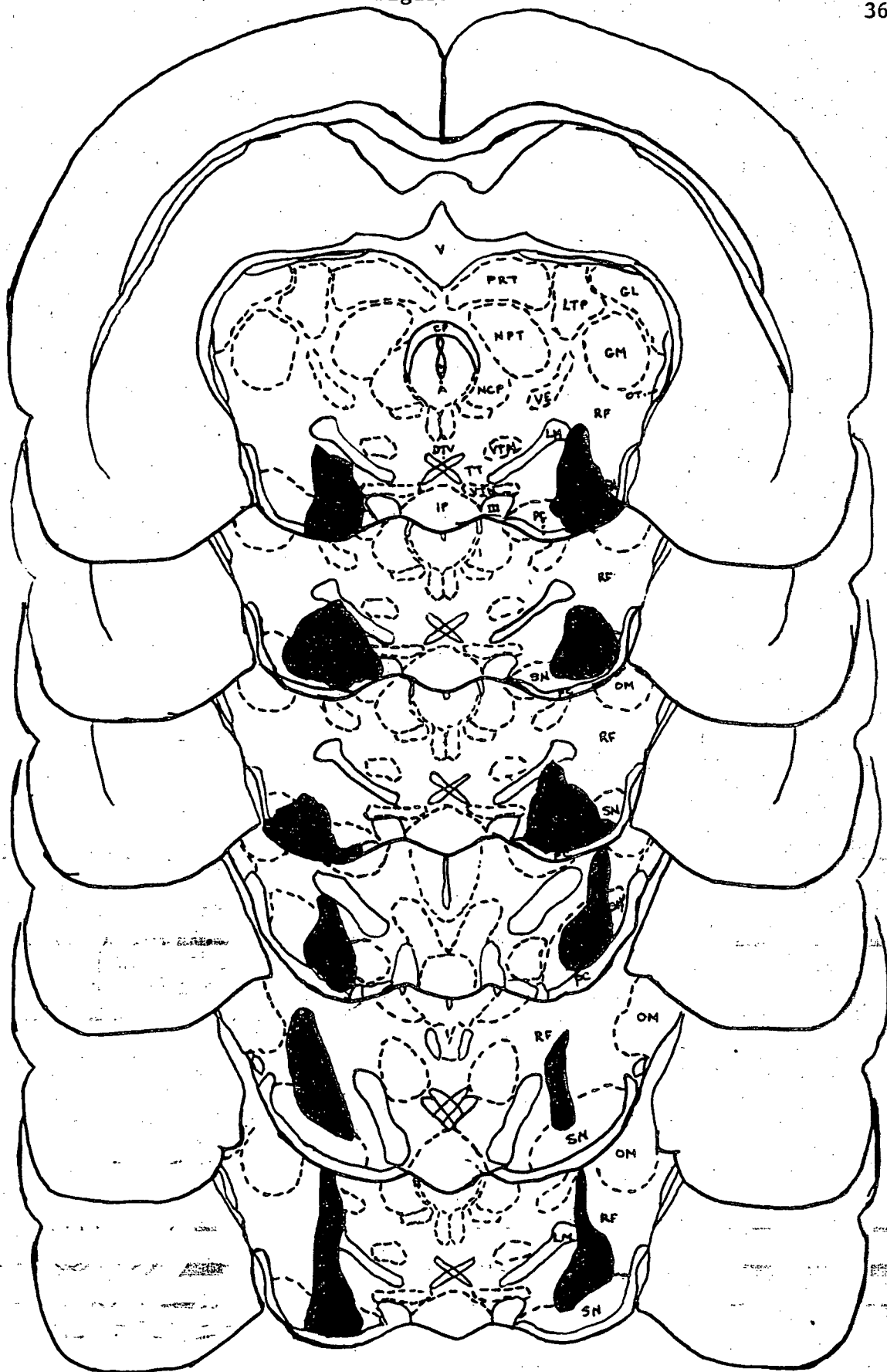
E 48

E 49

E 58

E 50

E 59



Discussion

The results of Experiment 2a clearly indicate that animals with damage to the nigro-striatal system have difficulty acquiring an active avoidance response if indeed they are able to acquire the response at all. Animals with electrolytic lesions to the SN did learn the task, but in more trials than controls ($\bar{X}$ 120 vs $\bar{X}$ 31.1). The 6-OHDA animals did not learn the response within the 200 trials of the experiment. Although the above results seem to indicate a deficit in active avoidance learning, they must be interpreted in light of Experiments 1 and 2b.

Experiment 1 revealed that prior to FS there were no differences in activity between any of the experimental or control groups as determined by counting the number of squares traversed in an open field apparatus over a 3-min period (see Table 1). However, when the animals were retested following FS, differences in activity were found. Both the electrolytic and 6-OHDA animals showed reduced activity following FS, but not to the same degree as control animals. These results demonstrate the importance of observing reactivity as opposed to activity. Experiment 1 has shown that the lesions do not result in changes in general activity or in increased tendency to freeze following FS, both of which could result in a deficit in active avoidance learning. However, Experiment 1 does not eliminate the possibility that the lesions are producing an alteration in the animal's reactivity to FS. Such an alteration could account for the deficits found in both CER learning and active avoidance learning.

Changes in reactivity to FS following various brain lesions have

been reported (Lints and Harvey, 1969; Yunger and Harvey, 1973; Dennis, 1972). Lints and Harvey (1969) found that lesions in the MFB, septal area, and dorsomedial tegmentum resulted in decreased pain thresholds as well as decreased levels of brain serotonin (5-HT). They also found that following lesions in the ventrolateral tegmentum the pain threshold as measured by reactivity to FS was increased. Fibers originating in the SN pass through this region and presumably would be affected by the lesion. Therefore it was essential to test for reactivity to FS between the groups in the present experiments.

The results of Experiment 2b indicated that the "jump" threshold of the 6-OHDA group was higher than that of the electrolytic group, while both groups had a higher threshold than controls. These results, together with those of Experiment 1 suggest that changes in reactivity to FS following nigro-striatal damage may account for at least part of the active avoidance deficit found in Experiment 2a.

As indicated in the introduction there is a great deal of indirect evidence implicating DA systems in active avoidance learning. Experiment 2a is important in that it provides direct evidence for the role of a specific DA pathway (nigro-striatal system) in this behaviour.

In Experiment 2a it was found that the 6-OHDA group was unable to learn the task within 200 trials, whereas the electrolytic group was able to learn the task but in a greater number of trials than the controls.

Following completion of a two-way active avoidance paradigm, Cooper et al. (1972) found that severe deficits in avoidance learning occurred in those animals treated with 6-OHDA plus pargyline which resulted in

a 95% reduction in brain DA and an 89% reduction in brain NA. The group treated for preferential depletion of brain DA did not show avoidance deficits although DA levels were reduced by 63%. However, it is clear from the present study that when DA levels are more significantly reduced (less than 2% of control level) profound avoidance deficits do occur. A direct comparison between the present findings and those of Cooper et al. (1972) is difficult for several reasons. First, these authors gave intracisternal injections of 6-OHDA resulting in a whole-brain depletion of DA. In the present study 6-OHDA was injected directly into the SN resulting in a specific DA depletion in the nigro-striatal system. Secondly, Cooper et al. (1972) employed a whole-brain bioassay technique which did not permit the measure of CA levels at any specific loci in the brain. In contrast, the present study employed an assay technique which specifically measured CA levels within the nigro-striatal system.

The results obtained with electrolytic lesions were consistent with the findings of Mitcham and Thomas (1972). They observed a large deficit in active avoidance learning following SN lesions. In their study the lesioned animals required approximately twice as many trials as controls (47 vs 20) to learn the task while in the present study the electrolytic group required about four times as many trials as the controls (120 vs 31; Table 2). The results of the present study differ from those of Mitcham and Thomas (1972) only in degree. However, the lack of biochemical data in the Mitcham and Thomas (1972) study and the asymmetry of the lesions in the present study makes direct comparison

difficult.

In the present study avoidance deficits were found with a depletion of DA ranging from 17-82% for the electrolytic group. One point made by Cooper et al. (1972) seems relevant to the present results. In their group treated with two injections of 250 mg of 6-OHDA, four animals of twelve tested did acquire the avoidance response although the degree of NA and DA depletion was the same for all animals. From this finding they suggest that "the anatomical location as well as the overall extent of brain catecholamine destruction is probably important (p. 729)". The present electrolytic results emphasize this point. One must recognize, however, that the lesions employed in the present study resulted in a great deal of non-specific damage which could be responsible for part of the behavioural deficit observed.

All previous attempts to examine the role of DA in learning have employed avoidance paradigms with their attendant methodological shortcomings. Experiment 3 provided a learning task with an alternate motivational state free of the confounding effects of FS. Deficits in response acquisition could therefore be more directly attributed to deficits in learning. The results of Experiment 3 reveal a large impairment in response acquisition. Neither the 6-OHDA nor the electrolytic groups learned the response within 140 trials (Table 6). The results of the food consumption test (Table 7) showed that all groups ate a comparable amount of food over the 3-hr daily feeding period indicating comparable levels of motivation. The results of Experiment 1 must also be reiterated here as they indicate that none of the groups displayed

decreased general activity as measured by open-field activity.

It appears from the results of the present experiments that general deficits in learning do occur following damage to the nigro-striatal system. However, the deficit must be interpreted with caution when employing an avoidance paradigm.

The results of Experiment 3 do not appear entirely consistent with those of Experiment 2a. In experiment 2a the electrolytic group did acquire the response although learning was retarded. In Experiment 3, however, the electrolytic group did not acquire the response and performed as poorly on the task as did the 6-OHDA group. The performance of the control animals in Experiment 3 indicates that this was a more difficult task than the avoidance task. Therefore, it is possible that the experimental groups of Experiment 3 could have learned the task in a greater number of test trials. If the ratio of trials to criterion for electrolytic and control groups was to remain the same as in Experiment 2a, the electrolytic group would require approximately 400 trials to learn the task. Unfortunately, the experiment had to be terminated after 140 trials because several of the experimental animals were becoming ill and the biochemical analysis was deemed essential.

The results of the present series of experiments do seem to indicate a learning deficit following damage to the nigro-striatal dopaminergic system. However, before one can confidently conclude that the animals with such damage were incapable of learning, more complex learning tasks should be employed. Nor should we conclude that learning deficits are exclusively related to DA depletion. In the present study a depletion

of hypothalamic NA was found in many animals suffering DA depletion (Tables 3 and 8). Studies by Anlezark, Crow, and Greenway (1973) have shown that noradrenergic systems are also involved in learning. They found that following ablation of the locus coeruleus and a subsequent depletion of cortical NA, animals took much longer to learn a runway task and did not attain the same level of performance as did the controls. Thus, it can be seen that the role of catecholamines in learning behaviour is both important and complex.

Bibliography

- Albert, M and Bignami, G. Effects of frontal, medial cortical and caudate lesions on two-way avoidance learning by rats. Physiology and Behavior, 3, 141-147, 1968.
- Anden, N.E., Dahlstrom, A., Fuxe, K., Larsson, K., Olson, L. and Understedt, F. Ascending monoamine neurons to the telencephalon and diencephalon, Acta Physiologica Scandinavica, 67, 1313-326, 1966.
- Anlezark, G.M., Crow, T.J. and Greenway, A.P. Evidence that the noradrenergic innervation of the cerebral cortex is necessary for learning. Proceedings of the Physiological Society, February, 1973.
- Arbuthnott, G.W., Crow, T.J., Fuxe, K., Olson, L., and Understedt, U. Depletion of catecholamines in vivo induced by electrical stimulation of central monoamine pathways. Brain Research, 24, 471-483, 1970. ◊
- Barrett, R.J., Leith, N.J. and Ray, O.S. A behavioral and pharmacological analysis of variables mediating active-avoidance behavior in rats. Journal of Comparative and Physiological Psychology, 82, 48-50, 1973.
- Bloom, F.E., Algeri, S., Groppetti, A., Revuelta, A. and Costa, E. Lesions of central norepinephrine terminals with 6-OH-dopamine: Biochemistry and fine structure. Science, 166, 1284-1286, 1969.
- Breese, G. L. and Traylor, T.D. Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: Evidence for selective degeneration of catecholamine neurons. Journal of Pharmacy and Experimental Therapy, 174, 413-420, 1970.
- Breese, G.R. and Traylor, T.D. Depletion of brain noradrenaline and dopamine by 6-hydroxydopamine. British Journal of Pharmacology, 48, 88-99, 1971.

- Breese, G.R. and Traylor, T.D. Developmental characteristics of brain catecholamines and tyrosine hydroxylase in the rat: Effects of 6-hydroxydopamine. British Journal of Pharmacology, 44, 210-222, 1972.
- Brodie, D., Moreno, O.M., Malio, J.L. and Boren, J.J.. Nonreversibility of the appetitive characteristics of intracranial stimulation. American Journal of Physiology, 199, 707-709, 1960.
- Cooper, B.R., Breese, G.R., Howard, J.L., and Grant, L.D. Effect of central catecholamine alterations by 6-hydroxydopamine on shuttle box avoidance acquisition. Physiology and Behavior, 99, 727-731, 1972.
- Crow, T.J. A map of the rat mesencephalon for electrical self-stimulation. Brain Research, 36, 265-273, 1972.
- Dahlstrom, A., and Fuxe, K. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brainstem neurons. Acta Physiologica Scandinavica, 62 (232), 1-55, 1965.
- Delgado, J.M.R., Roberts, W.W., and Miller, N.E. Learning motivated by electrical stimulation of the brain. American Journal of Physiology, 179, 587-593, 1954.
- Dennis, M. ~~Sex dependent and sex independent neural control of~~ reactivity to electric footshock in the rat. Experimental Neurology, 37, 256-268, 1972.
- Deutsch, J.A. Learning and electrical self-stimulation of the brain. Journal of Theoretical Biology, 4, 193-214, 1963.
- Faull, R.L.M. and Laverty, R. Changes in dopamine levels in the corpus striatum following lesions in the substantia nigra. Experimental Neurology, 23, 332-340, 1969.

- Fuxe, K., Hokfelt, T., and Ungerstedt, U. Morphological and functional aspects of central monoamine neurones. International Review of Neurobiology, 13, 93-126, 1970.
- Gibson, W. E., Reid, L. D. Sakai, M., and Porter, P. B. Intracranial reinforcement compared with sugar-water reinforcement. Science, 148, 1357-1358, 1965.
- Green, R. H., Beatty, W. W., and Schwartzbaum, J. S. Comparative effects of septo-hippocampal and caudate lesions on avoidance behavior in rats. Journal of Comparative and Physiological Psychology, 64, 444-452, 1967.
- Hess, W. R. Das Zwischenhirn: Syndrome, Lokalisationen, Funktionen, (2nd ed.). Basel: Schwabe, 1954.
- Kent, E. W. and Grossman, S. P. Elimination of learned behaviors after transection of fibers crossing the lateral border of the hypothalamus. Physiology and Behavior, 10, 953-963, 1973.
- Lints, E. E. and Harvey, J. A. Altered sensitivity to footshock and decreased brain content of serotonin following brain lesions in the rat. Journal of Comparative and Physiological Psychology, 1969, 67, 23-31.
- Lorens, S. The effect of lesions in the central nervous system on self-stimulation in the rat. University of Chicago, unpublished doctoral dissertation, 1965.
- Margules, D. L. Noradrenergic rather than serotonergic basis of reward in the dorsal tegmentum. Journal of Comparative and Physiological Psychology, 67 (1), 32-35, 1969.

- McGeer, E. G., Gibson, S., and McGeer, P. L. Some characteristics of brain tyrosine hydroxylase. Canadian Journal of Biochemistry, 45, 1557-1563, 1967.
- McIntire, R. W. and Wright, J. E. Parameters related to response rate for septal and medial forebrain bundle stimulation. Journal of Comparative and Physiological Psychology, 59, 131-134, 1965.
- Mitcham, J. C. and Thomas, R. K. Effects of substantia nigra and caudate nucleus lesions on avoidance learning in rats. Journal of Comparative and Physiological Psychology, 81 (1), 101-107, 1972.
- Nauta, W. J. H. Silver impregnation of degenerating axons. In: New Research Techniques of Neuroanatomy, W. F. Windle (Ed.,) Springfield, Illinois: Thomas, 1957.
- Olds, J. Physiological mechanisms of reward. In: Nebraska symposium on motivation. M. R. Jones, (Ed.,) Lincoln: University of Nebraska Press, 1955.
- Olds, J. A preliminary mapping of electrical reinforcing effects in the rat brain. Journal of Comparative and Physiological Psychology, 49, 281 - 285, 1956.
- Olds, J. Satiation effects in self-stimulation of the brain. Journal of Comparative and Physiological Psychology, 51, 675-678, 1958c.
- Olds, J. The limbic system and behavioral reinforcement. In W. R. Adey and T. Tokizane (Eds.,) Progress in brain research: (Structure and function of the limbic system) Vol. 27 Amsterdam: Elsevier, 1967.
- Olds, J. The central nervous system and the reinforcement of behavior. American Psychologist, 24(2), 114-132, 1969.

- Olds, J., and Milner, P. Positive reinforcement produced by electrical stimulation of the septal area and other regions of the rat brain. Journal of Comparative and Physiological Psychology, 47, 419-427, 1954.
- Olds, M. E. and Olds, J. Approach-escape interaction in rat brain. American Journal of Physiology, 203, 803-810, 1962.
- Olds, M. E. and Olds, J. Approach-avoidance analysis of rat diencephalon. Journal of Comparative Neurology, 120, 259-295, 1963.
- Olds, J. and Olds, M. E. The mechanisms of voluntary behavior. In R. G. Heath (Ed.), The role of pleasure in behavior. New York: Hoeber Medical Division, Harper and Row, 1964.
- Pellegrino, L. J. and A. J. Cushman. A stereotaxic atlas of the rat brain. New York: Appleton-Century-Croft, 1967.
- Phillips, A. G. and Fibiger, H. C. Dopaminergic and noradrenergic substrates of positive reinforcement: Differential effects of d- and l- amphetamine. Science, 179, 575-577, 1973.
- Pliskoff, S. S., Wright, J. E., and Hawkins, D. T. Brain stimulation as a reinforcer: intermittent schedules. Journal of Experimental Analysis and Behavior, 8, 75-88, 1965.
- Poirier, L. J. and Sourkes, T. I. Influence of the substantia nigra on the catecholamine content of the striatum. Brain, 88, 181-192
- Poschel, B. P. H. MAO inhibitor effects on self-stimulation: their measurement and meaning. Paper presented at the Drugs, Brain, and Behavior seminar, Department of Pharmacology, University of Michigan, November, 1967.

- Poschel, B. P. H. Mapping of rat brain for self-stimulation under monamine oxidase blockade. Physiology and Behavior, 4, 325-331, 1969.
- Poschel, B. P. H., and Ninteman, F. W. Norepinephrine: A possible excitatory neurohormone of the reward system. Life Sciences, 10, 782-788, 1963.
- Poschel, B. P. H., and Ninteman, F. W. Hypothalamic self-stimulation: its suppression by blockade of norepinephrine biosynthesis and reinstatement by methamphetamine. Life Sciences, 5, 11-16, 1966.
- Poschel, B. P. H. and Ninteman, F. W. Intracranial reward and the forebrain's serotonergic mechanism studies employing para-schorephenylalamine and para-chloroamphetamine. Physiology and Behavior, 7, 39-46, 1971.
- Routtenberg, A. Forebrain pathways of reward. Journal of Comparative and Physiological Psychology, 75, 269-276, 1971.
- Routtenberg, A., and Malsbury, C. Brainstem pathways of reward. Journal of Comparative Physiological Psychology, 68, 22-30, 1969.
- Seward, J. P., Uyeda, A. A., and Olds, J. Resistance to extinction following cranial self-stimulation. Journal of Comparative and Physiological Psychology, 52, 294-299, 1959.
- Spector, S. A. Gjoerdema, A., and Udenfriend, S. Blockade of endogenous norepinephrine synthesis by alpha-methyltyrosine, and inhibitor of tyrosine hydroxylase. Journal of Pharmacy and Experimental Therapy, 147, 86-95, 1965.
- Stein, L. Reciprocal action of reward and punishment mechanisms. In R. G. Heath (Ed.), The role of pleasure in behavior. New York: Harper and Row, 1964b.

- Stein, L., and Wise, C. D. Release of hypothalamic norepinephrine by rewarding electrical stimulation in the unanesthetized rat. Federal Proceedings, 26, 651 (Abstract), 1967.
- Stein, L., and Wise, C. D. Release of norepinephrine from hypothalamus and amygdala by rewarding medial forebrain bundle stimulation and amphetamine. Journal of Comparative and Physiological Psychology, 67(2), 189-198, 1969.
- Stein, L., Margules, D. L., Wise, C. D., and Morris, H. Noradrenergic inhibition of amygdala by the MFB reward system. Federal Proceedings, 27, 273, 1968.
- Terman, M., and Kling, J. W. Discrimination of brightness differences by rats with food or brain stimulation reinforcement. Journal of Experimental Animal Behavior, 11, 29-37, 1968.
- Thompson, R. L. Effects of lesions in caudate nuclei and dorsofrontal cortex on conditioned avoidance behavior in cats. Journal of Comparative and Physiological Psychology, 52, 650-659, 1959.
- Thompson, R. and Langer, S. K. Deficits in position reversal learning following lesions of the limbic system. Journal of Comparative and Physiological Psychology, 56, 987-995, 1963.
- Thompson, R., Rich, I., and Langer, S. K. Lesion studies on the functional significance of the posterior thalamomesencephalic tract. Journal of Comparative Neurology, 123, 29-44, 1964.
- Thompson, R., Kukaszewska, I., Schweigerdt, A. and McNew, J. J. Retention of visual and kinesthetic discriminations in rats following pretectodiencephalic and ventral mesencephalic damage. Journal of Comparative and Physiological Psychology, 63, 458-468, 1967.

- Trowill, J. A., Panksepp, J., and Gandelman, R. An incentive model of rewarding brain stimulation. Psychological Review, 76, 264-281, 1969.
- Valenstein, E. S. The anatomical locus of reinforcement. In: Progress in Physiological Psychology. Vol. 1, E. Stellar and J. J. Sprague (Eds.,) New York: Academic Press, 1966.
- Valenstein, E. S. and Campbell, J. F. Medial forebrain bundle-lateral hypothalamic area and reinforcing brain stimulation. American Journal of Physiology, 210, 270-274, 1966.
- Winocur, G. and Mills, J. A. Effects of caudate lesions on avoidance behavior in rats. Journal of Comparative and Physiological Psychology, 68, 552-557, 1969.
- Yunger, L. M. and Harvey, J. A. Effect of lesions in the medial forebrain bundle on three measures of pain sensitivity and noise-elicited startle. Journal of Comparative and Physiological Psychology, 83, 173-183, 1973.

APPENDIX A

A list of the animals used throughout experiments 1, 2a, 2b and three. A check beside the animal's number indicates in which experiment (S) the animal partook.

APPENDIX A - ANIMALS USED IN EXPERIMENTS 1 → 4

[illegible]

APPENDIX A - Continued

	<u>Electrolytic</u>				<u>6-OHDA</u>				<u>Control</u>			
	<u>1</u>	<u>2a</u>	<u>2b</u>	<u>3</u>	<u>1</u>	<u>2a</u>	<u>2b</u>	<u>3</u>	<u>1</u>	<u>2a</u>	<u>2b</u>	<u>3</u>
22												
23					x			x				
24												
25					x			x				
26												
27					x	x	x					
28								x				
29												
30					x	x	x					
31					x			x				
32												
33					x	x						
34					x	x						
35								x				
36					x	x	x					
37								x				
38					x			x				
39												
40								x				
41									x		x	
42									x			

APPENDIX A - Continued

	<u>Electrolytic</u>				<u>6-OHDA</u>				<u>Control</u>			
	<u>1</u>	<u>2a</u>	<u>2b</u>	<u>3</u>	<u>1</u>	<u>2a</u>	<u>2b</u>	<u>3</u>	<u>1</u>	<u>2a</u>	<u>2b</u>	<u>3</u>
43									x			x
44									x			x
45									x			x
46												x
47	x			x								
48	x			x								
49	x			x								
50	x			x								
51	x											
52	x											
53	x											
54	x											
55	x											
56				x								
57				x								
58				x								
59				x								
60									x			x
61									x			x
62									x			x
63									x			x

APPENDIX A - Continued

	<u>Electrolytic</u>				<u>6-OHDA</u>				<u>Control</u>			
	<u>1</u>	<u>2a</u>	<u>2b</u>	<u>3</u>	<u>1</u>	<u>2a</u>	<u>2b</u>	<u>3</u>	<u>1</u>	<u>2a</u>	<u>2b</u>	<u>3</u>
16b									x			
17b									x	x	x	
18b									x	x	x	
19b									x	x	x	
20b										x	x	

APPENDIX B

Source table for experiment 1 showing where significant differences were found.

APPENDIX B - SOURCE TABLE FOR EXPERIMENT 1.

<u>Source</u>	<u>Sum of Squares</u>	<u>df</u>	<u>Mean Square</u>	<u>F</u>	<u>Probability</u>
A Group	1.57	2	0.78	6.65	0.00147 ⁺
B Retest time	0.19	1	0.19	1.63	0.19802
C Pre-post	13.38	1	13.38	113.61	0.00000 ⁺
AB	0.39	2	0.19	1.68	0.18498
AC	0.58	2	0.29	2.48	0.03175 [*]
BC	0.06	1	0.06	0.47	0.50000
ABC	0.04	2	0.02	0.15	0.85923
Error	10.83	92	0.12		

* significant at .05 level

+ significant at .001 level