

EFFECTS OF SUBSTANTIA NIGRA AND CAUDATE NUCLEUS LESIONS ON AVOIDANCE LEARNING IN RATS

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Compared to operated and nonoperated controls, rats with small bilateral lesions in the anteroventral caudate nucleus or the rostral substantia nigra were significantly impaired in the acquisition of one-way active avoidance, passive avoidance requiring the inhibition of the previously acquired one-way response, and shuttle-box avoidance. The animals with nigral lesions took significantly more trials to criterion than the animals with caudate lesions on one-way avoidance. Results were considered in terms of the intimate anatomical and neurochemical relationships between these structures, and a circuit of structures involved in avoidance learning, differing from one previously described, was suggested.

Although the substantia nigra (SN) has been described as an important extrapyramidal motor nucleus (Jung & Hassler, 1960), relatively little is known about its functional significance. Most of the studies involving the SN have focused on its role in motor performance. Martin (1959) suggested that nigral damage resulted in the tremor and rigidity characteristic of Parkinsonism. However, Carpenter and McMaster (1964) subsequently reported that lesions destroying as much as 40% of the SN in monkeys produced no detectable physiological disturbances. These authors suggested that the tremor described previously as resulting from nigral damage was a consequence of the interruption of fibers of the brachium conjunctivum.

Those studies which have used behavioral tasks and which bear on the functions of the SN have generally included investigations of the functions of several structures and have not emphasized particularly the role of the SN. As a consequence, data concerning the role of the SN have often been tentative and based on small numbers of subjects. Thompson and his coworkers have variously suggested roles for the SN such as involvement in position reversal learning (Thompson & Langer, 1963), involvement in the integration of approach and avoidance behaviors concerned with simple learning situations (Thompson, Rich, & Langer,

1964), involvement in relearning brightness, visual pattern, and kinesthetic discriminations (Thompson, Lukaszewska, Schweigert, & McNew, 1967) and, most recently, involvement in the "visual memory system" of rats (Thompson, 1969). The SN has been implicated also in delayed-response performance (Cianci, 1965; Rosvold & Szwarcbart, 1964) and in the brainstem pathways of reward with intracranial self-stimulation (Routtenberg & Malsbury, 1969).

The role of the caudate nucleus has been extensively investigated in avoidance learning situations. However, considerable apparent disagreement has occurred among the results of these studies (Kirkby, 1970; Winocur & Mills, 1970). There have been reports with both one-way and two-way avoidance training procedures where deficits were found while in others no deficits were seen. Winocur and Mills (1970) suggested that deficits are usually the finding with relatively large and more posterior caudate nucleus lesions, but deficits typically are not seen with smaller anterodorsal caudate nucleus lesions. This explanation is consistent with the literature on one-way avoidance learning, but it will not account for discrepancies in results seen with two-way avoidance training. For example, Albert and Bignami (1968) reported no deficits in two-way avoidance learning following caudate nucleus lesions, while Neill and Grossman (1970) with comparable lesions reported deficits. However, the experiments of Albert and Bignami and Neill and Gross-

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man differed on other parameters, such as trials per day and US intensity; that might explain the different results. In spite of the apparent conflicts in the results of avoidance training following caudate nucleus lesions most of the literature suggests that such lesions produce deficits in avoidance learning.

The SN and the caudate nucleus are closely related anatomically. Both receive primary afferents from the same regions of the cerebral cortex (Diviac, 1968; Rinvik, 1966). In addition, the caudate nucleus is a source of primary projections to the SN (Szabo, 1962, 1970; Voneida, 1960). Both nuclei have been cited as projecting substantially to the globus pallidus (Carpenter & McMasters, 1964; Mettler, 1970; Szabo, 1962, 1970; Voneida, 1960), although some investigators (Carpenter & Strominger, 1967; Faull & Carman, 1968) have emphasized the nigra's projections to the ventral anterior and ventral lateral nuclei of the thalamus.

Based on the Nauta method there are questionable projections from the SN to the caudate nucleus (Carpenter & Strominger, 1967; Faull & Carman, 1968). However, based on fluorescent microscopy it has been suggested that there is a nigro-striatal projection composed of submicroscopic fibers which transport dopamine (Anden, Dahlstrom, Fuxe, & Larsson, 1966). Lesions of the SN drastically reduce the concentration of dopamine bilaterally in the corpus striatum (Faull & Laverty, 1969; Poirier & Sourkes, 1965), even when the nigral lesions are unilateral (Chandu-Lall, Haase, Zivanovic, & Szekely, 1970).

The data concerning the behavioral effects of lesions of the SN and the caudate nucleus are inconclusive and often seemingly contradictory. In view of the anatomic-chemical relationships between the caudate nucleus and the SN and the considerable available data on the caudate nucleus and avoidance conditioning, the present work compared the effects of lesions of the SN and the caudate nucleus on one-way and two-way active-avoidance conditioning and on passive avoidance where the animal had to learn to inhibit its response pre-

viously learned in the one-way active-avoidance task.

Lesions were placed in the rostral SN in order to involve the part of the nucleus shown to receive fibers primarily from the caudate nucleus in the monkey (Voneida, 1960). However, it should be noted that these connections have not been investigated in the rat. Caudate lesions were placed in the anteroventral region hopefully to involve the homologue of the area known to send abundant fiber bundles to the SN of the monkey (Voneida, 1960).

METHOD

Subjects

Seventy-one experimentally naive Sprague-Dawley male albino rats, age 90-120 days, were maintained in individual cages with food and water ad lib.

Surgery and Histology

The subjects were anesthetized with Equithesin (3.75 cc/kg of body weight)² and placed in a Kopf Model 1404 stereotaxic instrument with the level of the incisor bar set at 5 mm. above the interaural line. A midline incision was made, and after retraction of the skin the electrode positions were marked on the skull and drilled with a dental burr. Monopolar electrodes were constructed of stainless-steel wire .3 mm. in diameter and insulated to within 1 mm. of the tip. A Grass Model LM-4 radio-frequency lesion generator was used with lesion parameters being 22 v. for 30 sec. Stereotaxic coordinates taken from Pellegrino and Cushman (1967) for SN lesions were 3.0 mm. posterior to bregma, 2.5 mm. lateral to midline, and 8.2 mm. below the exposed dura. Coordinates for caudate lesions were AP 2.3 mm. (bregma), L 3.0 mm., and DV 5.5 mm. The same anterior-posterior and lateral coordinates were used for electrode track controls, but the electrode was lowered only to the edge of either the SN or the caudate nucleus. After surgery the scalp was sutured and dusted with tetracycline powder, and the animals were returned to their home cages.

Following completion of behavioral testing all operated rats were given an overdose of Equithesin and perfused intracardially with physiological saline followed by 10% Formalin. The brains were removed and embedded in celloidin. Coronal sections were taken at intervals of 40 μ . Alternate sections were stained with thionin and Weil stains.

²Subsequent to this experiment the authors have used Equithesin doses of 2.5 cc/kg following Wampler (1971).

Apparatus

The apparatus for both experiments was a 61.5 × 41 × 39.7 cm. stainless-steel shuttle box assembled from LaFayette Model A550 modules. The shuttle box was divided into two equal sections by a manually operated transparent Plexiglas door. One 15-w. bulb was located above a translucent ceiling in each shuttle-box compartment. The US was a .75-ma. shock supplied by a LaFayette constant-current shocker (Model A612) and was administered through a LaFayette Model 620 shock scrambler to the grid floor of the shuttle box.

Procedure

Task 1: One-way active avoidance. Ten rats received caudate lesions and 10 received SN lesions. Another 11 rats served as electrode track controls, and there were 9 unoperated controls. Ten days after surgery the animals were given a 15-min. adaptation period in the experimental chamber during which they had free access to both sides. Twenty-four hours later avoidance training was begun. The avoidance conditioning procedure was similar to that of Winocur and Mills (1969). The rat was placed in the right side of the experimental chamber with the Plexiglas door between the chambers closed. Five seconds later the door was raised simultaneously with light onset in both chambers. Movement of the animal to the left side of the experimental chamber within 5 sec. after CS onset terminated the CS and resulted in avoidance of the US. Failure to move to the left side within 5 sec. resulted in the presentation of the US, which remained on, along with the CS until the rat escaped to the left side. After 30 sec. in the left side the animal was picked up and returned to the right side for 5 sec. and the next trial began. Fifteen trials per day were given until an animal made nine avoidance responses on any 10 successive trials within a given day.

Task 2: Passive avoidance. Twenty-four hours after criterion was met in avoidance conditioning each animal was tested using a passive-avoidance procedure. The rat was placed into the right side of the apparatus and given one active-avoidance trial. After 30 sec. the rat was placed in the right side of the apparatus and 5 sec. later the CS was presented. Movement to the left side now resulted in the presentation of the shock until the rat returned to the right side of the apparatus. A correct passive-avoidance response was defined as the animal remaining in the right side of the apparatus for 60 sec. during continuous presentation of the CS. The animals received one trial per day until a criterion of four successive correct responses was met.

Task 3: Two-way active avoidance. Nine rats received SN lesions, while eight rats received caudate lesions. Ten animals were given electrode track control operations, and 5 animals served as unoperated controls. Ten days after surgery the

animals were given a 15-min. adaptation period in the apparatus. Twenty-four hours later avoidance training was begun. The rat was placed in one side of the experimental chamber with the door closed. Five seconds later the door was raised simultaneously with light onset into both chambers. Movement of the animal to the other side of the chamber within 5 sec. after the CS onset resulted in CS termination and avoidance of the US. Failure to move to the other side in 5 sec. resulted in the presentation of the US, which remained on until the rat escaped to the other side. After 30 sec. the CS was presented again, and the animal was required to reenter the other side of the chamber within 5 sec. or the US was presented. Fifty trials per day were given over a period of 5 days.

Before each test session all animals were screened for gross motor deficits. Each animal was placed in the center of a board 38 cm. long and 4.4 cm. wide which was suspended from two platforms 1 m. above the floor. If the animal failed to move to one of the platforms without difficulty or if it fell off, it was eliminated from the study. Three rats in the nigral group were eliminated on this basis.

RESULTS

Both SN and caudate animals were impaired on all measures, as both groups took more trials to criterion on both active and passive avoidance and neither group exceeded 20% correct responses during any stage of training in the two-way situation.

Anatomical

Caudate. All 18 animals received bilateral damage to the caudate nucleus. Figure 1 illustrates representative caudate and SN lesions. Representative lesions were selected in the following manner. For each animal the measures of lesion size (AP extent, DV extent, and diameter) were totaled. The mean of these totals was then computed and deviations of individual animals from the mean were recorded. Means were then computed for each measure of lesion size and deviations of individual animals from these means were recorded. All deviation scores were then totaled and the animals with the smallest totals were chosen as having representative lesions.

SN. Sixteen animals received bilateral damage to the SN, while 2 animals received unilateral damage to the SN.

Lesion size. Nigral lesion size was positively correlated ($r = .64, p < .05$) with trials to criterion on the passive-avoidance

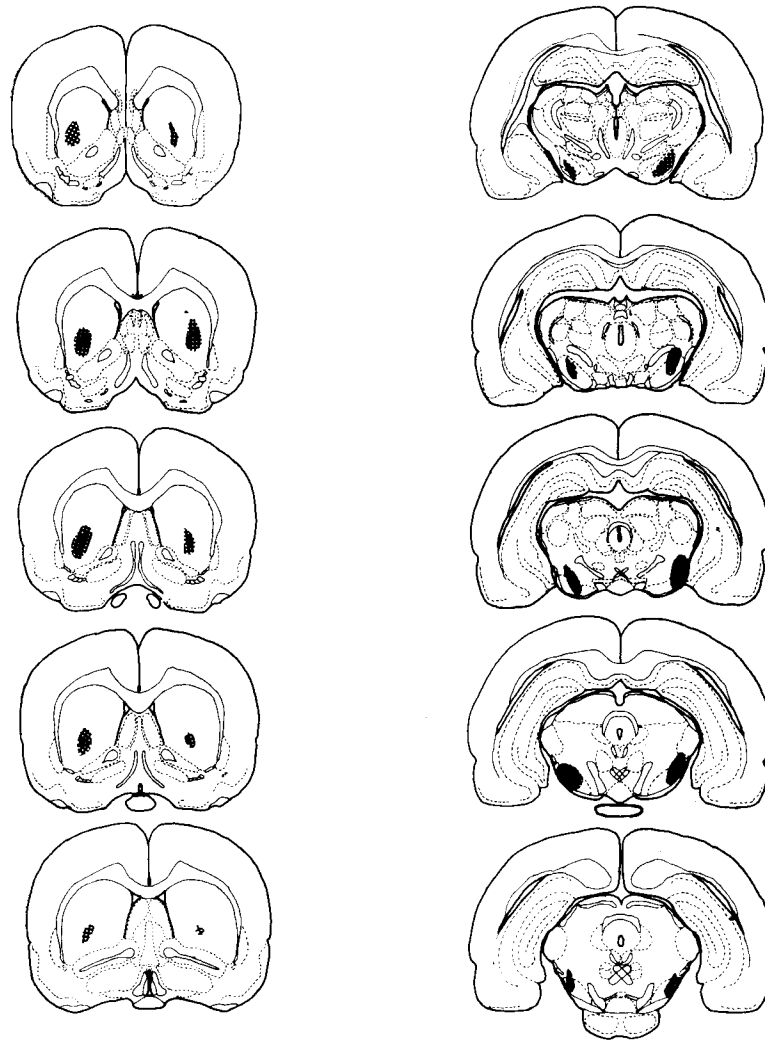


FIG. 1. Representative lesions (see text) of the caudate nucleus and the substantia nigra.

measure. Performance on the other measures was not significantly correlated with lesion size for either caudate or SN animals.

Behavioral

One-way avoidance. The nigral animals required an average of 47.1 trials to criterion, while caudate animals required 32.8. Normals, SN controls, and caudate controls required averages of 18.2, 23.5, and 15.8 trials, respectively. Caudate animals responded correctly on 57.4% of the trials, while SN animals responded correctly on 50.7% of the trials. For normals, SN controls, and caudate controls the percentages were 60.8, 59.6, and 68.4, respectively.

A one-way analysis of variance (Winer, 1962) revealed significant differences between the treatment groups ($p < .01$) with respect to trials to criterion on the one-way active-avoidance task, but the groups were not significantly different with respect to percentage of correct responses.

The Newman-Keuls procedure was used for individual comparisons. Caudate and SN animals performed significantly poorer than controls ($p < .01$). Nigral animals were significantly poorer than caudates ($p < .05$). The control groups did not differ significantly.

Passive avoidance. The SN animals required an average of 10.1 trials to criterion,

while caudate animals required an average of 10.2 trials. Normals, SN controls, and caudate controls required 5.6, 7.3, and 5.2 trials, respectively. Both caudates and SN animals responded correctly on 63.5% of the trials, while for normals, SN controls, and caudate controls the percentages were 71.6, 73.0, and 77.6, respectively.

A one-way analysis of variance revealed significant differences between the treatment groups ($p < .05$).

The Newman-Keuls procedure revealed significantly poorer performance ($p < .01$) by both caudates and nigral when compared with controls. The caudates and nigral did not differ significantly.

A one-way analysis of variance revealed no significant differences between the groups with respect to percentage of correct responses on the passive-avoidance task.

Two-way avoidance. Figure 2 shows the effects of caudate and nigral lesions on two-way avoidance responding. As may be seen, the control animals showed a negatively accelerated acquisition curve and had means between 70% and 80% correct re-

sponses on the final training day. Caudate and SN animals showed little improvement with training, and the mean number of correct responses for these groups never exceeded 20% correct responses at any stage of training.

A two-way analysis of variance with repeated measures on one variable indicated that there was a significant treatment effect ($p < .01$). There was also a significant trial-block effect ($p < .01$), as well as a significant Lesion \times Trial interaction ($p < .01$).

Tukey (*a*) tests were performed for each trial block in order to make individual comparisons between groups at each stage of training. For all trial blocks both caudates and nigral were significantly poorer ($p < .01$) than controls. Caudates and nigral did not differ significantly at any stage of training.

Tukey (*a*) tests were performed for each group to make comparisons of the effects of trial blocks within a group. After the second day of training all control groups had improved significantly ($p < .01$) over the first

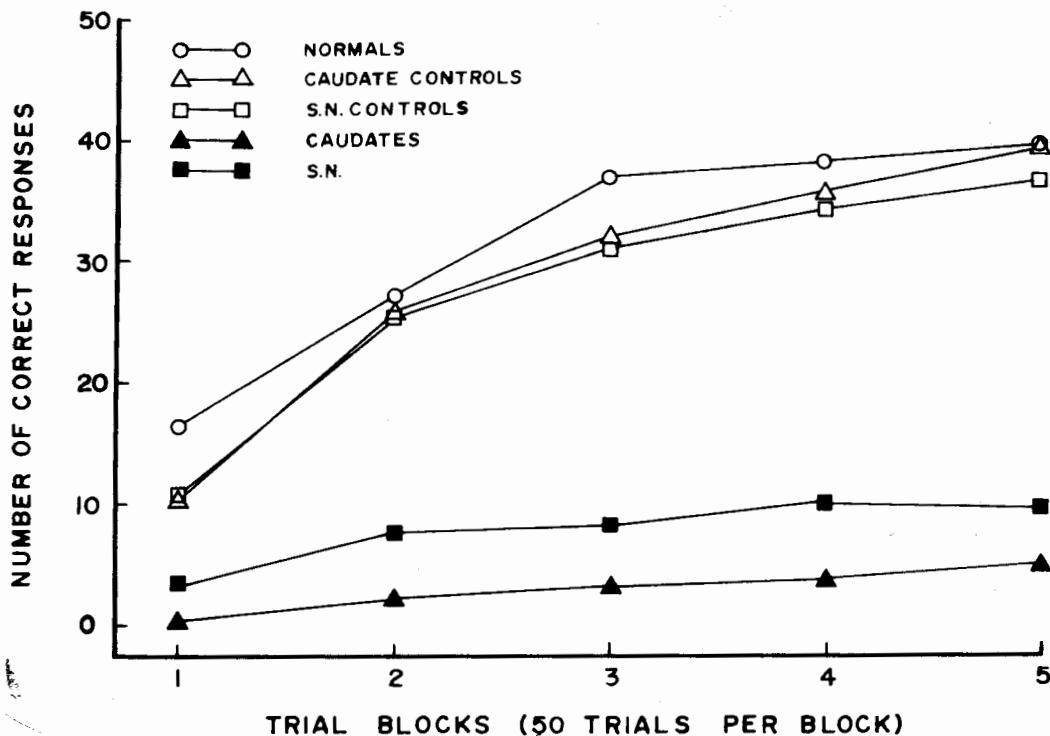


FIG. 2. Number of correct responses in successive blocks of 50 trials.

day's performance. Neither caudates or nigral showed significant improvement with increased training.

DISCUSSION

The results of the present work are consistent with those studies reporting deficits in one-way and two-way avoidance learning following lesions of the caudate nucleus (Kirkby, 1970). Lesions of the SN in the present study produced deficits in active- and passive-avoidance learning that were similar to the deficits associated with caudate lesions.

In view of the comparability of results seen in avoidance learning following ventral caudate and SN lesions, it may be useful to suggest a possible explanation. It has been established that the concentration of dopamine in the caudate nucleus is reduced following lesions of the SN (Faull & Laverty, 1969; Poirier & Sourkes, 1965) and that the SN may be the source of the caudate's dopamine (Anden et al., 1966). Neill and Grossman (1970) suggested a functional distinction between the dorsal and ventral caudate in the rat, and they implied that the ventral caudate might be selectively involved with the caudate's dopamine. Based on the present work, it is suggested that lesions of the ventral caudate or lesions in the SN affect the caudate's dopamine in a way that impairs avoidance learning.

Thompson et al. (1964) described a circuit of structures which may be critical for the performance of an avoidance response. This circuit does not include the caudate nucleus, yet it is apparent that any consideration of the anatomical substrates of avoidance conditioning must include this area. The similarity of effects seen with caudate or nigral lesions suggests an explanation for the disruption of avoidance conditioning following the caudal thalamic lesions seen in the Thompson et al. (1964) study without having to invoke an unknown pathway between the dorsomedial thalamus and the SN. The caudate nucleus, unlike the dorsomedial thalamus, sends clear-cut and abundant fiber bundles to the SN in a topographic fashion (Szabo, 1962). It is suggested that the transections de-

scribed by Thompson et al. (1964) which disrupted avoidance behavior and which were thought to sever a hypothetical pathway between the dorsomedial thalamus and the ventral mesencephalon may have damaged caudate-nigral projections in the internal capsule or nigra-striatal or nigra-thalamic projections.

The inclusion of the caudate-nigral components with the avoidance circuit provides additional possibilities for the role of other neural structures in avoidance learning. Divac (1968) has previously suggested that there are studies suggesting appropriate anatomical connections between and similar behavioral functions for discrete regions of the cerebral cortex and the caudate nucleus. The avoidance circuit to be emphasized in the present work includes neocortex to caudate nucleus and SN (Rinvik, 1966) and from the caudate nucleus to the SN (Nauta & Mehler, 1966; Szabo, 1962, 1967; Voneida, 1960). From the SN, the circuit may return rostrally to the caudate via the dopaminergic pathways (Anden et al., 1966) and to the ventral lateral and ventral anterior nuclei of the thalamus (Carpenter & Strominger, 1967) and/or globus pallidus (Mettler, 1970) via major anatomical projections. The thalamic nuclei project widely to the frontal neocortex (Truex & Carpenter, 1969) and, therefore, complete the loop of the avoidance circuit being emphasized here. In view of the questionable projections of the caudate nucleus and SN below mesencephalic levels, it is suggested that the responses in avoidance learning are mediated by the pyramidal tracts and the rubrospinal tract. Both tracts receive significant contributions from the frontal lobe (Brodal, 1969; Truex & Carpenter, 1969). The circuit described suggests the immediate usefulness of obtaining data on the effects of lesions in the ventral lateral and ventral anterior thalamic nuclei, the globus pallidus, the frontal neocortex, and, possibly, the red nucleus on avoidance learning.

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