THE DORSAL TEGMENTAL NORADRENERGIC PROJECTION: AN ANALYSIS OF ITS ROLE IN LEARNING

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ABSTRACT

THE DORSAL TEGMENTAL NORADRENERGIC PROJECTION: AN ANALYSIS OF ITS ROLE IN LEARNING

David C. S. Roberts

The hypothesis that the noradrenergic projection from the locus coeruleus (LC) to the cerebral cortex and hippocampus is an important neural substrate for learning was evaluated. Maze performance was studied in rats receiving either electrolytic lesions of the LC, or 6-hydroxydopamine (6-OHDA) injections into the region of the dorsal tegmental noradrenergic projection. In contrast to the results of an earlier report (Anlezark, Crow, and Greenway, 1973), LC lesions did not disrupt the acquisition of a running response for food reinforcement in an L-shaped runway, even though hippocampal-cortical noradrenaline (NA) was reduced to 29%. Greater telencephalic NA depletions (to 6 percent of control levels) produced by 6-OHDA also failed to disrupt the acquisition of this behaviour or impair the acquisition of a food reinforced position habit in a T-maze. Neither locomotor activity nor habituation to a novel environment was affected by the 6-OHDA lesions. Rats with such lesions were, however, found to be significantly more distractible than controls during the performance of a previously trained response. In another group of rats with identical 6-OHDA injections, the establishment of a lithium chloride-induced conditioned taste aversion was not affected by the lesions. The hypothesis that telencephalic NA is of fundamental importance in learning was not supported.

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INTRODUCTION

In 1953, Bein described the "tranquilizing" effect of rauwolfian alkaloids, and specifically reserpine. It was noted that this drug was capable of reducing motility of rabbits without the barbiturate-like sedation and disequilibrium. The behavioural suppression was later linked to the ability of reserpine to deplete the neuronal stores of catecholamines (CAs) and serotonin (5-HT) (Brodie et al., 1957). The finding that small quantities of DOPA, a metabolic precursor of dopamine (DA) and noradrenaline (NA), could reverse the reserpine-induced hypomotility suggested that CAswere important for this action of reserpine.

Other pharmacological manipulations of CA have since been shown to affect behaviour, and have sparked a widespread research interest into the functional importance of CAs in the central nervous system (CNS) and their relation to various response patterns. Specifically, a number of theorists have postulated that CAs are necessary to the learning process and the formation of memory. The present research report is an attempt to examine this relationship in several learning situations. A short introductory survey of the current state of knowledge concerning the localization and physiology of CAs in the CNS follows.

Localization and Metabolism of CAs in the Central Nervous System.

The presence of CAs in the CNS was demonstrated bio-

chemically by Vogt (1954) for NA and Carlson et al. (1958) for DA. However, a clearer understanding of the distribution and cellular localization of these monoamines awaited the development of the formaldehyde histochemical fluorescence technique introduced by Falck and Hillarp (1962). This procedure allowed direct visualization of catecholamine-containing cell bodies and highly fluorescent variocosites of the presume nerve terminals. Since the axons of CA containing cell bodies contain very low concentrations of amines, pharmacological and lesion techniques were necessary to map out the projections and pathways of these fiber systems. It was found that fluorescent variocosites accumulated proximal to the cell body at the site of a lesion, presumably due to orthograde axonal transport of the transmitter or its synthesizing enzymes. By making hemisections at various levels of the brain, the course of specific pathways could be established (e.g. Dahlstrom and Fuxe, 1964, 1965; Anden, et al., 1966). In 1971, Ungerstedt using this procedure provided a stereotaxic map of the known monoamine pathways.

Lindvall and Bjorklund (1974 a and b) have since been able to provide a more detailed description with the aid of the sensitive glyoxylic acid fluorescence method. These authors have identified five distinct NE fiber bundles. The central tegmental tract ascends (but also has descending components) from the medulla oblongata to the caudal diencephalon; contributing fibers orginate from

A1, A2, A5, A7 and the locus coeruleus (A6) (Dahlstrom and Fuxe, 1964, nomenclature). The dorsal tegmental bundle (formerly 'dorsal bundle' of Ungerstedt, 1971) originates solely in the locus coeruleus and projects principally to the thalamus, hippocampus and cortex. The periventricular system contains two components; the dorsal component contains cell bodies diffusely distributed along its extent from pons through posterior thalamus. The ventral component originates in the rostral mesencephalon and intermingles in the dorsomedial hypothalamic nucleus with the dorsal component to form an ascending hypothalmic CA system. The medial forebrain bundle system is an extension of the dorsal tegmental bundle and central tegmental tract. Fibers are seen to innervate the cingulum, hypothalamic nuclei, geniculate bodies, septum and olfactory bulbs among other forebrain structures.

Two major DA ascending systems have been described. The nigro-striatal bundle (NSB) originates in the pars compacta of the substantia nigra and terminates topographically in the caudate-putamen. The meso-limbic system has its cells of origin dorso-lateral to the interpeduncular nucleus, and projects to the nucleus accumbens, tuberculum olfactorium and nucleus interstitialis stria terminalis. DA terminals have also been identified in the frontal cortex, septum and anterior limbic cortex.

In 1939, Blaschko proposed the enzymatic sequence in

the biosynthesis of the catecholamines which is generally agreed upon at present. The amino acid, tyrosine, is converted to dihydroxyphenylalanine (DOPA) via the enzyme tyrosine hydroxylase. This is considered to be a rate limiting step in the synthesis of CA. The distribution of this enzyme roughly parallels the known DA and NE pathways with high levels found in terminal regions. The second enzyme in the synthesis is DOPA decarboxylase which yields dopamine. This enzyme is not specific to neural tissue but is present in many peripheral organs (e.g. kidney, liver). This enzyme is not specific to DOPA, and may more properly be referred to as L-aromatic amino acid decarboxylase. A third enzyme, dopamine-B-hydroxylase is not found in DA neurons, but is specific to adrenergic and NA neurons. This enzyme, which converts DA to NA, may also be rate controlling.

Two important enzymes have been shown to metabolize NA and DA. Catechol-O-methyltransferase is thought to function extraneuronally. The other, monoamine oxidase (MAO), is presumed to function intraneuronally to metabolize CA not confined to storage granules. MAO is found extraneuronally as well. Inactivation of CA in the synpatic cleft is not primarily via these metabolic enzymes, however, but is considered to take place mainly by a re-uptake process into the presynaptic cell.

Theoretical Consideration of NA Involvement in Learning

Several theories of learning involve the establishment

of new neural connections or at least a change in the conductivity of existing synapses (Roberts & Matthysse, 1970). Theorists have most often proposed that this plastic change takesplace at the cortical level and consists of a two stage process. The first phase is of short duration and labile to many types of external interference. This phase could involve an electrical reverberating pattern which is later consolidated by some physical change in the neural network. Theoretical refinements have drawn on various mechanisms which allow selective storage of adaptive response patterns, with the obvious advantage that maladaptive or unreinforced behaviors would not be as likely to reoccur.

One such mechanism proposed by Crow (1968) is termed a "discriminator". This is a system of fibers which have a widespread terminal distribution, and are able to affect a change in synaptic connections of those circuits carrying activity associated with a beneficial response. This proposed reward system is a neurophysiological analogue of Skinnerian reinforcement, since it increases the probability of reoccurrence of that particular neural response which led to the activation of the reward system.

Crow and Arbuthnott (1972) further speculated on the identify of such a pathway. On the basis of anatomical and behavioral evidence, these authors proposed that noradrenergic neurons from the nucleus locus coeruleus, with its

associated cortical distribution, may form the neural basis of reinforcement. The locus coeruleus has the characteristics of a visceral afferent nucleus (Russell, 1955) and may receive information from gustatory receptors. The presumed activation of this nucleus during feeding fits well with a theory of environmental activation of a neural reward pathway. This nucleus also possesses the required cortical terminal distribution which is predicted by this theory. Additional support for the implication of this nucleus being involved in a cortical reward system is the demonstration that this nucleus will support intracranial self-stimulation (Ritter and Stein, 1973).

Kety (1970, 1972) has also proposed a learning role for the adrenergic systems of the cortex. This theory is much like that put forward by Crow in that the release of NA "consolidates" neural associations.

Indirect support for the hypothesized role of the cortical NA projection in learning takes several forms. Pharmacological manipulations of CA synthesis have long been known to disrupt learned responses. An agent which inhibits the enzyme tyrosine hydroxylase, α-methyl-para-tyrosine, markedly interferes with the performance of a conditioned avoidance response (CAR) (Rech, Borys and Moore, 1966). Since the performance of the learned response is impaired, it is not surprising to find that this agent also disrupts the acquisition of the CAR (Essman, cited by Kety, 1970).

Inhibition of the enzyme dopamine-β-hydroxylase with diethyldithiocarbamate (DDC) has been shown to disrupt the formation of a passive avoidance response in mice (Randt, Quarterman, Goldstein and Anagnoste, 1971) and rats (Stein, Belluzzi and Wise, 1975). The fact that this effect of DDC can be reversed with intraventricular administration of NA (Stein et al., 1975) further indicates that NA synthesis plays an important role in memory function. Recovery from the amnesia produced by DDC has been reported to occur by administration of either of the monoamine oxidase inhibitors (MAOI), "Catron" or"Pargyline" (Botwinick and Quartermain, 1974) before the retest. These data suggest the possibility that while memory may be stored under the DDC drug condition, future recall is impaired. This facilitation of recall by MAOI is not specific to amnesia produced by DDC since an MAOI also attenuates amnesia in animals treated with acetoxycycloheximide (AXM), a protein synthesis inhibitor (Botwinick and Quartermain, 1974).

While the pharmacological data from peripherally administered drugs implicate NA in the acquisition of learned responses, clearly central manipulations are required to identify what, if any, specific NA pathways are involved.

Several studies have employed the neurotoxic agent 6-hydroxydopamine (6-OHDA) in an attempt to examine CA involvement in the acquisition of food-rewarded behaviors. This agent has been shown to produce selective destruction of

CA nerve endings and cell bodies in the CNS (Bloom et al., 1969). CA-neurons accumulate CA (Iversen, 1971). One analogue, 6-OHDA, is also actively taken up by these cells and is thought to cause neuronal damage via one of the oxidation products, hydrogen peroxide (Heikkila and Cohen, 1972). Cholinergic, serotonergic and gabanergic neurons are not damaged when 6-OHDA is used in appropriate dosages (Uretsky and Iversen, 1970); McGeer et al., 1973); however, some non-specific damage does occur at the site of injections (Hokfelt and Ungerstedt, 1973).

Howard, Grant and Breese (1974) administered 6-OHDA intracisternally and studied the effects of such treatment in rats on the acquisition and performance of a double T-maze. Each animal was required to choose between two levers at each end of a runway. Depression of one lever would deliver a food pellet twice, after which the animal was required to traverse the runway and choose the correct lever at the other end to obtain further food. The number of rewarded responses (lever presses) in each daily test session was the dependant variable. 6-OHDA treatment produced significant decrements in the response rate in rats which had previously been trained, and this reduction was clearly related to the depletion of CAs. 6-OHDA treatment prior to training also produced a profound impairment in the acquisition of the bar pressing response. Analysis of the whole brain content of NA and DA led the authors

to conclude that the behavioural deficits "were more related to the reduction in dopamine than they were to the depletion of brain norepinephrine (p. 995)."

Mason and Iversen (1974) employed the intraventricular route of 6-OHDA administration to reduce whole brain tyrosine hydroxylase activity to 8, 16, and 15% in the striatum, hypothalamus and cortex respectively. Animals with such lesions were found to be impaired on a complicated problem solving task. The rat was required to either push or pull a ball through a tunnel to gain access to a food reward. 6-OHDA treated animals required more trials to learn the response than did controls. The authors argued that motivational, motor or co-ordinational deficits were not involved, but that the deficit in acquisition of the task demonstrated that CAs were important to the learning process.

Attempts to test the involvement of specific NA pathways in the acquisition of appetitively motivated learning tasks have yielded contradictory results. Anlezark, Crow and Greenway (1973) reported that electrolytic lesions to the locus coeruleus (LC), which depleted cortical NA, also abolished the ability to acquire a running response in an L-shaped runway. Attempts to replicate this finding have failed. Amaral and Foss (1975) reported that electrolytic lesions to the LC which produced equal or greater depletions of hippocampal-cortical NA failed to produce deficits in

acquisition in either "T"- or "L"-shaped mazes.

The present investigation represents an attempt to determine the nature of the involvement of the NA pathway from the LC to the cortex. We have shown (Roberts et al., 1975) that extensive depletions of cortical NA can be achieved through intracerebral injections of 6-OHDA in the vicinity of the dorsal tegmental NA bundle (Lindvall and Bjorklund, 1974). Since this method is selective and more effective than electrolytic lesions in depleting forebrain NA, 6-OHDA lesions were utilized to examine further the involvement of forebrain NA in the acquisition of some learned behaviors.

GENERAL METHOD

Subjects

Male Wistar rats (Woodlyn Farms, Guelph, Ontario), weighing 290-320 gm, were used in all experiments. All animals were individually housed in stainless steel wire cages and received food and water ad libitum for three weeks postoperatively.

Treatments

All rats were anaesthetized with sodium pentobarbital (50 mg/kg) and prepared for surgery in a Kopf stereotaxic apparatus. In one group (LC), bilateral lesions of the nucleus locus coeruleus were attempted by passing 1mA for

30 sec through the bared tip of an electrode, fashioned from two twisted strands of .277-mm-diameter nichrome wire. The co-ordinates according to Fifkova and Marsala (1967) were AP 8.5; ML ± 1.2; DV - 6.5 mm.

Another group of rats (NA-6-OHDA) received injections of 6-OHDA in the dorsal tegmental NA bundle. Bilateral injections of 4 µg/2 µl/10 min of 6-OHDA hydrobromide (dose expressed as the free base) in 0.9% saline containing ascorbic acid (0.2 mg/ml) were infused through a 34-gauge needle. Co-ordinates, according to Konig and Klippel (1963), were AP + 2.6 mm: ML ± 1.1 mm; DV + 3.7 mm.

Control animals received burr holes through the skull, while some additional animals were unoperated.

Noradrenaline Assay

Extraction of catecholamines. Following the completion of the behavioral measures, the animals were killed by cervical fracture and the brains quickly removed. The hippocampus and cerebral cortex were dissected out on ice and combined. The tissue was weighed, then homogenized in 5 ml perchloric-acetic acid. The homogenizing tube was rinsed with a further 2 ml of acid which was added to the homogenate. This was allowed to stand in the cold for 1/2 hour and was then centrifuged at low speed. The clear supernatant was decanted. The residue was resuspended, centrifuged, and the supernatant decanted, twice more.

To the combined supernatants was added 0.5 ml, 0.1 M ethylenediaminetetracetic acid (EDTA). At this stage the samples were usually frozen overnight.

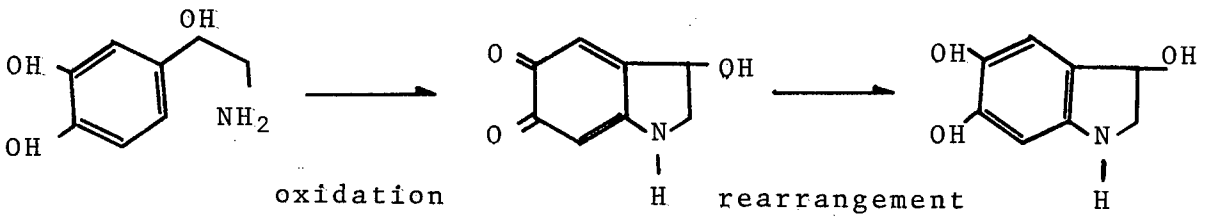
An alumina column was prepared as follows. A glass tube 20 cm in length was fashioned so that the upper portion of the tube consisted of a 2 cm diameter reservoir of about 15 ml capacity. The lower portion was a 4 mm diameter shaft, drawn out to a fine tip and plugged with glass wool. To the tissue extract was added 1 ml EDTA and 1.5 ml potassium phosphate (.35 M), and the pH adjusted to 9.2-9.4. A consistent measure of alumina (about 0.4 gm) was added and stirred for 3 minutes. This slurry was poured through the glass tube, the flow through which was adjusted by application of gentle suction. The alumina was washed with 25-30 mls of distilled water. The catecholamines were eluted with 0.5 ml acetic acid (0.5N).

Formation and estimation of fluorescent derivatives. To the 0.5 ml of eluant was added 0.5 ml of 1 M sodium acetate buffer (pH6) and the pH adjusted to 5.7-6.0 with 0.5N NaOH. Each sample was then brought to a volume of 3.0 ml with distilled H_2O. Into each of two test tubes was placed 0.5 ml of the sample solution, and 0.5 ml, 0.5 M sodium acetate buffer, pH 6.4 was added, followed by 0.5 ml iodine solution (0.254 gm I + 5.0 gm KI/227 ml H_2O). The mixture was shaken and allowed to stand. After 10 min 0.25 ml sodium thiosulphate (0.5 M) was mixed in. To one

of the two tubes was added 0.5 ml of a combination of ascorbic acid (5 mg/ml) and 5N NaOH in a ratio of 3:7. To the second tube was added 0.35 ml of 5N NaOH only. The samples were allowed to stand at room temperature, under fluorescent lighting for 90 - 120 min. To the second tube was added 0.15 ml ascorbic acid (5 mg/ml); this now constituted the "faded blank". All samples were read in a spectrophotofluorometer. The activation peak was set at 395 nm and the excitation peak was 505 nm. Both column and assay standards were employed to determine the percent recovery of amines of the column, and relationship of the fluorometer reading to amount of noradrenaline per sample.

Principle of the noradrenaline assay. While catecholamines will fluoresce (excitation peak, 285 nm; emission, 325 nm), this is no aid to their determination in small amounts. The reason lies in the fact that this fluorescence is a non-specific property due to the phenol ring present in the compound. The task then is to convert the NA, and only the NA, into substances which can be specifically identified fluorometrically. The assay method is based on the determination of a derivative of NA, and not NA itself. The derivative employed in the presently described assay technique is 3,5,6,-trihydroxyindole. The reactions required in the formation of this derivative are shown below (from Nagatsu, 1973).

noradrenaline noradrenochrome 3,5,6-trihydroxyindole



oxidation rearrangement

The oxidation is carried out by the iodine solution for 10 min after which time this reaction is stopped by the sodium thiosulphate. Rearrangement occurs in alkaline solution during exposure to light.

Statistics

Statistical comparisons of brain NA were done using Student's *t*-test. Behavioural data were analysed using repeated measures analysis of variance.

EXPERIMENT 1

Effect of Telencephalic NA Depletion on the Acquisition of an L-Maze Running Response

The importance of telencephalic NA to the acquisition of a food rewarded running response in an L-shaped runway was investigated. Both electrolytic and 6-OHDA-induced lesions were used in an attempt to replicate the finding of Anlezark et al., (1973). These authors reported that, unlike controls, rats with lesions of the LC which decreased cortical NA failed to decrease their running time over days.

Method

Four weeks post operatively, all rats were handled for 5 min/day and food deprived 22 hr/day. Training commenced the fifth week. On the first experimental day, each rat was allowed to explore the runway for 30 min without food present; for each of 10 subsequent days, each rat received five food-rewarded trials. On each trial, the rat was placed in the start box and after 3 sec a guillotine-style door was raised which allowed access to the runway. Another manually operated door prevented the animal from retracing after entering the goal box. The rat was removed after it had consumed the five 45 mg food pellets (Noyes). Purina Lab Chow was available for 2 hours after being returned to the home cage. The maze was constructed of wood, with dimensions as follows: height, 15 cm; width, 11 cm; runway, 140 cm. Two photo-cells, 120 cm apart, were utilized to measure running time over the initial long arm of the maze. The length of the runway conforms to the specifications of Anlezark et al. (1973).

Fifty rats were used, of which 22 were 6-OHDA treated, 10 were electrolytically lesioned, and 18 were controls.

Results

Four rats were excluded from the analysis (1 control, 1 NA-6-OHDA, and 2 LC). Each displayed aggressive and irritable behaviour characterized by freezing in the start

box, vocalizing when handled, and failing to consume food when placed in the goal box. The hippocampal-cortical NA depletions of the two LC animals were 18 and 87%, thus, the abnormal behavior does not appear related to destruction of the locus coeruleus per se.

Table 1 shows the effect of lesions to the locus coeruleus on NA content of the hippocampus plus cortex. Hippocampal-cortical NA was reduced to 29% of control (Range = 16-47%). In the NA-6-OHDA group, hippocampal-cortical NA was reduced to 6.7% of control values. Many of the readings from this group were at blank levels. The lower limit of the sensitivity of the assay was 0.01 μg. Histological examination of the locus coeruleus lesions revealed that in most cases the lesion destroyed the rostral portion of the nucleus. Some cells in the caudal portion of the nucleus appeared to be spared in most of the animals. Figure 1 shows the bilateral electrolytic lesion at the rostral aspect of the LC in an animal with NA depleted to 22% of control values.

No difference in running speed was found between the burr hole and non-operated control groups. These data were pooled in subsequent analyses. Figure 2 shows the reduction of mean running time over days. Statistical analysis failed to show any significant difference between these groups, $F(2,44) = 1.44$, $p > .05$. Single comparisons between

TABLE 1

Effect of 6-OHDA Lesions in the Dorsal Tegmentum and Electrolytic Lesions to the Locus Coeruleus on Hippocampus plus Cortex Noradrenaline.

Group	NA (ng/g) Cortex plus Hippocampus
Controls (n=28)	389 ± 30 (100%)
LC Lesions (n=8)	113 ± 20 * (29.0%)
6-OHDA Dorsal Bundle Lesion (n=33)	26 ± 7 * (6.7%)

Data represent means (± S.E.M.). The data from animals with 6-OHDA dorsal bundle lesions were combined from the different experiments.

* Significantly different from controls $p < 0.01$

Figure 1. Photomicrograph of bilateral electrolytic lesions of the n. locus coeruleus which caused a 79% reduction of hippocampus plus cortex NA. Abbreviations: Mes V = mesencephalic tract of the trigeminal nerve; MLF = medial longitudinal fasciculus; PVG = periventricular grey; SCP = superior cerebellar peduncle; IV = fourth ventricle.

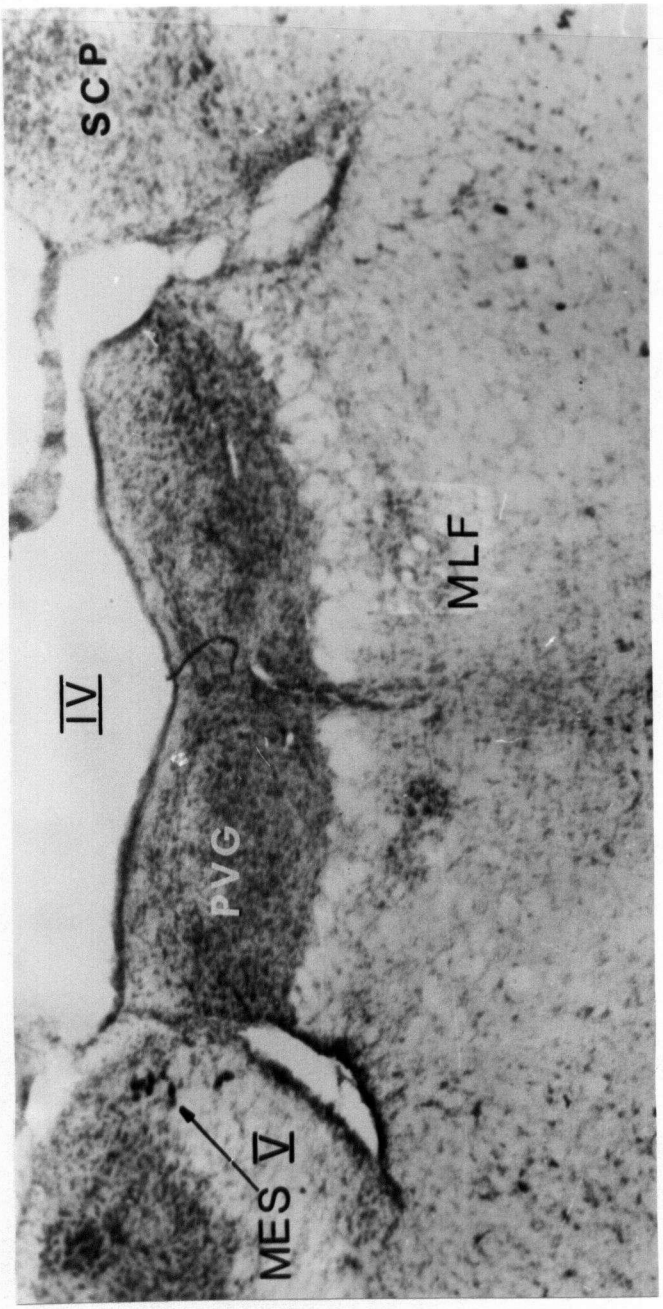
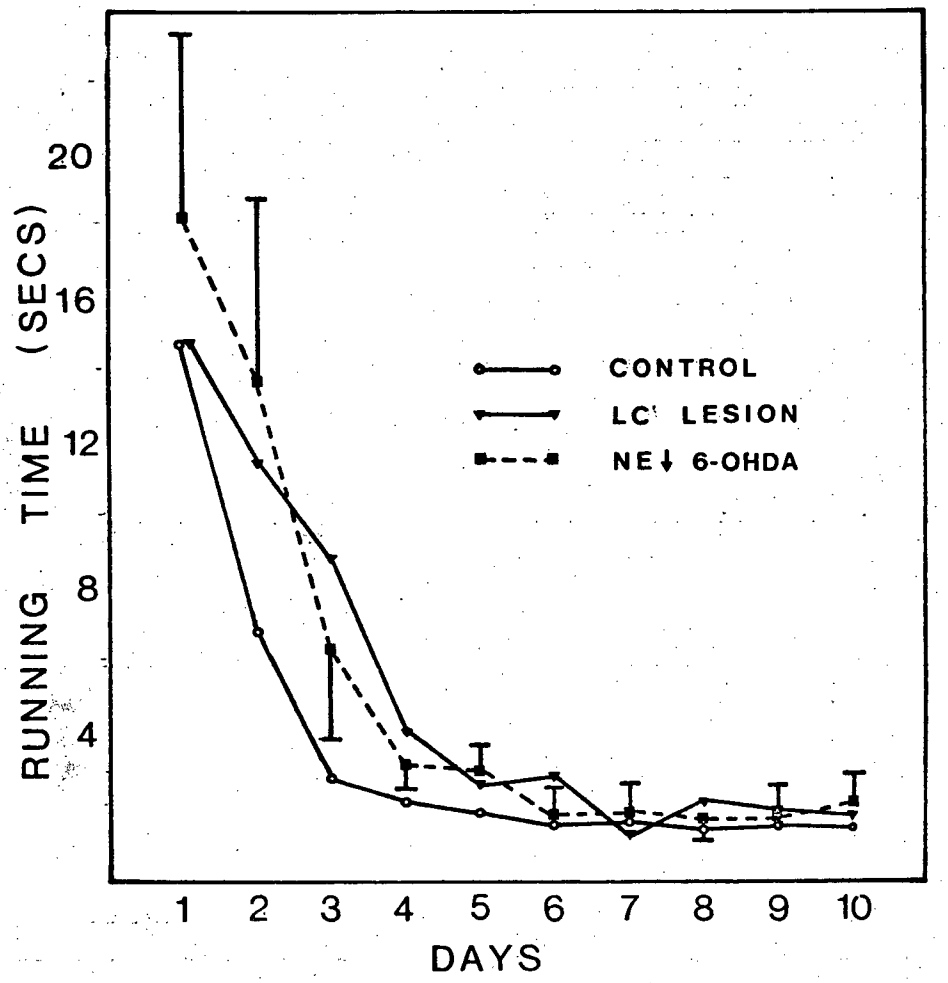

SCP
IV
PVG
MLF
MES V

Figure 2. Running times measured between two photocells 120 cm apart on the initial arm of an L-shaped maze. Animals were tested for 5 trials/day and each point represents the mean of 5 trials. For clarity S.E.M.s are shown for one group only; variances were homogeneous on each day. See methods for details.


RUNNING TIME (SECS)
20
16
12
8
4
1
2
3
4
5
6
7
8
9
10
DAYS
CONTROL
LC LESION
NE↓ 6-OHDA

experimental groups against controls also failed to reach statistical significance at $p < .05$ [NA-6-OHDA vs Cont. $F(1,37) = 2.71$: LC vs Cont. $F(1,24) = 2.30$]. Analysis restricted to the initial three days was performed to test whether a possible difference at this stage was obscured by the similarity of the following days. This comparison also failed to reach significance, $F(2.44) = 1.10$, $p > .05$.

Two animals in the LC group displayed a urogenital disorder. Nine of fourteen rats used to confirm lesion co-ordinates also showed identical symptoms. These were: blood in the urine, 1-3 days postoperatively; polydipsia and polyuria which subsided in 5-14 days. Several animals died, and autopsy revealed that the bladder had ruptured in each case. This disorder did not appear related to the degree of locus coeruleus damage, as assessed histologically, nor depletion of cortical NA. It was never observed in animals with 6-OHDA lesions of the dorsal tegmental NA projection.

Discussion

Anlezark et al. (1973) reported learning, as assessed by increasing running speed in an L-shaped runway, was absent in animals with complete electrolytic lesions to the locus coeruleus. Because these lesions also significantly reduced telencephalic NA, it was concluded that

these noradrenergic neurons were important substrates for learning. The present results are not consistent with the conclusions of this earlier work. Comparable NA depletions were achieved with electrolytic lesions, without significant impairment on this task. It should be noted, however, that our LC lesions appear to have been more rostral than those utilized by Anlezark et al. (1973). To what extent this variable may have contributed to the discrepant results is not known. 6-OHDA lesions to the dorsal tegmental bundle caused a greater NA depletion, yet also failed to alter significantly the response pattern as compared with controls.

The running times differed in another respect from those reported by Anlezark et al. (1973). Running times at the beginning of training in the present experiment were shorter than the times reported by those investigators. Procedural differences may explain this discrepancy. We handled the rats extensively and allowed exploration of the runway before beginning training. It is not known whether Anlezark et al. followed a similar procedure. Our shorter times may reflect a greater degree of adaptation to handling and to the runway. This discrepancy does not detract, however, from the major conclusion, i.e., that contrary to previous observations, hippocampal-cortical NA does not seem to be critical for

the demonstration of learning in an L-maze, as assessed by running times.

The four animals which were excluded from the analysis deserve comment. They would typically behave normally on the first trials in a day; however, with increased handling, freezing, defecation, urination, and vocalization became evident. These animals were run through day 5, with a cutoff value of 5 min per trial. If average running times were used as the sole criterion for learning, then it might be concluded that these animals had a "learning deficit". However, since some running times decreased to as little as 1.2 sec, we concluded that learning did occur, but that its demonstration was obscured by a competing behaviour, namely freezing. It is not clear from the report of Anlezark et al. (1973) whether the LC lesion animals which did not show decreases in running speed behaved in a normal manner or displayed unusual behaviors as were sometimes observed in the present experiments.

The present results are in close agreement with those of Amaral and Foss (1975). These authors also failed to show a deficit in runway learning after lesions to the LC. Urogenital disorders similar to those reported here were also observed. These disorders have recently been studied in detail by Osumi, Oishi, Fimiwara and Takaori (1975).

EXPERIMENT 2

The Effect of Telencephalic NA Depletion on the Acquisition and Reversal of a T-Maze Postion Habit

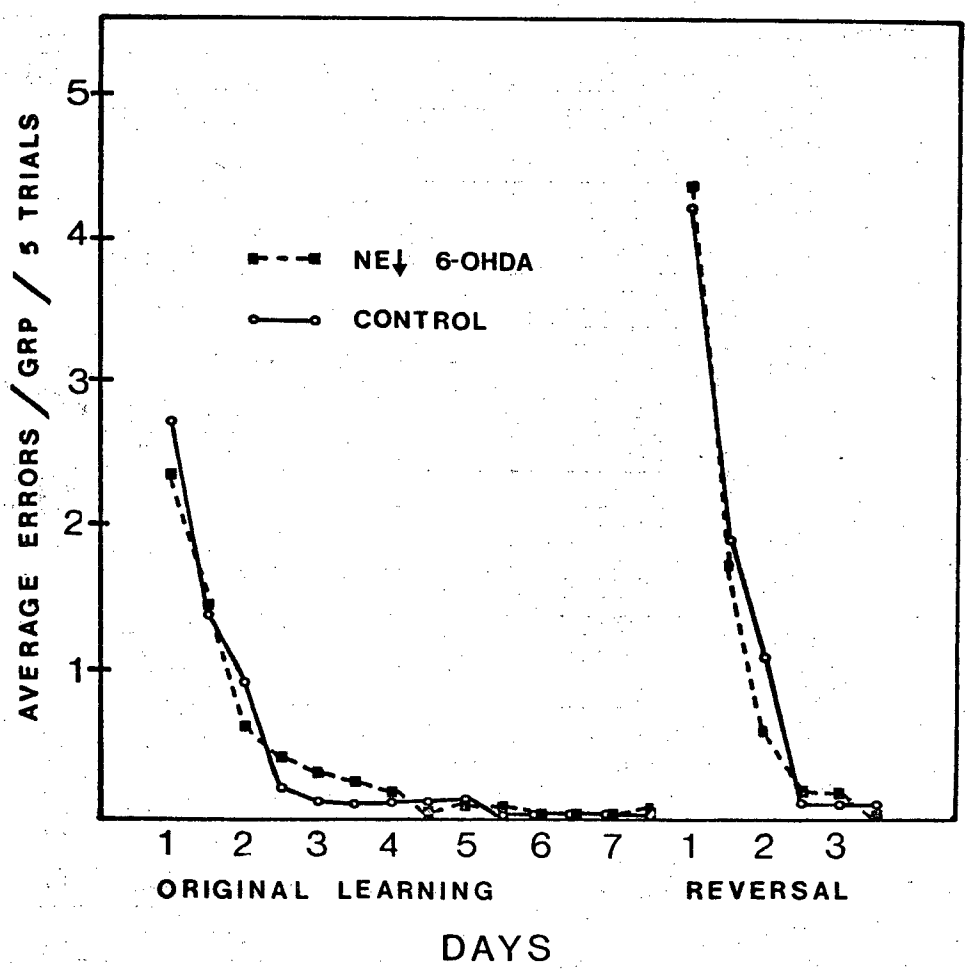
Decreased running times in an L-shaped runway may indicate that an animal has learned that food is present in the goal box. However, in this procedure food is available regardless of running speed,and reinforcement is not contingent on a specific behavior, other than eventually finding the food. In addition, changes in running speed may reflect motivational, motor or sensory factors rather than learning. For these reasons, the acquisition of a T-maze position habit was selected as a situation more suited to an analysis of learning. In this situation, only appropriate responses are reinforced, i.e., choosing the correct goal box. In the following experiment, the hypothesis that cortical NA is critical for learning was therefore investigated using 6-OHDA lesioned animals in a T-maze position task. 6-OHDA lesions were performed prior to the training of a position habit after which the animals were trained to run to the opposite arm of the T-maze.

Results

Depletions of hippocampal-cortical NA comparable to Exp. 1 were obtained and are shown in Table 1.

Figure 3 shows the average error per group in blocks of 5 trials. Statistical analysis revealed no significant difference between the two groups on errors, $F(1.20) < 1.0$;

Figure 3. Average errors of two groups of animals in blocks of 5 trials during the acquisition of a position habit in a T-maze. Each animal was tested for 10 trials/day with food reward available in one goal box only. On days 8-10 food reward was presented in the goal box on the opposite side from original training. See methods for details.


AVERAGE ERRORS / GRP / 5 TRIALS
5
4
3
2
1
NE↓ 6-OHDA
CONTROL
1 2 3 4 5 6 7 1 2 3
ORIGINAL LEARNING
REVERSAL
DAYS

or running speed, $F(1.20) = 1.32$, $p > .05$. Analysis of errors on the reversal task also failed to yield a significant difference, $F(1,20) < 1.0$.

Discussion

The hypothesis that telencephalic NA is important for the learning of a food-rewarded response was not supported. Examination of Figure 3 shows essentially identical learning curves for the experimental group and controls, despite near total depletions of hippocampal-cortical NA. A previous report (Amaral and Foss, 1975) indicated electrolytic lesions to the locus coeruleus, which depleted cortical NA to 18%, were also ineffective in producing a learning deficit. These authors employed an olfactory stimulus to identify the correct goal box of a T-maze. The present results extend these findings to more complete depletions of hippocampal-cortical NA. The reversal data indicate that extensive lesions of the dorsal NA bundle do not significantly affect the ability of the animal to alter a position habit as the reinforcement contingencies are modified.

EXPERIMENT 3

The Effect of Telencephalic NA Depletion on Exploratory Locomotor Activity

Exploratory behavior and habituation to a noval environment are two important processes in the establishment of an

appetitive response. Sufficient investigation of a maze is required for the animal to find the reward; further, habituation to non-reinforcing aspects of the environment must occur for the appropriate response to predominate eventually over the exploratory behavior. The following two experiments represent an attempt to examine the degree of involvement of hippocampal-cortical NA in these processes. In the first experiment the amount of locomotor activity and the time course of habituation to a noval environment were measured in activity cages.

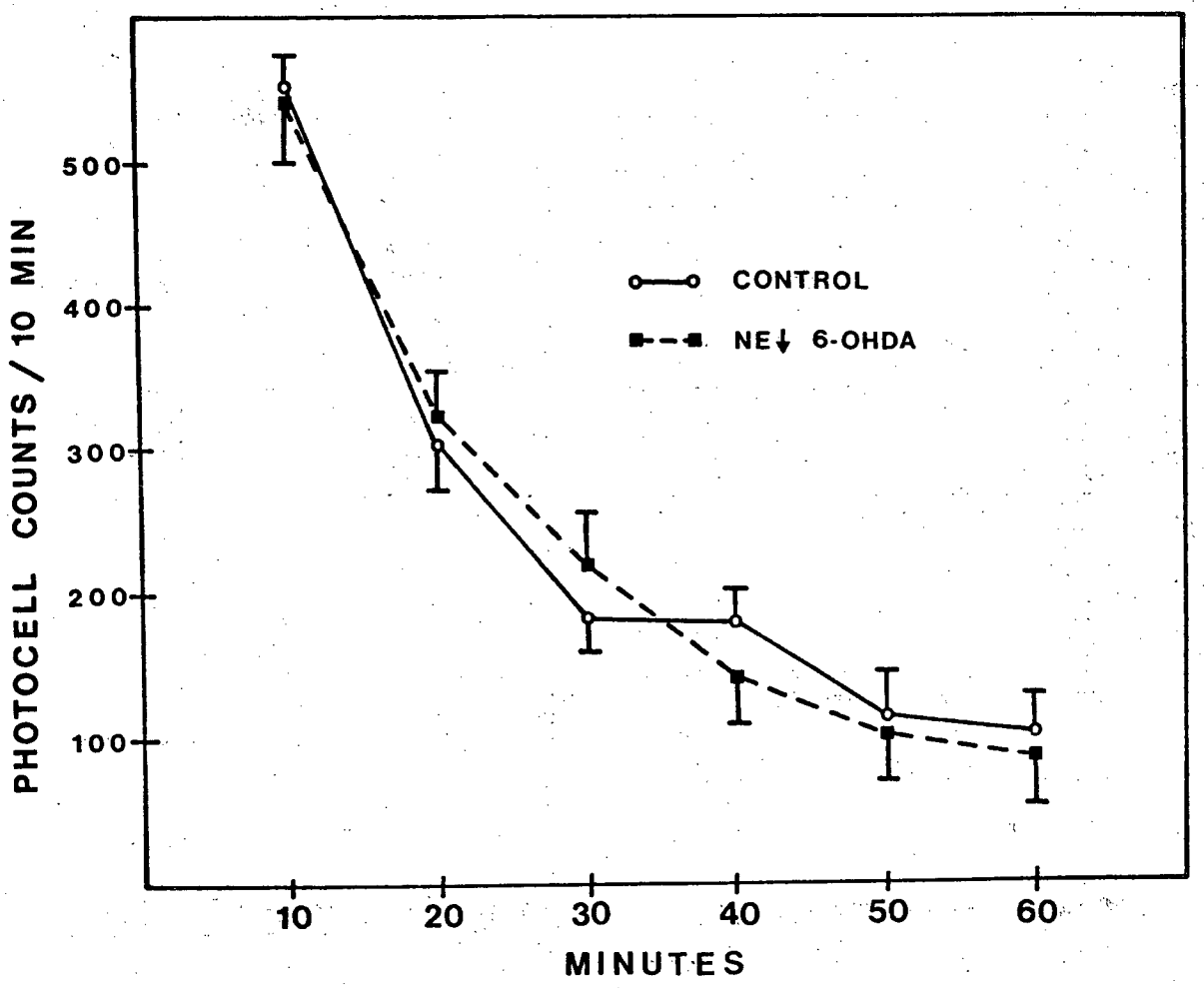
Method

Subjects were those used in Experiment 2. Activity was measured in six circular 61-cm-diameter activity cages (Lehigh Valley Electronics) housed in an illuminated, sound attenuated room. Each cage was equipped with 12 photocell sensor units, mounted on the outer wall at equal distances, 2 cm above the wire mesh floor. Interruptions of the light beams were recorded on a 10-min timed printout counter for 1 hr. Activity was measured both before and after 4 days of a 22 hr/day food deprivation schedule at the same time of day for each animal.

Results

Statistical analysis failed to reveal a significant difference between control animals and those with 6-OHDA lesions to the dorsal NA bundle, in either the deprived

Figure 4. Locomotor activity of two groups of animals measured during 1 hr in photocell activity cages. Each point represents the mean (+S.E.M.) photocell interruptions over 10 min. See methods for details.


PHOTOCELL COUNTS / 10 MIN
500
400
300
200
100
CONTROL
NE↓ 6-OHDA
10
20
30
40
50
60
MINUTES

F (1, 20) < 1.0 or non-deprived condition, F (1, 20) < 1.0. The time course of the decline of motor activity over the 1 hour test period also did not differ between the groups in the deprived, F (5,100) < 1.0, or non-deprived condition, F (5,100) < 1.0.

Figure 4 shows the activity measures of the two groups in the non-food deprived state over 1 hour in 10 minute segments. The scores following 4 days of food deprivation yielded essentially the same habituation curve except that a significantly lower response pattern was seen, F (1, 20) = 7.63, $p < .05$. Testing order was not counterbalanced and therefore this effect might have been due to having been in the activity cages previously.

Discussion

In as much as activity cages can measure habituation to a noval environment, cortical-hippocampal NA does not appear to play a significant role in this behavior. These results are in agreement with earlier results from this laboratory (Roberts, Zis and Fibiger, 1975). Anlezark et al. (1973) also found no difference in exploratory behavior between LC-lesioned animals and controls

in an open field test. In contrast, Amaral and Foss (1975) reported that activity, measured over a 90-minute test period, correlated positively with NA content following electrolytic LC lesions. Motor disturbances were also noted, however, and may have accounted for the decreased activity in lesioned animals which was observed by these workers. Differences in test apparatus may also account for this apparent discrepancy. Amaral and Foss used animex activity cages which are more sensitive to small body movements than are photocell cages.

EXPERIMENT 4

The Effect of Telencephalic NA Depletion on the Distractibility of Rats during the Performance of Trained Response

In Experiment 3, it was shown that the exploratory activity of animals with depletions of hippocampal-cortical NA declined at the same rate as that of controls, and thus

might indicate the same rate of habituation to the novel test environment. However, in activity cages, no response is specifically reinforced. The following experiment was designed to measure the effect of novel tactile and visual stimuli on a trained food-rewarded response.

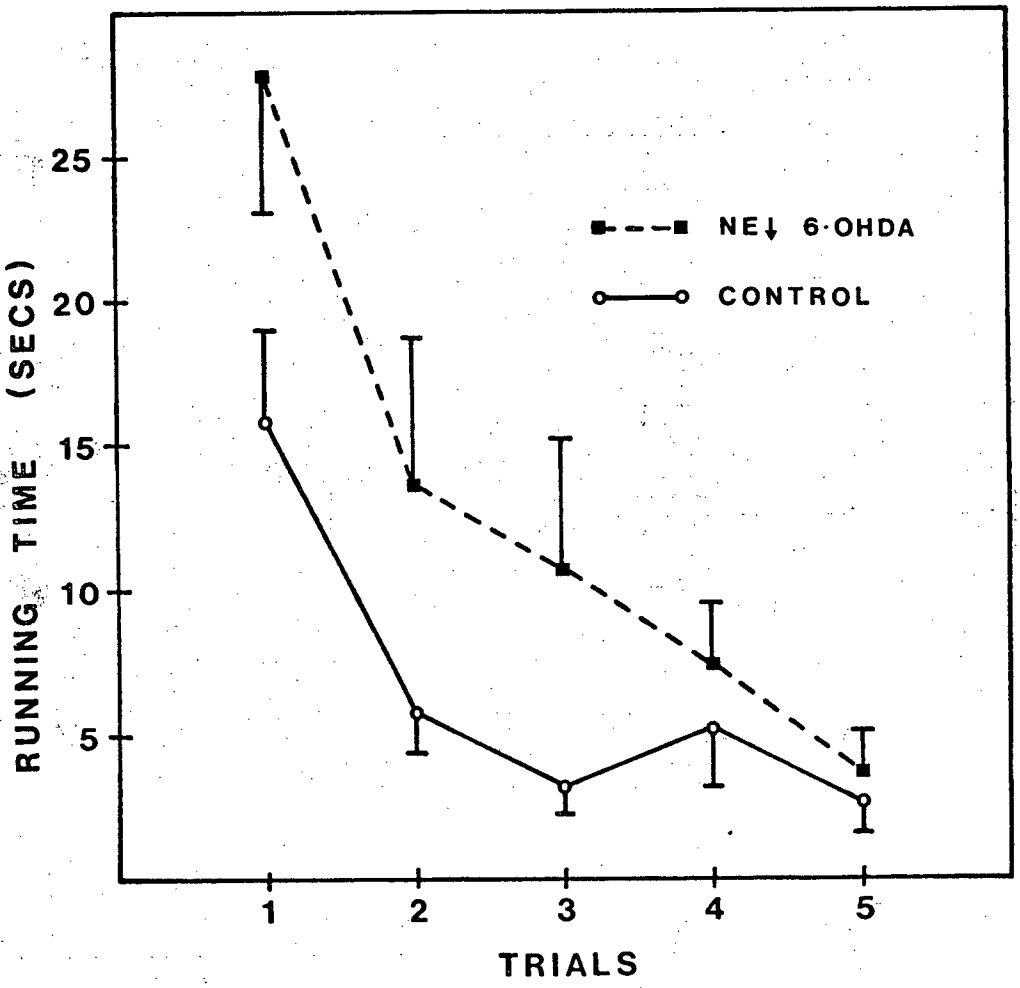
Methods

Dorsal tegmental bundle 6-OHDA animals and controls from Experiment 1 were used. The L-shaped runway as described above, was fitted with heavy grade sandpaper on the runway floor between the two photocells. Two 10-watt light bulbs were fixed, 30 cm apart, to the wire mesh ceiling of the runway, directly above the sandpaper. These were programmed to flash at 10 Hz commencing when the first photocell was activated and terminating when the rat reached the second photocell; that is, the lights flashed during the timing interval. The animals were trained over a 10-day period. On experimental day 11, the tactile and visual stimuli were added, and running speeds were recorded over five trials.

Results

Figure 5 represents the average group running speeds on each of the five trials. Statistical analysis revealed a significant difference between groups. $F(1,37) = 4.68$, $p < .05$. Individual comparisons yielded a significant difference on the first trial only, $t(37) = 2.21$, $p < .05$. No qualitative difference was seen in the response to the

Figure 5. Running times in the presence of novel tactile and visual stimuli measured between two photocells 120 cm apart on the initial arm of an L-shaped maze. Animals were pretrained for 10 days before exposure to the novel stimuli on the 11th day. Each point represents the group mean (± S.E.M.) on each trial. See methods for details.


RUNNING TIME (SECS)
25
20
15
10
5
1
2
3
4
5
TRIALS
NE↓ 6-OHDA
CONTROL

novel stimuli. On the first trial, the subjects typically would momentarily freeze at the onset on the flashing lights, followed by exploratory sniffing behavior of the floor and ceiling. By the fifth trial, running speeds were comparable to those prior to the distracting stimuli, i.e., day 10 on Figure 2.

Discussion

Rats with lesions to the dorsal tegmental NA bundle were more distractible than controls in a situation which required the performance of a previously trained response. This effect, though statistically significant, was transient; after five trials both groups had completely habituated to the novel stimuli.

The similarity between the decrease in running time in the present experiment and the decrease in running time over days in Experiment 1 is noteworthy. While the 6-OHDA lesion group was not significantly different from controls in the original L-maze learning task, the group means for this group tended to be above those of controls on the first six days (Figure 1). This may have been due to increased distractibility during the acquisition phase of this experiment. However, this tendency was not statistically significant. Due to the inherent variability in the running speeds on the first few days of training, large groups of animals would be required to test such differences. In any event, lesions to the dorsal NA bundle appear to affect the rate of habituation to novel stimuli during the performance of a trained response.

EXPERIMENT 5

The Effect of Telencephalic NA Depletion on the Acquisition of a Conditioned Taste Aversion

Many experimental learning paradigms have been developed as an aid to the investigation of processes involved in the formation of learned associations. Most of these have employed stimuli or situations which are unnatural to the animal. That is, the learning situation would not be encountered in the normal environment of a non-experimental rat. One exception is the conditioned taste aversion procedure.

In 1955, Garcia et al. demonstrated that rats will avoid ingesting a solution which has formerly been associated with illness. They did this by subjecting the animals to gamma radiation during their first exposure to saccharin flavored water. Two days later, both the saccharin solution and tap water were made available simultaneously and the amount of each fluid consumed was measured. Control (non-irradiated) rats drank 85% of their daily fluid intake from the saccharin container, while the x-irradiated animals completely avoided the flavoured solution for the first week and still showed an aversion at the end of the two month experiment.

Notice that the illness was not brought about by the novel tasting solution, but was under the direct control of the experimenter. The suppression of intake (henceforth

referred to as conditioned taste aversion, CTA) has since been accomplished using a wide variety of stimuli which cause illness. LiCl (2% BW, .15 M) causes sickness in the rat within 15 min (Nachman, 1970) of i.p. administration as adduced by unresponsiveness and reduced activity. Such injections, following ingestion of novel tasting solutions will cause CTAs much like that seen in the irradiation paradigm (Barker and Smith, 1974).

Other distress-inducing agents which have been shown to produce CTAs are apomorphine and emetine (Revusky and Gorry, 1973) hypertonic saline (Braveman and Capretta, 1965) and insulin (Lovett, Goodchild and Booth, 1968). These stimuli are presumed to have different loci of actions in the body, however, all produce a general malaise which has been postulated as essential to the conditioning of aversions to novel tastes (Garcia et al., 1967).

The present experiment was undertaken to examine the ability of rats with decreased telencephalic NA to learn an association analogous to that required of an animal in the natural setting.

Method

6-OHDA injections (n=12) were made into the area of dorsal tegmental NA bundle as previously described (Experiment 1). Control animals (n=12) received sham

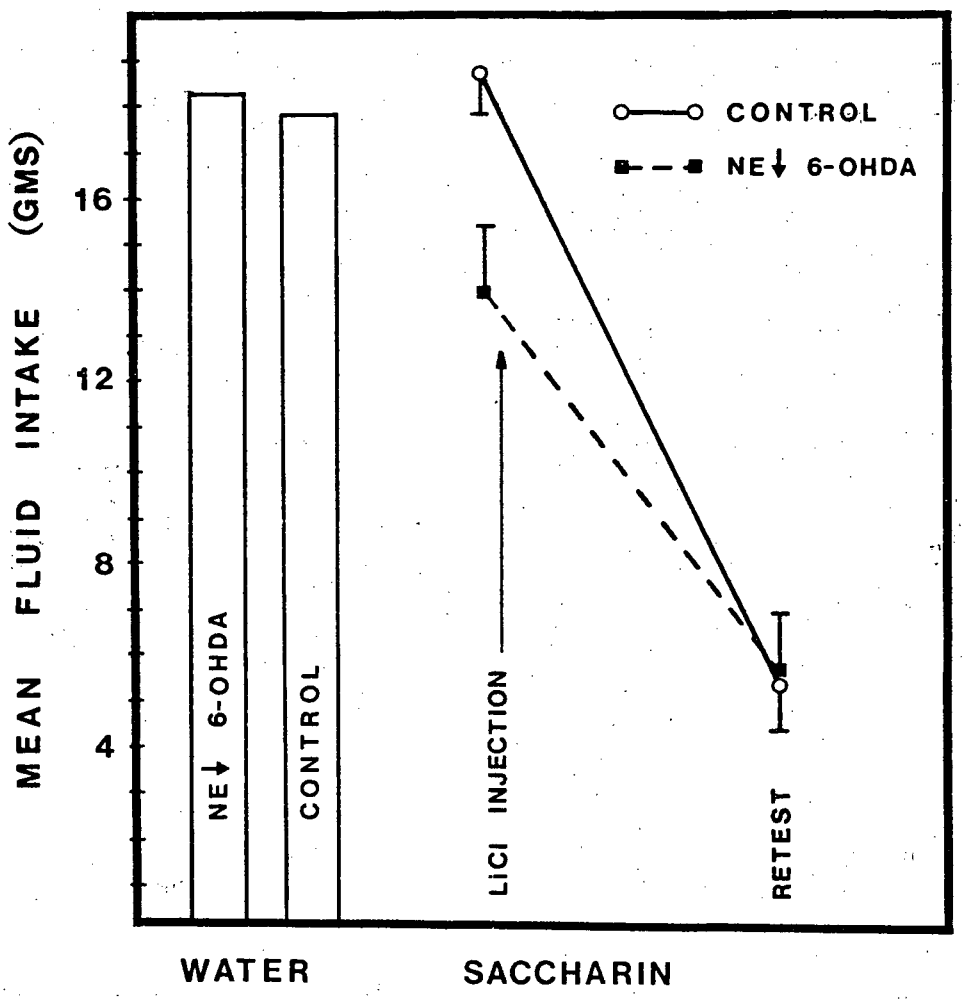
operations. All animals were housed individually and, three weeks post operatively, were placed on a 23.5 hr/day water deprivation schedule. Food was available ad libitum at all times. Fluid intake was measured by the weight difference of the water bottle before and after presentation. This measure was uncorrected for spillage, which was assumed to be constant across groups. After water intake had stabilized, a 0.1% saccharin solution was made available to each rat in lieu of their daily water presentation. Five min following the removal of the saccharin solution, each rat was injected i.p. with LiCl (2% Body Weight, 0.15 M). For the next three days water was again made available for 0.5 hr/day. On the fourth day following the LiCl injection, saccharin was again offered to the rats, and the amount consumed measured.

Results

Injections of 6-OHDA into the dorsal tegmental NA bundle produced depletions of telencephalic NA similar to those produced in the previous experiments. Control values for NA content of the hippocampus plus cortex were calculated to be 487 $\pm$ 40 ng/gm. Rats which received injections of 6-OHDA were found to have a mean NA content of 16 $\pm$ 1 ng/gm. This represents a mean group reduction of 97% from control values.

Figure 6 shows the effects of the 6-OHDA treatment on the fluid consumption during this experiment. The bar graph

Figure 6 Effect of 6-OHDA lesions on the acquisition of a conditioned taste aversion induced by an i.p. injection of LiCl. The two bars on the left of the figure show mean daily water intake during the 1/2 hour drinking period. The points on the graph show mean (± S.E.M.) group saccharin intake before and three days after the LiCl injection.


MEAN FLUID INTAKE (GMS)
16
12
8
4
NE↓ 6-OHDA
CONTROL
LiCl INJECTION
RETEST
CONTROL
NE↓ 6-OHDA
WATER
SACCHARIN

on the left of the figure shows no difference in the water intake between these two groups. This measure was taken on three separate days; on no day was a statistically significant difference observed. The middle point on the graph shows the intake of saccharin of each group on their initial exposure to this solution. A significant difference was observed between the two groups on this day ($t = 2.93$, $df = 21$, $p < 0.01$).

No statistical difference was found between control animals and experimentals on saccharin intake on the retest day. Significant differences were observed, however, within each group from initial exposure to retest day. That is, both groups displayed a significant aversion to the novel taste following LiCl injection.

Discussion

These data are consistent with those reported by Roberts and Fibiger (1975) who showed that 6-OHDA administered intraventricularly had no effect on a taste aversion to saccharin induced by repeated injections of LiCl. In the present experiment the only difference observed between the two groups was in the reluctance of the NA depleted animals to consume the saccharin on their first exposure. This neophobia has been observed previously in this laboratory in other groups of animals with identical lesions. While

this effect appears reliable, the difference is transient and is not found in subsequent non-punished presentations.

The present experiment offers no support to the hypothesis that cortical NA is necessary to the learning of a conditioned taste aversion.

GENERAL DISCUSSION

Depletions of hippocampal-cortical NA were found to have little effect on the acquisition of food-rewarded maze-running responses. Neither electrolytic lesions to the nucleus locus coeruleus, nor 6-OHDA lesions of the dorsal tegmental buncle affected the acquisition or performance compared to control animals in an L-shaped runway. These data do not support the hypothesis proposed by others (Crow, 1968; Kety, 1970), which contends that noradrenergic terminals in the cerebral cortex mediate synaptic changes during learning.

The first experiment was undertaken in an attempt to replicate previously reported results (Anlezark et al., 1973) indicating that animals with lesions to the NA cell bodies of the LC were impaired in the learning of a food-motivated running task. Further, if this impairment was due to the decrease in telencephalic NA, then lesions of the ascending pathway should also result in such an impairment. Electrolytic lesions to the LC, which caused depletions of cortical

NA comparable to those of Anlezark et al. (1973), were found to have no effect on L-maze running when compared to controls. Near total forebrain NA depletion, caused by intracerebral injections of 6-OHDA also failed to affect this running response for food.

Changes in running speed may possibly supply an indirect measure of learning in the runway situation, but also reflect motor ability, motivation, habituation, and distraction. A more direct assessement of learning is available, however, in the T-shaped maze by measuring the development of a position habit to the food-reinforced side of the maze. Animals with near total depletions of forebrain NA learned a position discrimination in this paradigm, and were not significantly different from controls. These results agree with a previous report (Amaral and Foss, 1975) in which LC electrolytic lesions had no effect on a T-maze discrimination task.

Mason and Iversen (1974) found that intraventricular administration of 6-OHDA disrupted acquisition of a complicated problem solving task. The animal was required to either push or pull a ball through a tunnel to gain access to a food reward. 6-OHDA treated animals required more trials to learn the response. It was argued that this deficit was not a result of motor impairment. It is interesting to note that no differences were observed

between these animals and controls when the task was reversed; i.e. pushing the ball, when formerly trained to pull. The possibility exists therefore that a learning deficit after 6-OHDA lesions becomes evident only in very complicated tasks. The reversal of the present T-maze problem may not have been sufficiently difficult to demonstrate the disruption in acquisition of learned responses. The present finding that animals with telencephalic NA depletions were more distractible supports this notion. This distractibility may become debilitating only when the appropriate response is difficult to learn.

Alternatively, the deficit observed by Mason and Iversen (1974) following intraventricular 6-OHDA administration disrupted learning through a CA pathway other than the LC projection. Deficits in acquisition and performance of a double T-maze food-rewarded response have been reported after intracisternal administration of 6-OHDA (Howard et al., 1974). A clear relationship was noted between these impairments and whole brain depletions of CAs; however, these correlated more with DA than NE depletions. This is consistent with observations from this laboratory that 6-OHDA lesions of the nigro-neostriatal dopaminergic pathway produce a profound impairment in the acquisition of a conditioned avoidance reponse or a simple approach response for food (Fibiger, Phillips and Zis, 1974). Subsequent experiments have indicated however that these impairments are due

to a specific motor disability rather than a learning deficit (Price and Fibiger, 1975; Zis, Fibiger and Phillips, 1974; Fibiger, Zis and Phillips, 1975).

While the present research report offers no support for the necessity of NA to the learning process, a considerable amount of data exist whichare consistent with this hypothesis. These data come in the form of pharmacological manipulations of NA synthesis, using the dopamine-β-hydroxylase inhibitor, diethyl-dithiocarbamate (DDC). Intraperitoneal injections of this either immediately before or after training on a passive avoidance task will prevent the formation of a "long-term" but not a "short-term" memory. This is demonstrated by the retest latencies to step down from a safe platform either immediately after or several hours or days following training. While this drug may have many pharmacological effects, Stein et al. (1975) have presented data which implicate central NA in the action of DDC. These workers showed that intraventricular injections of NA are capable of restoring the capacity of a DDC treated animal to learn a passive avoidance task if these injections follow immediately after training. This finding has since been confirmed by Meligeni et al. (1975). Peripheral mechanisms have also been implicated in the amnesic effect of DDC. Post training intraperitoneal injections of either NA (Méligini et al., 1975) or adrenaline (Van Buskirk and Gold, 1975) have been

reported to reverse the DDC-induced deficit. This effect may be due to the NA gaining access to central NA receptors, although this possibility appears unlikely. Peripheral administration of epinephrine has been shown to facilitate time-dependent memory storage processes (Gold and Van Buskirk, 1975), thus demonstrating that peripheral adrenergic systems may well interact with the CNS in the storage of memory. The inhibition of synthesis in peripheral adrenergic systems may thus be one explanation for the amnesic effect of DDC.

Some authors have questioned whether DDC either impairs the consolidation of memories or disrupts the subsequent ability of the animal to "retrieve" the memory which was stored. Botwinick and Quartermain (1974) have reported that pre-test injections of monoamine oxidase inhibitors (Catron or Pargyline) reverse the DDC amnesia. These data demonstrate that because recall is possible under some conditions, some learning must have been consolidated during the original training. Cohen et al. (1975) have reported that DDC impairs passive avoidance retention when given prior to the re-test, although subsequent testing while not under the drug condition showed that learning had occurred. These authors interpreted the data as indicating that noradrenergic systems are necessary for"memory access".

If disruption of NA synthesis by DDC is the critical pharmacological factor involved in the disruption of memory, then other drugs which effect NA synthesis should also affect memory. Dismukes and Rake (1972) showed that reserpine, an agent which depletes CA impaired the consolidation of a passive avoidance task memory, and that this was reversed by DOPA. While these data certainly support the NA hypothesis, other pharmacological agents do not yield such evidence. Alpha-methyl-para-tyrosine (AMT), an effective inhibitor of CA synthesis does not produce memory failure. This drug however does produce state dependent learning (Overton, 1968). This refers to an apparent amnesia which is seen if the retest is performed under a drug condition different to that of the original learning. Administration of AMT does not produce a decrement in memory as long as the retest is performed after an additional injection of AMT. A similar effect has been reported for FLA-63, a DBH inhibitor, (Alhenius, 1973). This drug had no effect on the acquisition or performance of a conditioned avoidance response. It also, however, was shown to possess the potential of producing state dependency in learning. This state dependency may be similar to the retrieval or memory access failures proposed as the deficit produced by DDC.

From the pharmacological data, one thing is clear. Manipulations of CA through peripheral administration of

drugs, almost always induces a learning, consolidation, or retrieval deficit. The question as to whether these are specific and exclusive to CA systems remains unanswered. Assuming they are, there is a considerable likelihood that peripheral adrenergic mechanisms play a large role in the learning process, particularly in conditioning paradigms using aversive stimuli. The present research report has dealt only with central NA manipulations. Concommitant peripheral manipulations may allow a better understanding of the nature of NA involvement in the learning process.

CONCLUSIONS

The present experiments offer no support to the hypothesis that the dorsal tegmental noradrenergic projections is of fundamental importance to the learning process, and pharmacological data which support such a theory might be explained by drug action on other, possibly peripheral, adrenergic mechanisms.

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