THE NEURAL ASSOCIATIONS OF NUCLEUS ACCUMBENS SEPTI

IN THE ALBINO RAT

by

Richard Dale Wilson

SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF

SCIENCE

at the

MASSACHUSETTS INSTITUTE OF

TECHNOLOGY

Signature of Author_____

Department of Psychology May 12, 1972

Certified by_____

Thesis Supervisor

Accepted by_____

Chairman, Departmental Committee on Graduate Students

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The term nucleus accumbens septi refers to that part of the mammalian striatum which extends under the ventral tip of the lateral ventricle and bulges into the septal region. The efferent connections and some of the afferent connections of this striatal region in the rat were analyzed, using various modifications of the Nauta technique.

After ablation of the hippocampus, but not after hemidecortication, terminal degeneration was found in nucleus accumbens. After lesions in nucleus accumbens degenerating fibers can be traced to their termination in the substantia innominata, which on the basis of its cellular structure and synaptology, has been interpreted as a ventral part of the pallidum. There are no projections from accumbens to globus pallidus, as usually defined, to the entopeduncular nucleus, or to the substantia nigra. These results indicate that despite its structural similarity to the caudoputamen, nucleus accumbens has different neural connections -- connections which establish it as a component of the limbic system rather than the extrapyramidal motor system in the usual sense.

On the basis of these and other studies a new formulation of the relationships among accumbens, the caudoputamen, and the olfactory tubercle is proposed. In this formulation the corpus striatum is divided into a dorsal striatum and a ventral striatum. The dorsal striatum, or caudoputamen, is principally associated on the afferent side with neocortex and on the efferent side with the globus pallidus and substantia nigra. By contrast, the corresponding associations of the ventral striatum (nucleus accumbens, olfactory tubercle, ventrolateral striatal area), are with allocortex (hippocampus and piriform cortex) and the substantia innominata.

It is suggested that the psychoactive properties of drugs which alter dopamine metabolism may be the manifestation of a functional disturbance in the dopaminergic pathway from the ventral tegmental region to the ventral striatum.

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TABLE OF CONTENTS

BIOGRAPHICAL NOTE

The author was born on July 13, 1949 in Oakland, California and spent the subsequent 18 years in Butte and Billings, Montana. He will receive simultaneous Bachelor of Science and Master of Science degrees from M. I. T. in June 1972. In September of 1972 he will enter Harvard Medical School.

ACKNOWLEDGEMENTS

I would like to express my thanks to Miss May Chen, Miss Pat Waterman, Miss Lydia Sultzman, and Miss Elizabeth Jones for their help in the critical and chaotic final stages of the preparation of this thesis.

Thanks also must go to my many teachers in the Department of Psychology, particularly to:

Mrs. W. J. H. Nauta, Mrs. Clare Kerr, and Miss Diane Major, from whom I have learned the techniques of neuroanatomy;

Drs. Ann Graybiel and Harvey Karten, from whom I have learned many principles of neuroanatomy;

Dr. Lennart Heimer, from whom I have learned a style of neuroanatomy;

Dr. Walle J. H. Nauta, from whom I have learned more than he or I realize.

INTRODUCTION

Terminology.

During the first third of the twentieth century, the zenith of the descriptive era of Comparative Neurology in America, there was a great deal of conflict among the major laboratories concerning the subdivision and homologization of cell groups both lateral and medial to the lateral ventricle. However, nucleus accumbens, situated at the base of the lateral ventricle, was a point of agreement among the major investigators of the period. The term, nucleus accumbens, was first used by Ziehen (1904-1908) to describe a cell group at the base of the most rostral part of the lateral ventricle of the marsupial and monotreme forebrain. Koikegami (1967) reports that a similar cell group had been described earlier, but not named, by Meynert, by Zukerkandl, by Obersteiner, and by Ganser. Ariëns Kappers and Theunissen (1908), in an often-quoted and vastly influential monograph on the phylogeny of the rhinencephalon and corpus striatum, applied the term nucleus accumbens septi ("nucleus leaning against the septum") to a cell group which curls around the base of the lateral ventricle of the vertebrate forebrain. Crosby (1917) and Hines (1923) recognized a nucleus accumbens in amphibia and offered a description of its cellular morphology which might well serve as a description of the rat's accumbens.

J. B. Johnston (1913) initially rejected the term nucleus accumbens septi (and many other terms which included the word septi) because he thought it implied false homologies with the human septum pellucidum.

In his 1913 paper, Johnston suggested a new system of terminology in which accumbens was included in the nucleus lateralis parolfactorius. (In mammals he seems to have meant the terms accumbens and nucleus lateralis parolfactorius to be equivalent.) By 1923, his concept of the striatal region had become greatly modified by his study of the embryological development of vertebrate brains (Johnston, 1923). He proposed that the striatum of mammals is of dual embryological origin and that a fundamental distinction can therefore be made between the caudate nucleus and the other striatal structures -- the bed nucleus of the stria terminalis, nucleus accumbens, and his ill-defined anterior olfactory nucleus. Having abandoned his earlier terminology, he again referred to accumbens as accumbens.

The recognition of accumbens in reptilian and amphibian forebrains, which were thought to be dominated by the olfactory sense, was a principal factor in the formulation of a then-unnamed concept according to which Johnston linked accumbens to the olfactory system. Even the brains of mammals were thought to receive "olfactory impulses" in all rostral basal regions, including nucleus accumbens. Moreover, accumbens was seen to be predominantly related to the medial forebrain bundle, which was considered to consist largely of fibers of the olfactory system.

Herrick (1926) was the first to formalize this concept with the term olfacto-striatum. In a discussion of the corpus striatum of mammals he defined his implications for the term:

> Medially there is the olfacto-striatum (part of the caudate nucleus)[in mammals, accumbens and the caudate have identical cellular structure] which borders the lateral ventricle for almost its entire length from far into the temporal lobe.

> It receives fibers directly or indirectly from the olfactory bulb, and also fibers from the somatic sensory centers of the thalamus. The efferent fibers go in part to globus pallidus and thence to cerebral peduncle, and in part directly into the medial forebrain bundle. This region probably serves for the correlation of olfactory with tactual and other exteroceptive stimuli and maybe concerned chiefly with locomotor and facial reflexes involved in feeding.

Various authors have included different cell groups in different brains in their definitions of olfacto-striatum, but accumbens is invariably included. Gurdjian (1928) equates the olfacto-striatum with accumbens in his description of the striatum in the albino rat. After Gurdjian there appears to have been very little further consideration of this concept, and the fundamental duality of striatal origin and connections has been largely ignored.

The term, accumbens, has come to refer to the ventromedial portion of the corpus striatum which curves under the lateral ventricle and bulges into the septum (Figure 1). The term, fundus striati, (base of the striatum) refers to the region of continuity of the head of the caudate and the putamen. Accumbens is not generally included as part of the fundus striati.

The comparative embryological work of Källen (1956) and the earlier descriptions of Herrick indicate that the cells of the non-mammalian accumbens are probably homologous to those of the mammalian accumbens, but the histochemical and hodological studies necessary to firmly establish the homology have not been done. Therefore, the term nucleus accumbens septi will in this paper refer only to the mammalian brain, specifically to the rat's, unless otherwise stated.

Boundaries.

The boundaries of accumbens have been discussed in previous literature only in vague terms. On the basis of cell type and arrangement, the medial and lateral boundaries are easy to establish, but the dorsal and ventral boundaries are nearly impossible to delimit precisely.

On its medial side, accumbens is sharply bounded in its rostral part by fibers of the parolfactory radiation. Farther caudally the lateral and medial septal nuclei are easily distinguishable from accumbens: the cells of the medial septal nucleus are larger, of different shape, and much less densely packed; those of the lateral septal nucleus are also slightly larger and less densely packed. In every mammalian species the middle portion of this medial border is accentuated by the insula magna of Calleja -- the largest of the insular conglomerates of granule cells characteristic of the olfactory tubercle. (Figure 1)

Laterally accumbens is bounded by the large cells of the deep layers of olfactory cortex.

The ventral boundary is much more difficult to draw because there is no clear distinction between the cells of accumbens and the medium-sized cells that compose the polymorph layer of the olfactory tubercle. In fact, the relationship between accumbens and the tubercle appears to be very similar to that existing between caudate nucleus and putamen. Just as the internal capsule more or less coincidentally, and incompletely, separates the latter two structures, so the fibers of the most rostral portion of the medial forebrain bundle (the so-called olfactory radiation) divide a single population of cells into a dorsal region termed

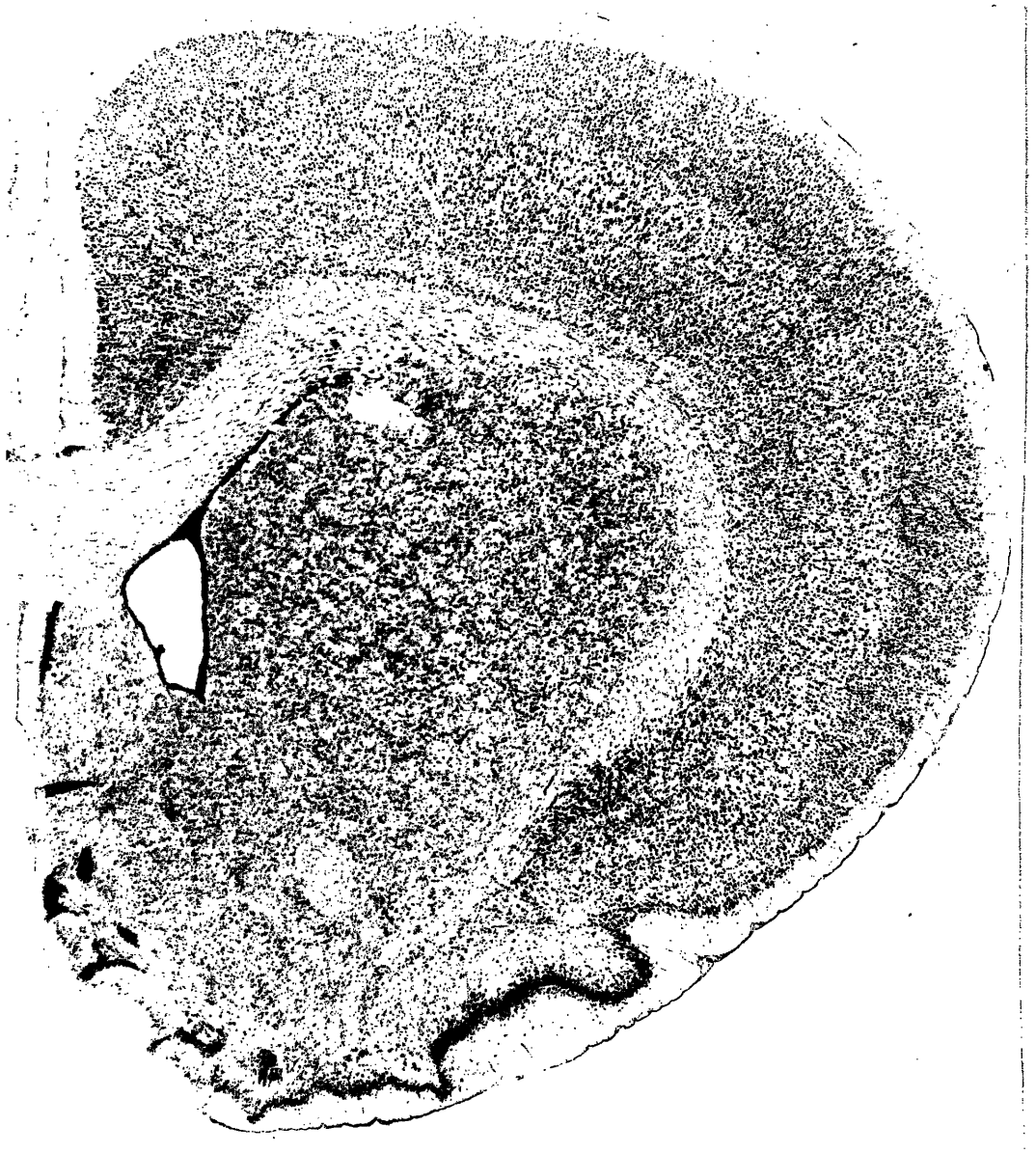

Figure 1. Transverse section through the striatum of the rat, stained for cell bodies by the Nissl method. Nucleus accumbens is that part of the striatum which is ventral and medial to the lateral ventricle. Cellular bridges from accumbens to the olfactory tubercle are visible. (This photograph was kindly supplied by Dr. Harvey Karten.)

nucleus accumbens, and a more ventral territory commonly considered the deepest stratum of the olfactory tubercle. The division, however, is not complete -- there remain numerous cellular bridges from accumbens to the deep layers of the tubercle. Previous investigators (Fox, 1940; Gurdjian, 1928) noted these bridges, but, quite curiously, they referred to them as areas of "strio-tubercular fusion." That is, they interpreted the bridges as areas of conjunction of two distinct cell groups rather than as the remaining threads of connection of a homogeneous mass cleaved by fibers of passage.

Accumbens is bounded dorsally, in medio-lateral succession, by the lateral septal nucleus, the lateral ventricle, and the caudoputamen. The boundaries with the lateral septal nucleus and the lateral ventricle are quite clear. On the other hand, accumbens and the caudoputamen are indistinguishable on histological or histochemical grounds. In sections stained by the Nissl method, the entire striatal area is predominantly characterized by small, palely staining neurons that are densely packed and uniformly distributed. Golgi stains reveal that these neurons have short, varicose axons which do not extend much beyond the dendritic field of the neuron. Approximately 2% of the cells of the caudoputamen are larger cells with axons that extend outside the nucleus. Although such quantitative statements so far have not explicitly included accumbens, a similar ratio of small to large cells appears to apply to this region of the striatum. (Figure 2) Histochemically the striatum appears equally homogeneous. The entire region has a high concentration of dopamine-containing nerve terminals (Dahlstrom and Fuxe, 1969; Ungerstedt, 1971).

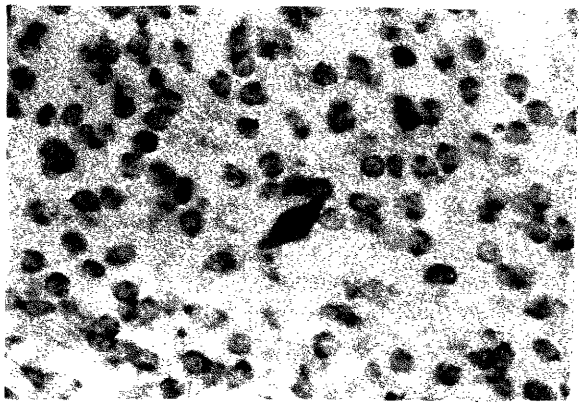

Figure 2. Cell types in nucleus accumbens. As in the caudoputamen, a few large neurons are embedded in a field of densely-packed smaller neurons. 500X.

Accumbens has, therefore, generally been considered to be a ventromedial portion of the caudoputamen rather than a separate nucleus. A convention of unknown origin specifies that the rostral and the ventromedial portion of the corpus striatum which is not traversed by the fascicles of Wilson's pencil bundles, particularly the part medial to ventricle, is call nucleus accumbens. Thus, the histological criterion for the demarcation of nucleus accumbens and the caudoputamen is a very tenuous one -- the presence or absence of perforating fiber bundles.

In summary, nucleus accumbens is well delineated from the septal nuclei but appears virtually identical in its histology and histochemistry to the adjacent caudoputamen and dorsal olfactory tubercle. In view of this similarity, it is surprising that the tubercle was considered an unrelated structure, but it is quite understandable that nucleus accumbens was considered a subdivision of the caudoputamen. It was only when the fiber connections of this region were examined that a dichotomy began to appear between the caudoputamen and nucleus accumbens.[1]

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[1]Actually Källén (1956) had already observed the dichotomy in his studies of embryology. He discovered that nucleus accumbens, the tubercle, and the bed nucleus of the stria terminalis derive from the same embryonic migration zone, different from that which gives rise to the caudoputamen.

Afferent Connections.

The first demonstration of an afferent connection of accumbens came in 1940 when Fox recognized fascicles of the stria terminalis extending toward a small region of the lateral septal nucleus and the caudal parts of accumbens (Fox, 1940). Valverde, by the Golgi method (1965), has verified this connection.

More powerful evidence for the duality of the striatum came from studies of the fornix by Powell, Cowan, and Raisman (1966) with the Nauta method. This study revealed that large areas of accumbens in the rat receive projections from the hippocampus via the precommissural fornix. More recently it has been shown that in the rabbit one can draw a sharp line demarcating accumbens on the basis of differential afference: the caudoputamen receives neocortical but not hippocampal projections; accumbens receives hippocampal but not neocortical projections (Carman, Cowan and Powell, 1963).

The dopaminergic projections from substantia nigra to caudoputamen do not encroach upon nucleus accumbens (Hedreen, 1971). However, a parallel dopaminergic pathway has been identified that arises in the ventral tegmental region of the mesencephalon and follows the medial forebrain bundle forward to terminate in accumbens, the tubercle, the bed nucleus of the stria terminalis, and nucleus centralis of the amygdala (Dahlstrom and Fuxe, 1969; Ungerstedt, 1971). The terminal degeneration which Guillery (1957) found in accumbens after lateral hypothalamic lesions was probably due to the interruption of this pathway. Additional fibers from the rostral intralaminar thalamic nuclei

have been reported to terminate in accumbens (Scheibel and Scheibel, 1967). After lesions of the accumbens of the cat, Koikegami (1967) identified retrograde cell changes in midline thalamic nuclei, the thalamic anterior medial nucleus, and the ventral-anterior nucleus, the hypothalamic paraventricular nucleus, and the septal region. Siegel and Tassoni (1971) suggested that the septal nuclei may project to accumbens in the cat.

In conclusion, the pattern of afferent connections of nucleus accumbens shows certain parallels with those of the caudoputamen, but differ from the latter in details of origin. A series of neuroanatomical experiments employing anterograde degeneration methods, currently in progress in this laboratory, has been designed so as to determine if the hippocampal and amygdaloid projections are complementary or overlapping in accumbens, and to establish the accumbens-caudoputaminal boundary in the rat.

Efferent Connections.

Although the region of accumbens is quite free from the massive cortex-related fiber bundles which penetrate the caudoputamen in the rat, it contains many smaller fascicles which join the MFB below the anterior commissure. These fascicles were interpreted by Gurdjian as efferents from accumbens, and named tractus strio-hypothalamicus. Guillery (1959) and Powell (1963) mentioned fibers from the region of accumbens in the rat which terminated in the medial part of the mediodorsal nucleus of the thalamus. Leonard and Scott (1971) suggested

that accumbens efferents in the rat distributed widely in the mid-lateral hypothalamus. Koikegami (1967) traced efferent fibers from accumbens to the subthalamic region, the adjacent ventral thalamus, and the mesencephalic tegmentum.

In order to clarify the picture of the efference of the nucleus accumbens, a series of neuroanatomical experiments was performed by the aid of anterograde degeneration methods.

METHODS

Lesions.

Electrolytic lesions were placed stereotaxically in the nucleus accumbens septi of 50 male albino rats ranging in weight from 150 g. to 550 g. Two stereotaxic approaches were employed in order to minimize the difficulties in interpreting degeneration caused by the electrode track. A vertical approach minimally involves the septal nuclei or efferent septal fibers. An angular approach from the contralateral side minimizes damage to the caudoputamen and involvement of cortical afferents and efferents. Some lesions were made using the stereotaxic coordinates of König and Klippel (1967); with animals too large for stereotaxic accuracy with this atlas, the lambda and bregma points were used as references with coordinates derived through experimentation.

The rats were anesthetized by intraperitoneal injections of Equithesin (0.25 ml per 100 g body weight) and mounted in Stoelting stereotaxic instrument. A hole was made in the skull at the proper coordinates with a hand-held dental burr. The dura was incised, and the electrode (an insect pin coated with Insulex except for a tip of 1 mm or less) was lowered into the brain to the desired depth. Anodal DC current (commonly 0.5 ma for 15 seconds) was applied through the electrode, with an injection cannula inserted under the skin of the tail serving as the cathode.

In a series of control animals, lesions were placed in the hippocampal rudiment, the neocortex of the frontal pole and medial wall of

the hemisphere, or the lateral septal nucleus, following the same operative procedures.

Aspiration lesions of the neocortex, hippocampus, and amygdaloid complex were made under direct vision with the aid of a Zeiss operating microscope. For the hippocampal lesions a large cortical exposure was made, the dura reflected, and the overlying cortex removed. The hippocampus was then removed until the dorsal surface of the thalamus was visible. As much of the hippocampus as possible was removed, but care was taken not to damage the stria terminalis. Nearly total hemidecortications were performed by aspirating the superficial layers and fiber tracts. Lesions of the amygdaloid complex were made via a lateral approach, with the lateral olfactory tract serving as a landmark.

Histology.

After survival times ranging from 2 to 5 days the rats were again anesthetized and then perfused transcardially or intra-aortally with 0.9% saline followed by at least 300 cc's of 10% formalin in 0.9% saline. The brains were removed immediately following perfusion, immersed in 10% formalin for at least 5 days, and subsequently placed in a formol-sucrose solution (30% sucrose w/v in 10% formalin) for 3 days or more. The brains were then blocked and embedded in a gelatin-albumin mixture; the blocks were hardened in formalin fumes for 24 hours, then immersed in 100% formalin for periods ranging from 3 days to 1 month. The blocks were finally sectioned on a freezing microtome at 26 micra, and the sections stored indefinitely in 5% formalin.

Sections were stained using the Fink-Heimer (1967) methods for degenerating axons and boutons, and a modified Nauta method (Nauta and Ebbesson, 1970, page 150) for heavier impregnation of degenerating axons. Variability in staining characteristics of each brain, particularly pronounced when the Fink-Heimer method No. 2 was used, made individual adjustments of the procedure necessary. All variations were within the limits outlined in the descriptions of the techniques. Ordinarily sections were stained at 130μ intervals; occasionally closer spacing of sections through a region of interest was necessary. For precise topographic identification of the lesion and the sites of degeneration, adjacent sections were stained with cresylecht violet.

General.

Representative brains were selected for illustration. The terminology employed is generally that of König and Klippel.

OBSERVATIONS

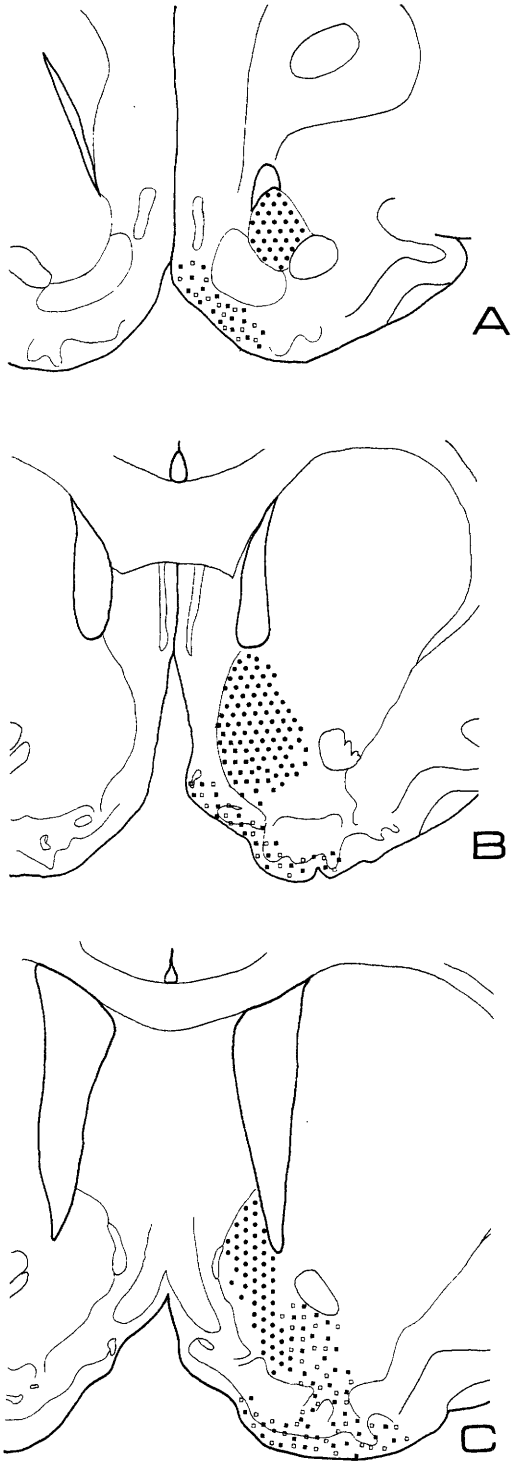
Afferent Connections.

Experimental results. After ablation of nearly the entire hippocampus (Case RHp-1, survival time 5 days), it is possible to trace fibers of the precommissural fornix which turn rostrally to travel along the interface of nucleus accumbens with the lateral septal nucleus. The fibers diverge laterally from this bundle and terminate in the rostral and medial parts of accumbens. These results are in complete agreement with those of Raisman, Cowan, and Powell (1966). In this case the terminal degeneration in accumbens was relatively sparse and especially difficult to stain. This probably indicates that a shorter survival time would be more appropriate for the demonstration of this pathway.

Comments. It is interesting to compare this pattern of terminal degeneration in the striatum with those resulting from lesions of the piriform cortex and the entire amygdaloid complex. Such a comparison is illustrated in schematic form in Figure 3[2]. Case RHp-1 is one of massive lesion of the hippocampus with only slight involvement of the stria terminalis. The pattern of terminal degeneration was previously described. Case P6, kindly supplied by Dr. Lennart Heimer, is one of large lesion of the piriform cortex with very little damage to the nuclei of the amygdala. Nearly all of the rostrally directed degenerating fibers travel via the ventral amygdalofugal pathway. The slight

[2]Because the brains which are compared are not cut in exactly the same plane of section, it has been impossible to do a precise comparison.

Figure 3. A schematic representation of the pattern of terminal degeneration in the ventral striatum after allocortical lesions. The hippocampus projects to the rostral and medial parts -- nucleus accumbens (closed circles). The piriform cortex projects to the ventral and ventrolateral parts -- the olfactory tubercle and the extreme ventrolateral striatum (open squares). A lesion of the entire amygdaloid complex reproduces the pattern of piriform-related degeneration and adds degeneration in the bed nucleus of the stria terminalis (closed squares).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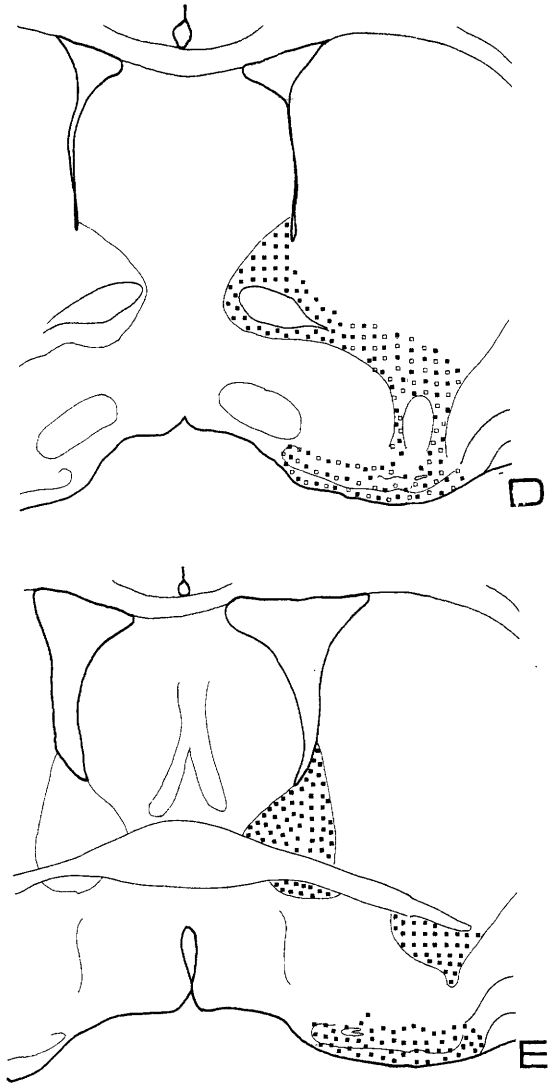
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damage to the subcortical amygdaloid complex is reflected in a few degenerating fibers in the stria terminalis. There is dense terminal degeneration in the entire olfactory tubercle, the ventrolateral parts of the striatum, and the cellular bridges which connect these two regions. Case A8, also made available by Dr. Heimer, is one of ablation of the entire amygdaloid complex. Both the ventral amygdalofugal pathway and the stria terminalis are heavily degenerated. The pattern of degeneration in Case P6 is reproduced exactly. The additional degeneration in the rostro-medial cellular bridge and the caudal parts of the tubercle and striatum (Fig.3b & e) is probably the result of a more complete lesion of piriform cortex than was obtained in Case P6. The bed nucleus of the stria terminalis is filled with degeneration resulting from the destruction of the amygdaloid nuclei (Heimer and Nauta, 1968; Leonard and Scott, 1971).

Efferent Connections.

Experimental results. Nucleus accumbens seems to project exclusively to the substantia innominata, a well defined cellular region which is located ventral to the anterior commissure and the lentiform nucleus. Nearly identical results were found in seven cases of lesion of accumbens. Three representative lesions are illustrated in Figure 4. The following case is described in some detail.

Case RACC-24 (survival time 2 days), (Figure 6). An attempt to produce a selective lesion of the accumbens by lowering the electrode vertically through the lateral ventricle was unsuccessful, as the electrode grazed the medial edge of the caudoputamen. However, by far

the largest portion of the lesion was confined to nucleus accumbens.

There is massive terminal degeneration in the region of the substantia innominata, between the striatum and the nucleus of the diagonal band of Broca. (A short discussion of what is known about the substantia innominata is presented in the section of the Discussion entitled: The Ventral Striatum and Dopaminergic Limbic Pathways.) As indicated in Figure 6, the region of termination is distinct from both the striatum and the nucleus of Broca. Fibers emerge from the lesion and sweep ventrally to terminate just ventral to the caudal part of nucleus accumbens and the bed nucleus of the stria terminalis (Figures 6C, 7). The area of termination expands underneath the anterior commissure (Figures 6D, E) but never includes the nucleus of Broca, the medial preoptic region, or any part of the lentiform nucleus, as usually defined. The dorsal boundary of the field of degeneration is extraordinarily sharp and corresponds exactly to the ventral edge of accumbens or the bed nucleus of the stria terminalis (Figure 8). The terminal degeneration is illustrated photographically in Figure 9.

In cases with lesions placed more rostrally (RACC-1, RACC-15, RAi) degenerating fibers emerge from the lesion and as they run caudally toward the substantia innominata condense into the bundles Gurdjian (1927) called tractus strio-hypothalamicus. No terminal degeneration in this area results from the control lesions illustrated in Figure 5. In addition, complete transection of the olfactory peduncle, behind the anterior olfactory nuclei, leaves this region free of terminal degeneration. Case RHR-4 revealed that fibers originating in the infraradiate cortex (Rose, 1929) pass through the zone of accumbens' terminations but do not terminate there themselves.

Figure 4. Lesions of nucleus accumbens septi, each of which resulted in accumbens-related terminal degeneration only in the substantia innominata.

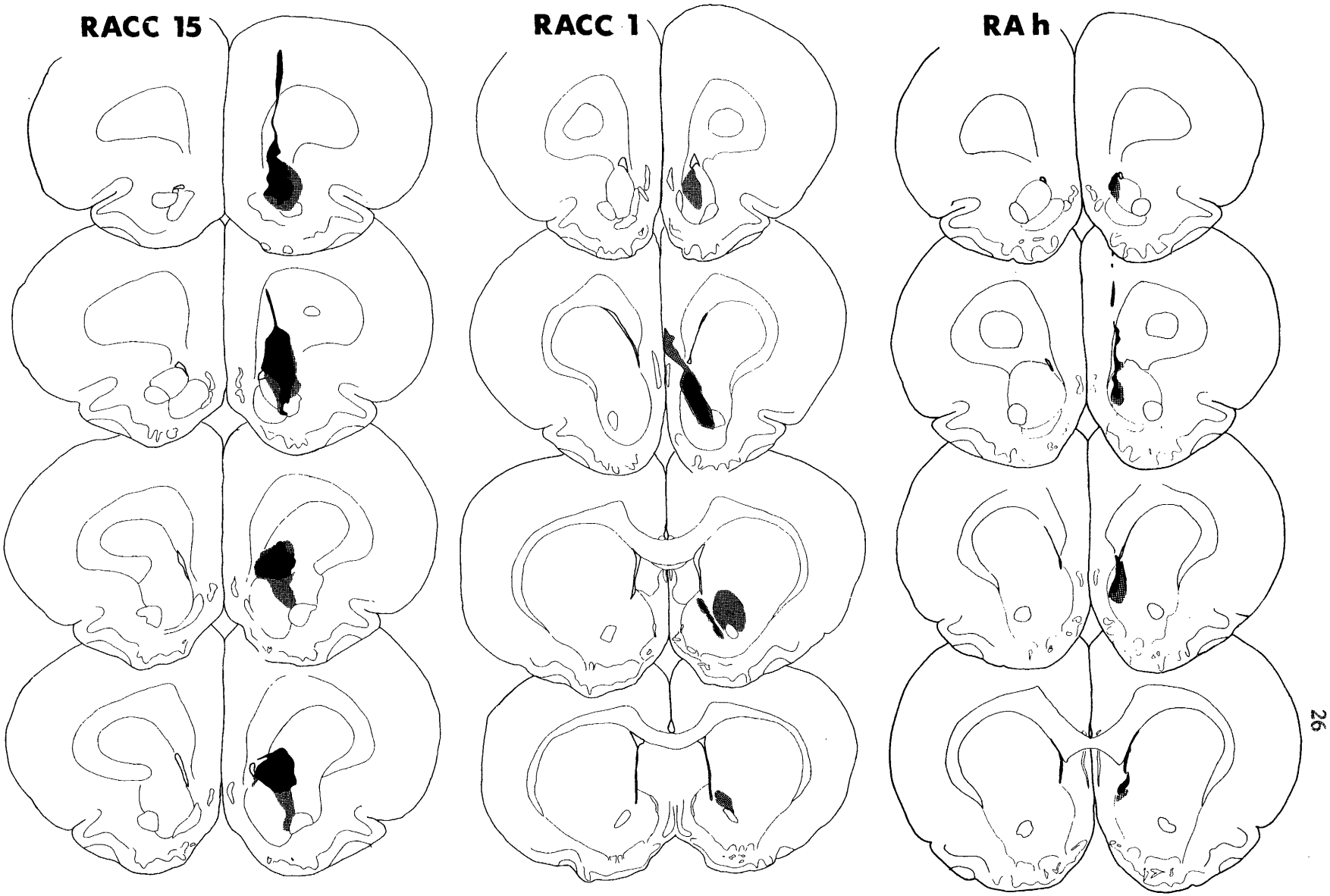
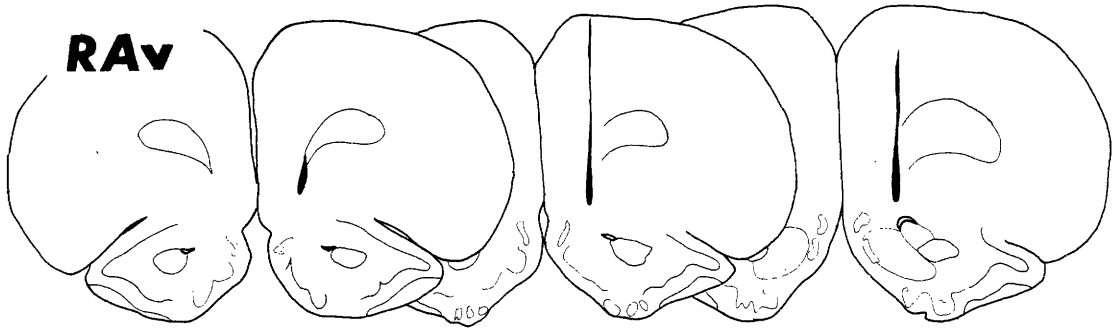

RACC 15
RACC 1
RA h

Figure 5. Control lesions, none of which resulted in terminal degeneration in the substantia innominata. The extent of the lesions in Cases RD-1 and RD-2 is illustrated compositely. Neither of these cortical lesions produced terminal degeneration in the ventral striatum.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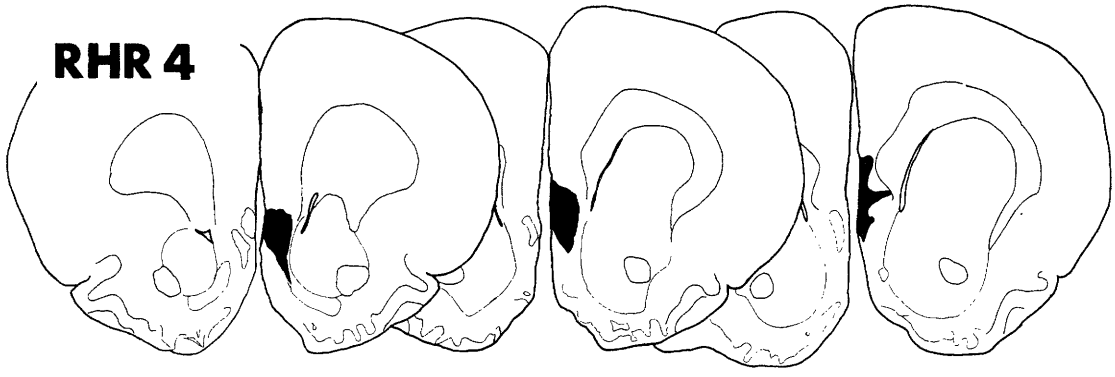
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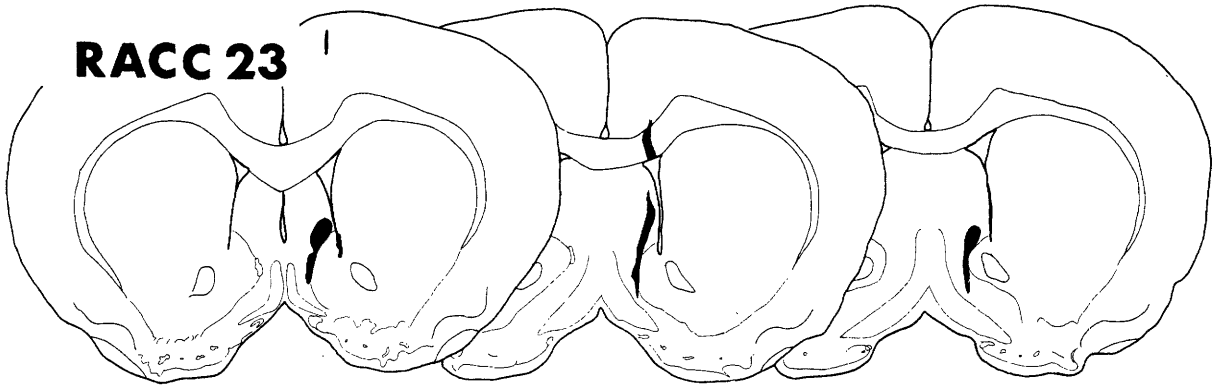
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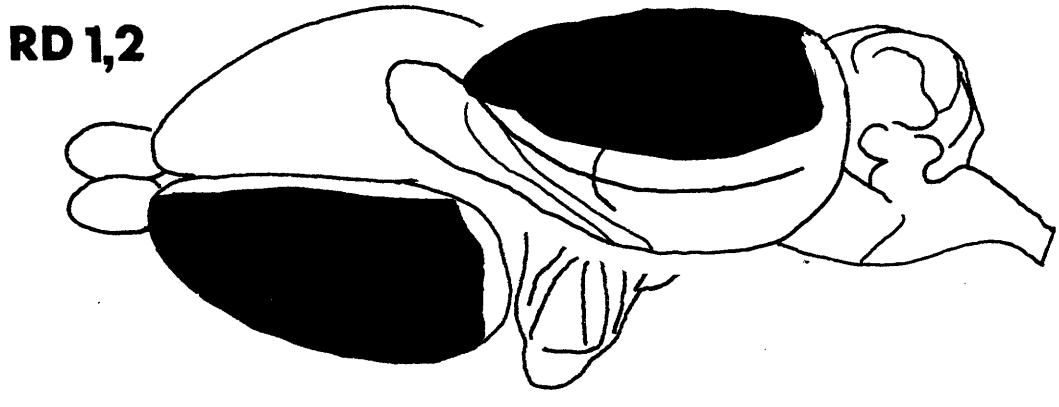
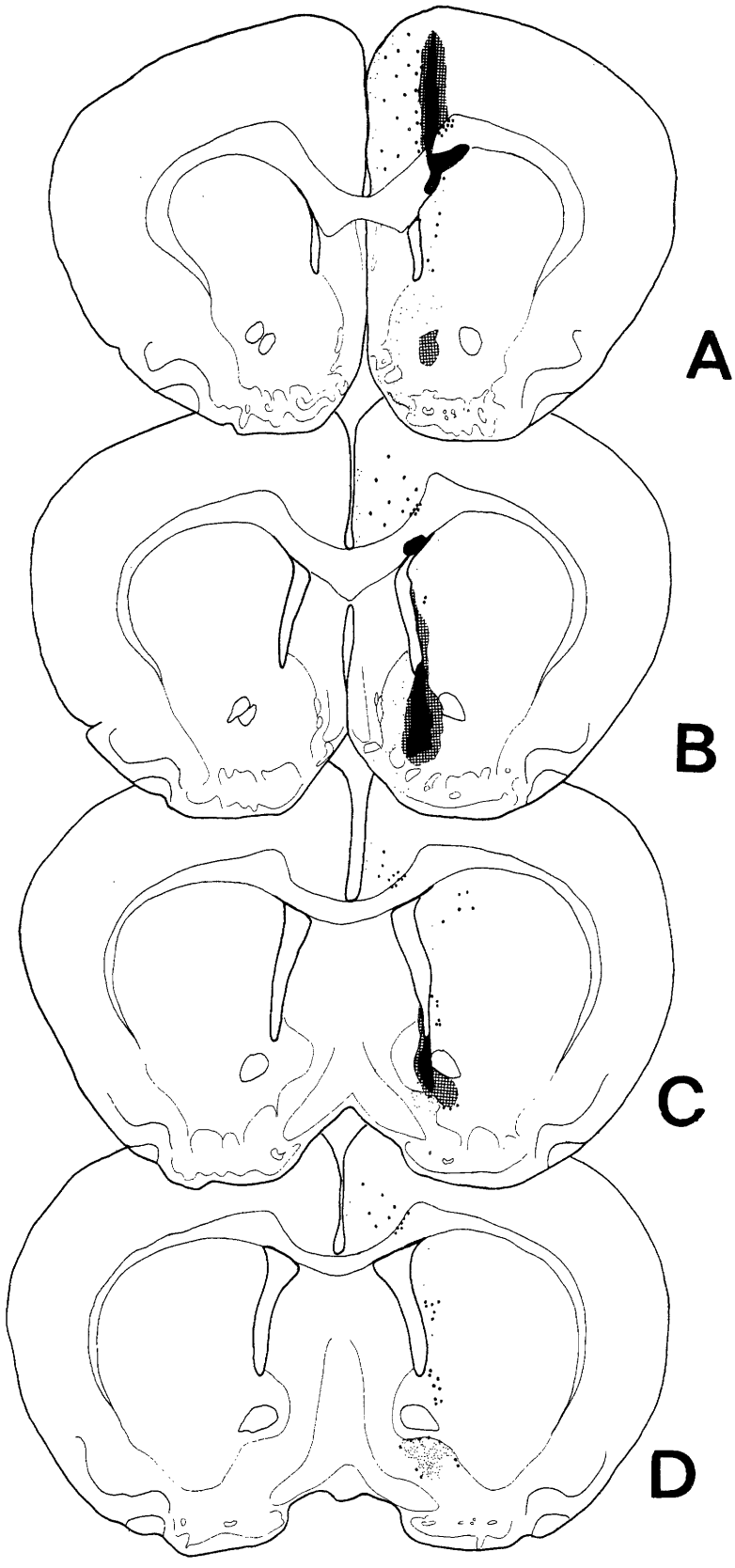

RD 1,2

Figure 6. Case RACC-24, a lesion of nucleus accumbens septi. Degenerating axons are represented by large dots, degenerating terminals by fine dots. Areas of extreme gliosis and cell loss are indicated by cross-hatching.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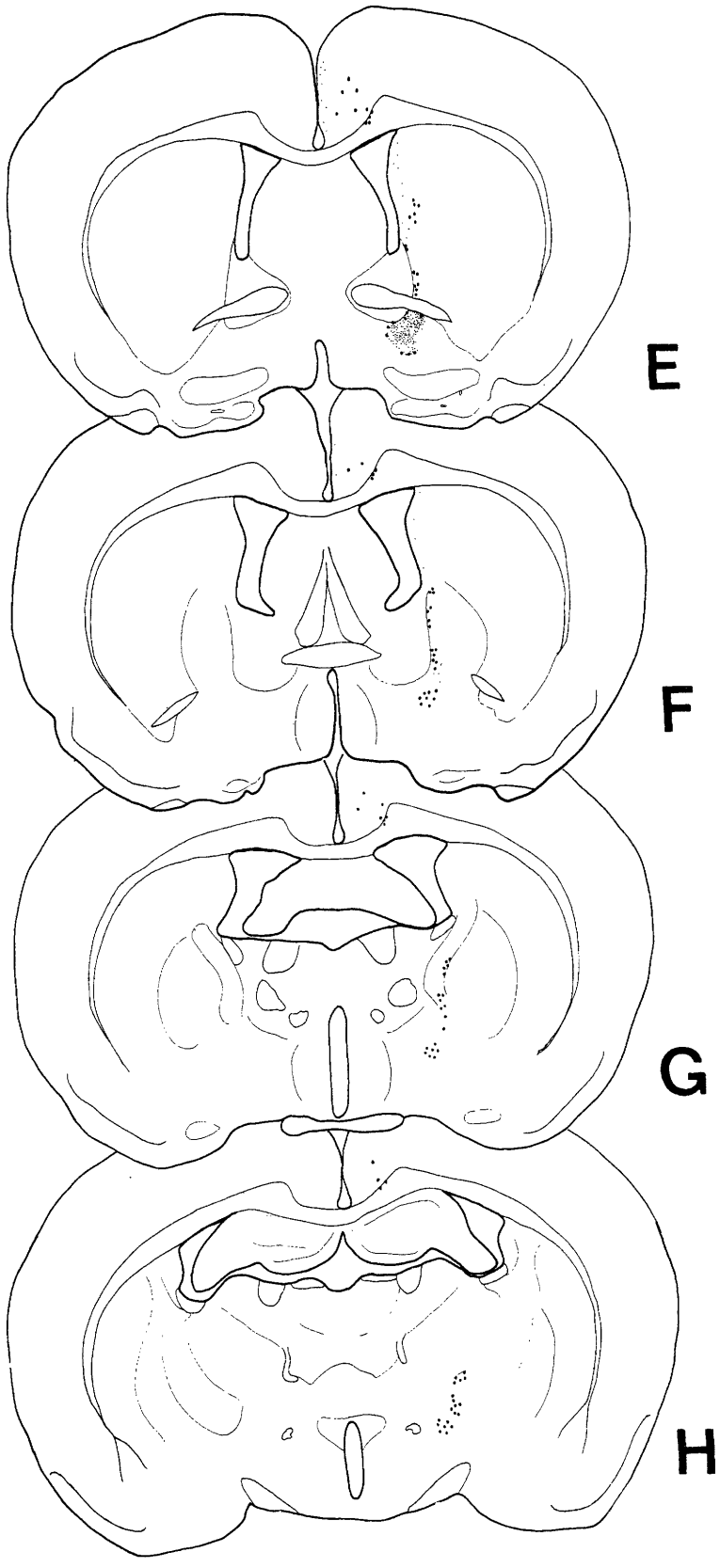
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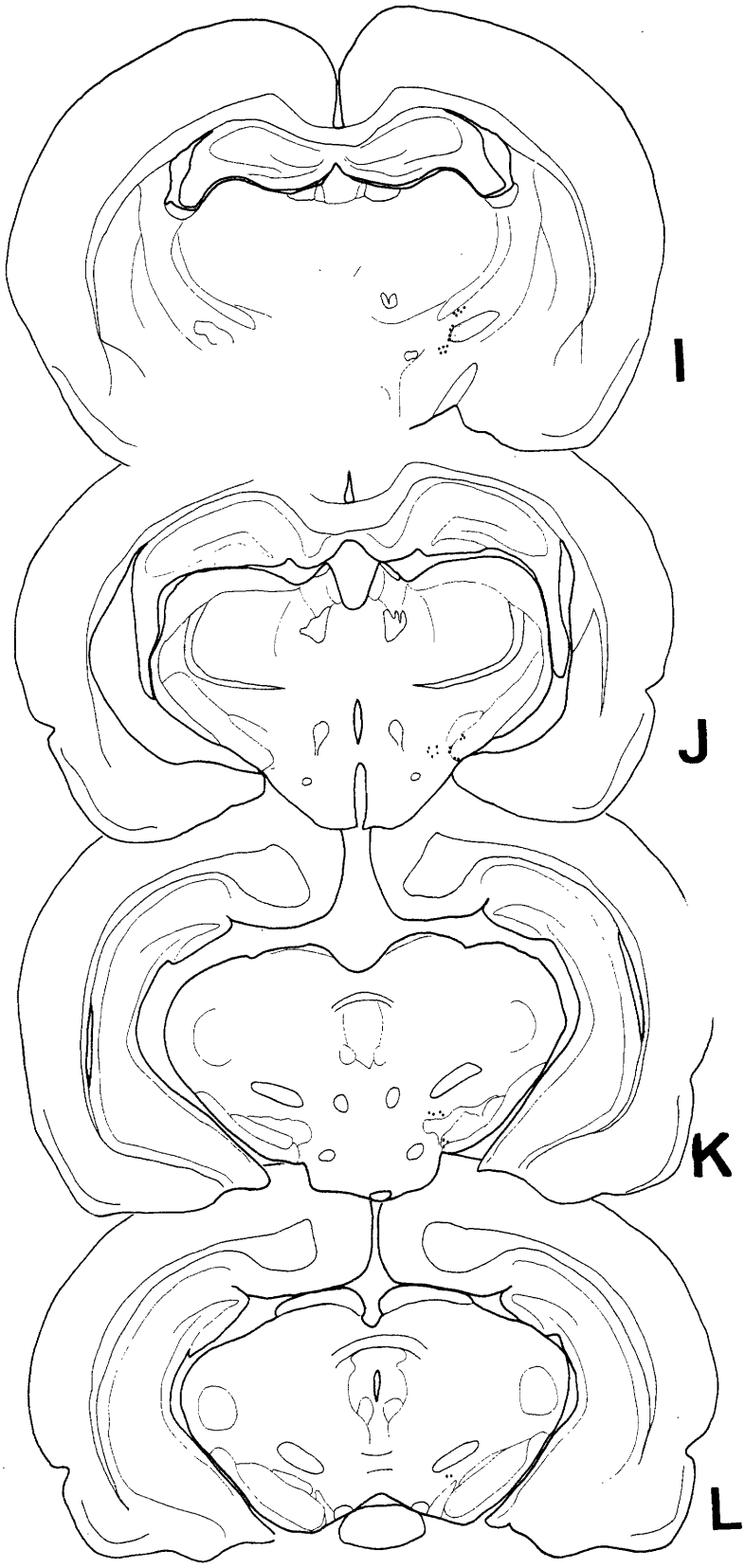
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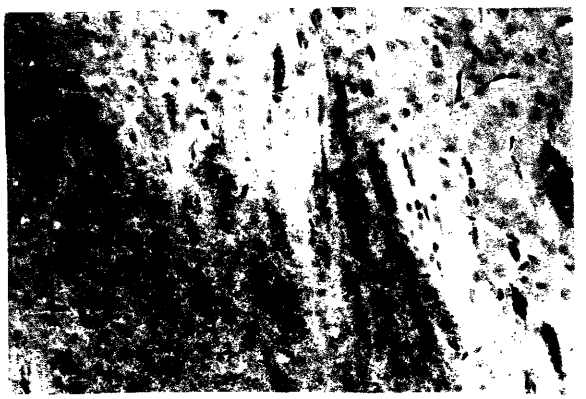
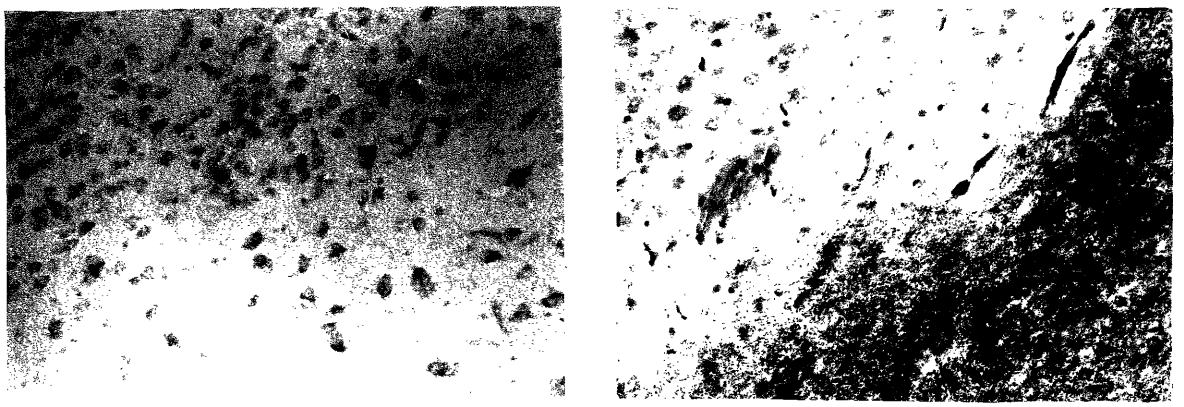


Figure 7. Degenerating fibers moving through the bed nucleus of the stria terminalis and terminating in the substantia innominata. Fink-Heimer method 1. 250X.

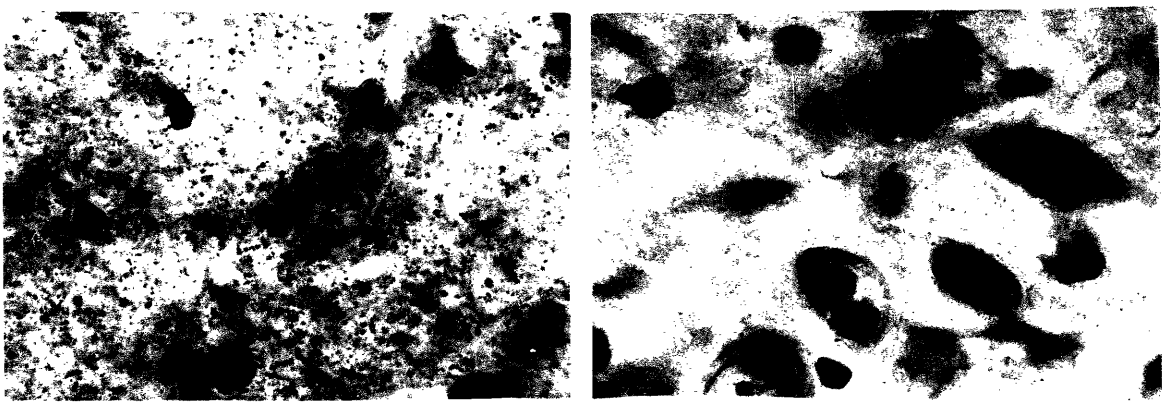


a. b.

Figure 8. An illustration of the sharpness of the boundary between the bed nucleus of the stria terminalis and the substantia innominata.

a. Nissl method, 250X.

b. Fink-Heimer method 1, 250X.

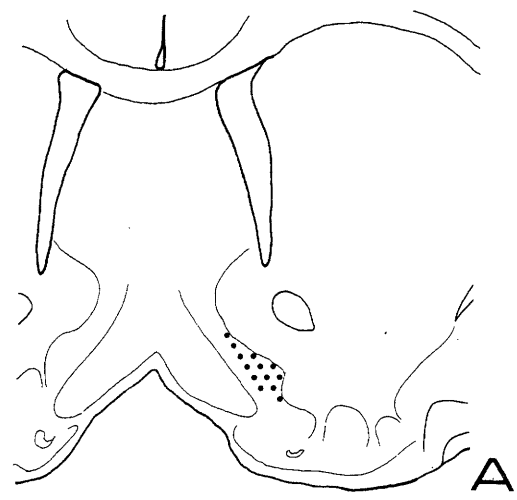


a. b.

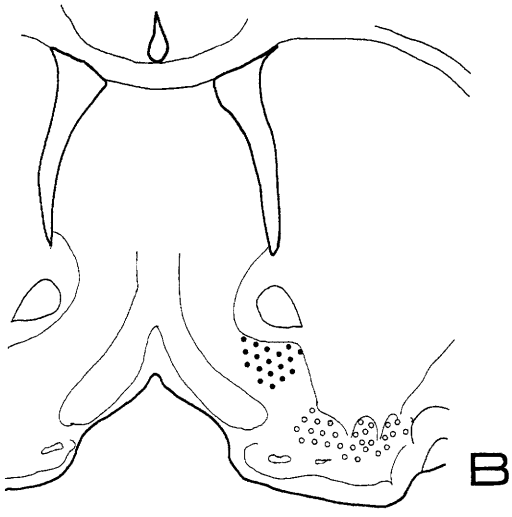
Figure 9. a) Massive terminal degeneration in the substantia innominata after a lesion of nucleus accumbens. Fink-Heimer method 1, 1000X.

b) The contralateral substantia innominata, free of degeneration.

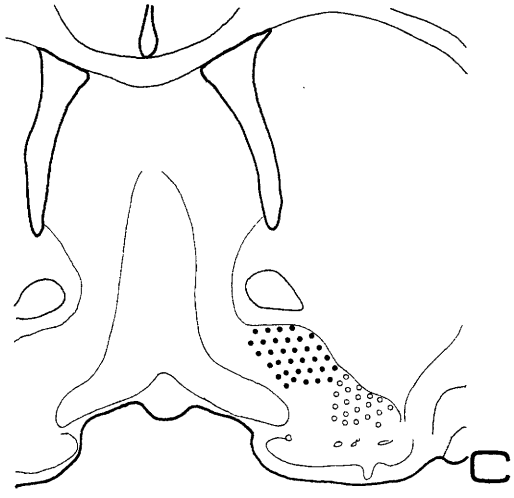
Figure 10. A schematic representation of terminal degeneration in the substantia innominata after lesions of nucleus accumbens (closed circles), and the olfactory tubercle (open circles).



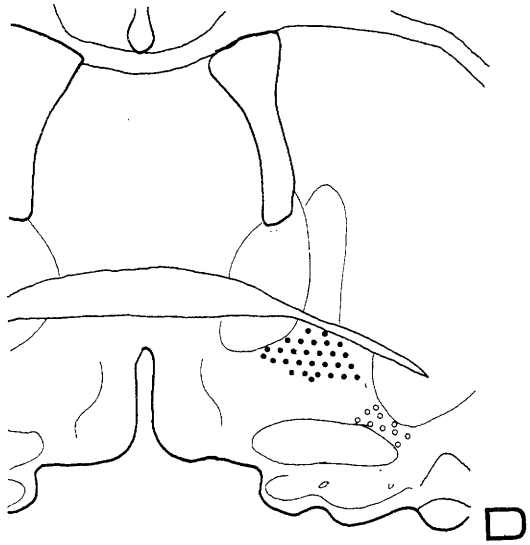
A



B



C



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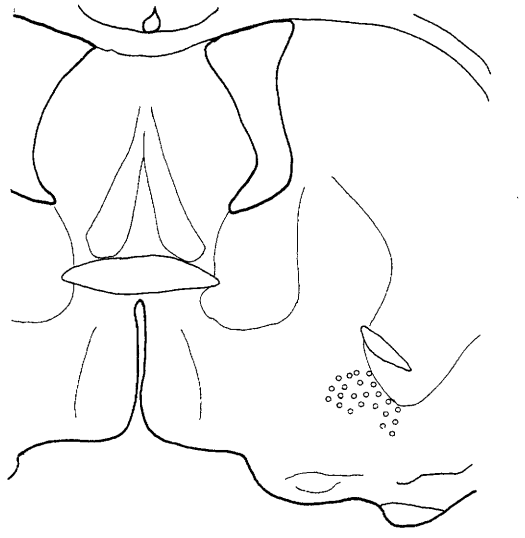


E

Four other components of degeneration are illustrated in Figure 6. The terminal degeneration in accumbens and the tubercle medial to the lesion (Figures 6A, B) may result from either the interruption of brainstem or thalamic afferents or the disruption of intra-accumbens connections.

Degenerating fibers pass from the lesion in caudoputamen (Figure 6C) and run caudally to terminate in globus pallidus (Figure 6F), the entopeduncular nucleus (Figure 6I), and the substantia nigra (Figure 6L). The size of this component of degeneration in other cases of accumbens lesion is proportional to the degree to which the lesion extends in the dorsolateral direction, i.e. into the caudoputamen. Thus, the component is large in Case RACC-1 but completely absent in Case RAi.

Degeneration in the fasciculus cinguli and cingulate cortex can be attributed to the electrode track disrupting fibers en route from the anterior thalamic nuclei to cingulate cortex (Domesick, 1970). This component is minimal in Case RACC-1, but because the thalo-cingulate fibers pass through the striatum before looping around the genu of the corpus callosum, a small number of them were interrupted by the lesion itself.

A very small number of fibers, apparently of cortical origin, can be traced as they pass through the dorsal caudoputamen and into the internal capsule. They disappear caudal to the entopeduncular nucleus, but neither their exit from the internal capsule nor their site of termination could be determined.

Cases RHR-4 and RHR-2 (RHR-2 is not illustrated; it shows a lesion of infraradiate cortex not involving the hippocampal rudiment)reveal that fibers originating in the infraradiate cortex pass through accumbens and must unavoidably be involved in all lesions of accumbens. These fibers pass through accumbens and through the substantia innominata without terminating. In the illustration of Case RACC-24 their presence in substantia innominata is obscured by the extreme density of the terminal degeneration. A few fibers are visible at the ventral boundary of the field of degeneration (Figures 6D, E). Caudal to the zone of accumbens projection these fibers of small diameter can be seen traveling diffusely in the medial forebrain bundle, just ventral and medial to the internal capsule. At the level of the mammillary bodies the fibers run just dorsal to the pars compacta of the substantia nigra (Figures 6K, L). The bundle dwindles rapidly in this region, but neither the dispersion of its fibers nor its site of termination could be determined.

Comments. In cases of superficial lesion of the olfactory tubercle, Heimer (unpublished results) has found terminal degeneration principally in the SI, adjacent to the zone projected upon by accumbens. The projections from accumbens and the tubercle appear to be contiguous, and to outline a somewhat discrete, unitary region (Figure 10). Although the center of this field of degeneration is just below the anterior commissure, the tubercular projection extends far rostrally between accumbens and the multiform layer of the tubercle and further caudally, behind the anterior commissure. The parts of the SI which remain free of degeneration in Figure 10 are probably projected upon by areas of accumbens, the tubercle, and the ventro-lateral striatum which have not been involved in lesions.

DISCUSSION

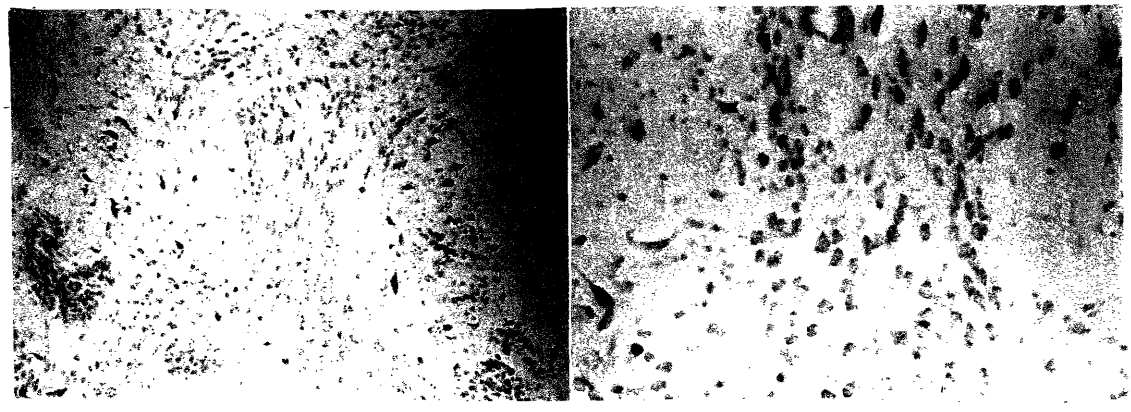
Nucleus Accumbens and the Olfactory Tubercle.

The results of these studies of nucleus accumbens reveal a pattern of connectivity strikingly similar to that of the adjacent olfactory tubercle. This similarity, combined with a sharing of histochemical and morphological characteristics, provides the foundation for a new view of the basal forebrain region, in which accumbens and parts of the tubercle are seen as a homogenous mass divided by the fibers of the medial forebrain bundle.

In the rat, the corpus striatum and tubercle are to a large extent separated by fibers of the deep olfactory radiation, but their continuity is revealed at some points by columns of cells which remain uninterrupted by the olfactory radiation. In Nissl-stained sections the cells of these bridges are indistinguishable from either the cells of the striatum or the medium-sized cells of the multiform layer of the tubercle, suggesting that the bridges are areas of continuity of a single cellular mass rather than areas of conjunction of two distinct masses. (Figure 11) These cellular bridges are present in a variety of other mammals.

The comparability of the cellular morphology is further revealed by the Golgi method. The caudoputamen, nucleus accumbens, the striotubercular bridges, and the multiform layer of the tubercle are all characterized by a predominance of relatively small neurons with extremely spiny dendrites and very short, tortuous axons. The medium-sized pyramidal cells of the tubercle have a similar size and morphology, except for the orientation of their dendrites perpendicular to the pial surface.

a.



b. c.

Figure 11. Cellular bridges between nucleus accumbens and the multiform layer of the olfactory tubercle.

a. Fink-Schneider method for normal fibers, 25X.

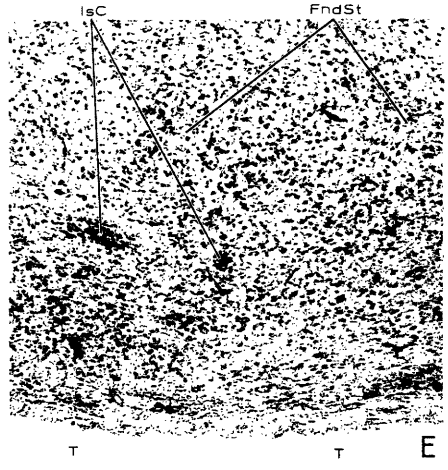
b. Nissl method, 100X.

c. Nissl method, 250X.

The brain of the dolphin (Tursitops truncatus) provides an illuminating natural illustration of the unity of this region. Most of the fibers that comprise the rostral (pre-septal) part of the MFB of terrestrial mammals are efferents from the paleocortex and olfactory tubercle en route to the nuclei gemini of the hypothalamus and the medio-dorsal nucleus of the thalamus; it is principally these fibers which cleave accumbens from the tubercle. In the dolphin these fibers must either be absent or follow a different route, because there is complete cellular continuity of the striatum from the corpus callosum to the pial surface at the base of the brain. The ventral striotubercular region is entirely homogeneous except for the granular islands of Calleja, which, as in every other mammal, are dispersed near the surface of tubercle and on the medial edge of nucleus accumbens.

The anosmatic dolphin, without olfactory receptors, bulb, or tracts, has an enormous tubercle (bulge) at the base of the anterior forebrain, but the characteristic three layers -- plexiform, pyramidal, and multiform -- that in other species cover the ventral surface of the region are absent (Figure 12). The plexiform layer, or striatum zonale, composed primarily of tubercle afferents from the bulb, is absent in an animal without an olfactory system. As a result, the tubercle of the dolphin ("lobule désert" of Broca) is grey at the surface rather than white. The cells of the pyramidal layer of a terrestrial mammalian tubercle seem different from the cells of the multiform layer not in type but rather in arrangement and orientation: their arrangement in a tightly-packed layer and the orientation of their apical dendrites into the plexiform layer are really the only distinguishing morphological

Figure 12. A sagittal section through the lobule désert of the dophin, illustrating the absence of the deep olfactory radiation, pyramidal cell layer, and plexiform layer, and the resulting extension of the striatum all the way to the base of the brain. Fnd.Str., fundus striati (ventral striatum); IsC, islands of Calleja; T, tubercular area. (This photograph was kindly supplied by Dr. Myron Jacobs.)



characteristics of these cells. One final difference between the lobule désert and the olfactory tubercle is the lack in the dolphin of the large cells of the multiform and pyramidal layers. Their absence suggests that the tubercle may contain a quite heterogeneous population of cells. Lacking the large cells as well as the perforating MFB and the layered arrangement, the lobule désert of Broca seems to represent the striatal matrix which, in terrestrial mammals, is variously modified into the olfactory tubercle.

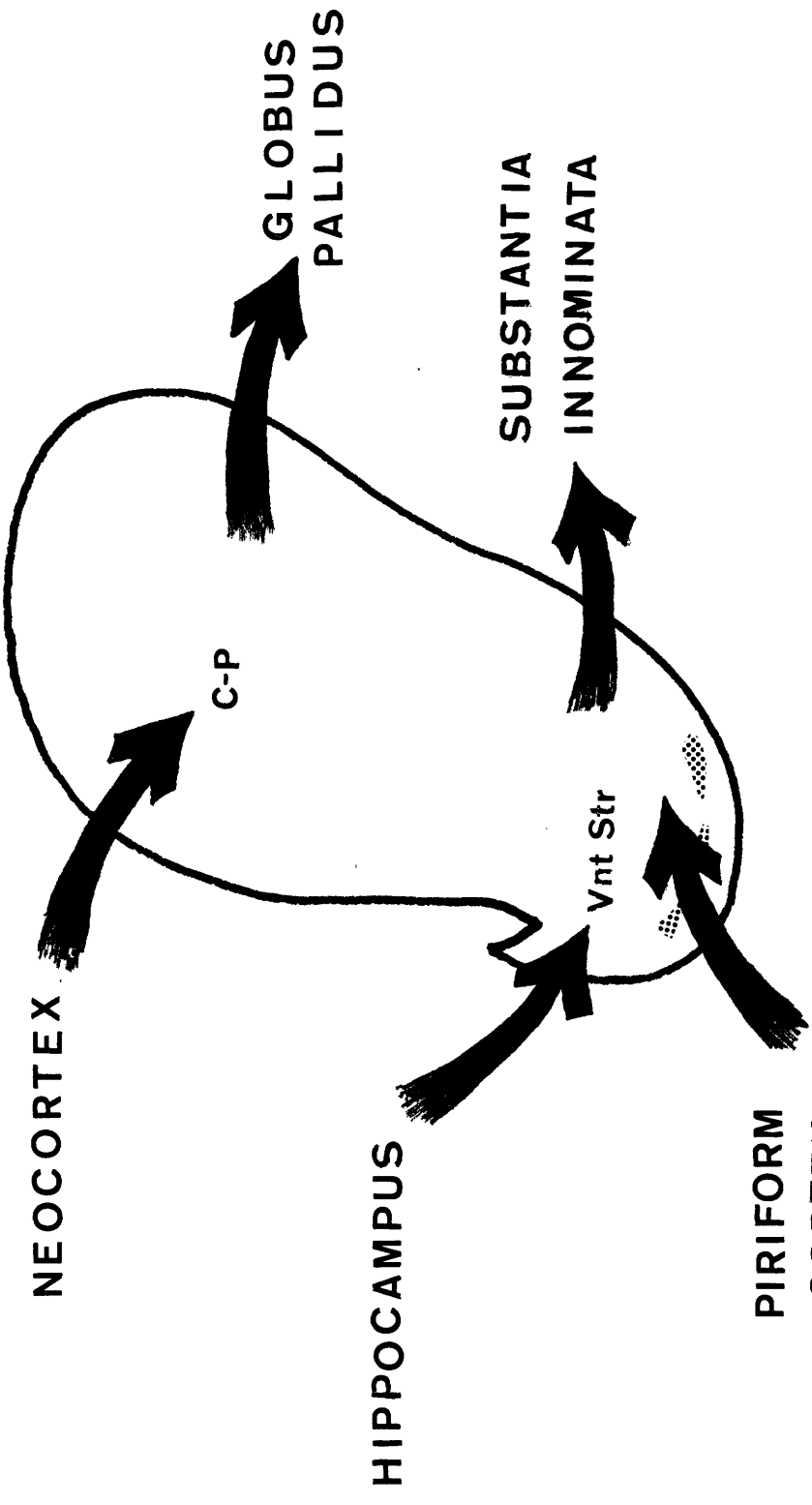
No boundary can be drawn between nucleus accumbens and the olfactory tubercle on histochemical grounds either. Both contain high concentrations of dopamine containing nerve terminals.

The efferent pathways from tubercle have two sets of targets -- one in common with accumbens efferents, and one in common with the efferents of the piriform cortex. In addition to the afore-mentioned projection to the substantia innominata, the olfactory tubercle also gives rise to less massive projections to the medio-dorsal nucleus of the thalamus (MD), the nuclei gemini of the hypothalamus, the plexiform layer of the olfactory peduncle, and hippocampal rudiment (Heimer, personal communication). According to Cajal (1911) the hypothalamic pathway from piriform cortex tubercle region is composed of collaterals of axons which ascend via the stria medullaris and inferior thalamic peduncle to MD. This fact, combined with the known olfactory nature of the hypothalamic pathway (Scott and Leonard, 1971), suggests that the cells of origin of the diencephalic (i.e. tuberculo-hypothalamic and tuberculo-thalamic) pathways are the cells which receive direct input from the olfactory bulb and are, therefore, the truly olfactory cells of the

tubercle. The observation that the time course of degenerative changes differs for the diencephalic and innominata projections from the tubercle (Heimer, 1972) suggests that the cells which project to the substantia innominata may not be the olfactory cells but rather the cells of the tubercle which resemble those of nucleus accumbens. One is thus led to speculate that the olfactory elements of the tubercle project to MD, nuclei gemini, the olfactory peduncle, and the hippocampal rudiment, and that the non-olfactory tubercle cells project to the substantia innominata.

Although no lesions have been made in the extreme ventro-lateral striatal region, it seems likely that cells which receive input from piriform cortex would have efferents to the SI rather than to globus pallidus. On the basis of an expanding body of experimental neuroanatomical data it is now possible to offer a new formulation of the relationships among accumbens, the caudoputamen, and the olfactory tubercle. In the light of these data, the striatum seems divisible into a dorsal and a ventral part (Figure 13). The dorsal part, related to neocortex and globus pallidus, is the caudoputamen. The ventral part, related to allocortex and the SI is best described by the term ventral striatum. This region can be subdivided into nucleus accumbens, a ventrolateral striatal area, and the multiform region of the olfactory tubercle. Just as neocortex projects topographically onto the dorsal striatum,so allocortex projects topographically onto the ventral striatum: hippocampus projects to the rostral and medial parts (accumbens), and piriform cortex projects to the ventral and ventrolateral parts (tubercle and extreme ventrolateral striatum).

Figure 13. A schematic representation of the neural associations of the mammalian striatum, with emphasis on the dichotomy which exists within this structure. (C-P), caudoputamen; (vent. str.), ventral striatum.


NEOCORTEX
HIPPOCAMPUS
C-P
Vnt Str
GLOBUS PALLIDUS
SUBSTANTIA INNOMINATA
PIRIFORM CORTEX

The Ventral Striatum as a Part of the Limbic System.

Although studies of fiber connections can offer no more than a hint of the precise neurophysiological role of accumbens, they can at least establish the functional realm in which it should be considered. To that end, a brief description of some of the structures to which the ventral striatum is synaptically related may provide an implicit characterization of nucleus accumbens and the other subdivisions of the ventral striatum.

Hippocampus. The hippocampus has been implicated in various aspects of viscero-endocrine regulation and many facets of the behavior of man and experimental animals. The multifarious influences of this unique paleocortical structure are exerted by means of the fornix system, a massive fiber bundle which distributes principally in the septal region, the anterior thalamic nuclei, and the mammillary body. The septal nuclei, in turn, give rise to fibers which terminate widely in the lateral hypothalamus. The efferent connections of the anterior thalamic nuclei and mammillary body are links in the so-called Papez circuit, which also includes the cingulate and parahippocampal cortices.

Piriform cortex. Because of its afference from the olfactory bulb, the 3-layered cortex ventral to the rhinal sulcus has generally been considered "olfactory" cortex; however, its presence in a well developed form in anosmatic mammals (Jacobs et al., 1971) makes it extremely unlikely that this cortex is only a link in olfactory pathways. The most revealing indication of the nature of piriform cortex is probably that it is the most massive single source of afference to the amygdaloid nuclei (Valverde, 1965). It may therefore be assumed that the

functional state of neurons of the amygdala is influenced to a large extent by the piriform cortex.

Ventral tegmental region. The dopaminergic pathway from the midbrain to the ventral striatum seems to arise from the ventral tegmental area of Tsai (Ungerstedt, 1971). This ill-defined cell group, situated between the interpeduncular nucleus and the substantia nigra, merges almost imperceptively with the lateral hypothalamic region, and is a principal target of post-mammillary fibers of the fornix system (Nauta and Haymaker, 1969). The nucleus also receives caudally-directed fibers from the MFB and rostrally-directed fibers from the mammillary peduncle. The latter connection led Papez (1932) to name this cell group the nucleus of the mammillary peduncle.

Substantia innominata. The region of termination of ventral striatal efferents has been tentatively labelled substantia innominata because it seems to correspond topographically to the substantia innominata of the cat and monkey brains. However, in the absence of any comparative studies of this region, no homologies should be inferred from this terminology.

Recent unpublished observations by Heimer reveal that the histological appearance of the substantia innominata is comparable in every major respect to that of the overlying globus pallidus. The large fusiform cells are rather loosely arranged, and the neuropil in which they are embedded is composed of a dense network of unmyelinated axons. The long, radiating dendrites have very few spines and are encased by a nearly continuous sheath of boutons. Aside from the fiber bundles of the internal capsule which perforate the globus pallidus, there is

virtually no histological difference between the globus pallidus and the entire substantia innominata. Thus, the substantia innominata should be considered to be a ventral part of the pallidum, related to the ventral striatum in the same way that the globus pallidus is related to the dorsal striatum.

Nothing is known of the efferent connections or other afferent connections of this region.

Certainly the major conclusion to be drawn from this study is that there are fundamental anatomical and probably functional differences between the caudoputamen and nucleus accumbens. Although it receives fibers from the entire neocortical mantle, the caudoputamen has as its principal efferent target the globus pallidus, a structure predominantly concerned with movement (Nauta and Mehler, 1966; DeLong, 1971). Dysfunctions of the caudoputamen or the cells to which it is synaptically related are most distinctly manifested as disorders of movement such as hypo- and hyperkinesias, tremor, and abnormal muscle tonus.

In contrast, the efferent connections of accumbens establish it as a central structure of the limbic forebrain. As such, accumbens is probably more involved in the affective facets of behavior than in the control of movements. Some possible effects of dysfunctions of accumbens will be discussed in a following section.

The most notable physiological demonstration of the limbic nature of accumbens was given in a series of experiments by Rioch and Brenner (1938), who found that local electrical stimulation of the caudate nucleus in freely-moving decorticate cats produced no consistent behavioral changes. On the other hand, stimulation in accumbens

produced behavioral effects which are unique to this region but which nevertheless suggest strong association with the limbico-hypothalamic axis:

> The second response consistently obtained was evoked by prolonged stimulation of the floor of the remaining portion of the frontal horn of the ventricle, between the head of the caudate and the septum, at or just in front of the anterior commissure (b, Figure 3). After a latent period of 1 to 5 seconds, the pupils dilated moderately, following which respiration became much more rapid, the depth and rate increasing with the duration of the stimulus. The animal showed no other signs which could be interpreted as pain, fear or rage, but sat or stood rather tensely. When the stimulation had continued for 5 to 10 seconds, there was a sudden burst of activity in the form of violent springing and running movements which continued for 1/2 minute or more after the stimulation ceased and then subsided gradually. At the end of the outburst the animal sat or stood on the table as quietly as before, the respirations and pulse rapidly returning to normal. The whole reaction could be repeated by stimulating the same region (on either side) again. In cat 1 there were frequent twitches and jerks appearing irregularly in one or another of the legs, which momentarily interrupted the running movements at the height of the activity. Apart from these isolated twitches, there was nothing resembling an epileptiform seizure or convulsion. These results were comparable to those obtained by Schaltenbrand and Cobb ('31) from their 'point 2' and by Ranson and Magoun ('33), in cats under pentobarbital sodium, from the lateral hypothalamic area. Taken as a whole, however, this reaction was entirely distinct from that of pseudo-affective rage which was elicited from the posterior hypothalamus (p. 500).

The power of this illustration has not been surpassed, but the proposed striatal dichotomy has been substantiated by several subsequent studies. In contrast to sites in the overlying caudato-putamen, nucleus accumbens has been identified as a potential site of self-stimulation of the "rhinencephalic" type (low frequency of stimulation, hypo-activity, lack of cortical desynchronization) (Rolls, 1971). Bilateral lesions of accumbens cause changes in

avoidance and feeding behavior which are more similar to the effects of septal lesions than to the effects of caudoputaminal lesions (Lorens, et al., 1970). Finally, stimulation in accumbens, and probably the entire ventral striatum, thus should be considered among those regions of the brain which, by virtue of their connections with the hypothalamus and/or their apparent involvement in affective behavior and the maintenance of visceral and endocrine homeostasis have come to be known as the limbic system.

The Role of the Ventral Striatum in the Limbic System.

Although accumbens' membership in the limbic system appears quite firmly established, the details of its role in that system are completely unknown. It is ironic, however, that accumbens' structural similarity to the caudoputamen -- the factor which has obscured its autonomy -- may eventually provide the key to its function.

The distinctive cellular morphology and synaptic organization shared by the caudoputamen and ventral striatum (see below) suggests that some unique form of input-transformation is being carried out by both of these striatal components. It would seem conceivable that the same role played by the caudoputamen in processing neocortical outflow might be played by the ventral striatum in the processing of allocortical efflux. Such a generalized "striatal transformation" could be postulated as a conceptual parallel to the distinctive transformation of many diverse inputs which is performed by the uniform structure of the cerebellar cortex.

Recalling the structural similarities between the globus pallidus and the substantia innominata, one might further speculate that there is also a generalized "pallidal transformation" characteristic of cell regions receiving striatal afferents. It cannot be determined whether or not this parallelism in the associations of neocortex and allocortex extends one step further because the efferent connections of the substantia innominata remain unknown.

Because the caudoputamen is ultrastructurally identical throughout its extent, and because the dorsal part of the ventral striatum is indistinguishable from the caudoputamen on the light microscopic level (Nissl, Golgi)[3], it is probable that the ventral striatum has basically the same cell types and synaptic organization. The major characteristics of the caudoputamen, as emphasized by Kemp and Powell (1971) are: 1) 96% of the cells of the caudate are true interneurons, with branching axons which do not extend out of the dendritic field of the neuron (Figure 14). These classic Golgi-type II neurons have extremely spiny dendrites, and most afferent contacts on them are axo-spinous. There are a number of other cell types in the caudate, but the only cells which project out of the nucleus are large fusiform cells with smooth, long (1 mm radius) dendrites (Figure 14). Kemp and Powell, using the Golgi method, estimate that these efferent cells comprise approximately 1% of the total neuron population; Foix and Nicolesco (1923) using the Nissl method, estimate 2%. 2) All of the afferents to the

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[3]In preliminary Golgi-Cox observations only the interneurons with very spiney dendrites and the large fusiform neurons (see below) have been recognized so far.

Figure 14. Neurons characteristic of the striatum. The type illustrated in the upper right, with spiny dendrites and a short, tortuous axon, predominates. Approximately 2% of the cells are of the type illustrated in the lower left. Not drawn to scale. Golgi method. (This figure reproduced from Kemp, 1968.)

caudoputamen, whether from cortex, thalamus, or substantia nigra, converge on the same cells, without any apparent preference for a particular segment of the dendrite. 3) Axons tend to travel perpendicular to dendrites, contacting many cells by synapses en passage. The implication of these structural characteristics is that the output of the caudate, as a reflection of the activity of a large number of local interneurons, must be a highly abstracted and compounded version of the input.

In view of the foregoing anatomical description, it is not surprising that electrophysiological studies have revealed that the caudoputamen is characterized by an unusual mode of neural activity, namely, a very low level of spike activity and highly complex postsynaptic potentials (Hull, et al., 1970). These physiological characteristics, combined with the anatomical indication that the cells of the striatum have multiple local connections en passage, suggest that sub-threshold activity may be of primary importance in the mode of interaction of striatal neurons.

Unfortunately the conclusion we must come to is that the significance of the synaptic events in the caudoputamen (and by inference, in the ventral striatum) is at present completely unknown. Just as in the case of the cerebellum, it seems that it is the long-known manifestations of pathology, rather than anatomical and physiological details, which must still provide the basis of our conceptions of the functions of the caudate. Attempts to approach the question of the physiological role of the caudate through the study of its dysfunctions is hindered by, to quote Denny-Brown, "the very general nature of the types of pathology that affect [the basal ganglia] and the kaleidoscopic variations in

resulting symptomatology. . . ." Perhaps the most precise statement which can be made is that the caudate is somehow involved in the coordination of muscular activity.

Nucleus Accumbens and Dopaminergic Limbic Pathways.

A possible clue to the nature of dysfunctions of nucleus accumbens (and perhaps the nature of the physiological role of accumbens) is provided by the common experience of clinicians that drugs which either relieve or produce Parkinsonian motor symptoms also produce changes in motivational and emotional states. Parkinsonian patients treated with L-dopa often develop psychotic tendencies; conversely, schizophrenics treated with phenothiazines, which are thought to block dopaminergic synapses, often develop Parkinsonian motor disorders. Thus, the following hypothesis emerges: there are two dopamine (DA) pathways rising from the midbrain, parallel but independent. Attempts to replenish DA in the "motor dopamine pathway" (nigra to caudoputamen) result in overloading the "limbic dopamine pathway" (ventral tegmentum to accumbens, the tubercle, the bed nucleus of the stria terminalis, and the central amygdaloid nucleus). Conversely, attempts to reduce dopaminergic transmission at the terminals of the limbic pathway similarly affects the motor dopaminergic pathway and thus may result in Parkinsonian dyskinesia.

Unfortunately, the field of Neuropsychopharmacology is filled with transient "truths", and before one can accept an hypothesis which attempts to relate behavior, chemistry, and anatomy in a precise way, one must evaluate thoroughly all the evidence on which the concepts are based. Accordingly, the following hypothesis is put forth and supported

as strongly as possible, but with full realization that some of the support is weak and that many details of evidence are likely to be proven invalid. Nonetheless, the proposal seems essentially sound, and the available pattern of evidence warrants consideration.

Outline of the hypothesis of the involvement of the ventral striatum in affective behavior. The basic hypothesis is that either direct or indirect interventions upon synaptic transmission mediated by DA can modify neural activity in limbic dopaminergic pathways and can thereby elicit significant changes in affective behavior. Two points must be made with respect to this hypothesis. First, changes in affect -- whether exaggeration, as in the manic-depressive syndrome, or "flattening" as in schizophrenia -- seem to be a hallmark of the major psychoses. Although schizophrenia is not considered to be primarily an "affective disorder," the etiology of the many manifestations of schizophrenia is undoubtedly very complex, and it seems possible that the pathognomonic symptom -- a "disorder of thought"-- might be a compensatory reaction to a more fundamental derangement, viz. the loss of appropriate affective responses to normal situations. Second, it should be recognized that the tendency for modifications of dopaminergic transmission to produce or abolish psychotic behavior does not necessarily imply that the natural lesions in psychoses (if, indeed, there are such) directly involve DA systems. However, this possibility connot be overlooked. There are four points which must be established in order to substantiate this hypothesis.

1. DA is the neurotransmitter of the ventral tegmental DA-containing neurons which project to accumbens, the tubercle, the bed nucleus of the stria terminalis, and the central amygdaloid nucleus.

2. Psychoactive phenothiazines block synaptic transmission mediated by DA. L-dopa increases the synthesis of DA and thereby the impulse transmission to neurons normally receiving dopaminergic inputs.

3. The anti-schizophrenic effects of phenothiazines are produced by a decrease in dopaminergic transmission to postsynaptic neurons (more, specifically, by a receptor blockade at dopaminergic synapses), and not by any other effect of the drug.

4. It is the limbic dopamine pathways rather than the nigrostriatal or infundibular pathways which are principally involved in the modification of affective behavior.

Examination of the hypothesis.

1. DA is the neurotransmitter of the ventral-tegmental DA-containing neurons which project to accumbens, the olfactory tubercle, the bed nucleus of the stria terminalis, and the central amygdaloid nucleus. DA has now met most of the criteria for recognition as a neurotransmitter. No experimental work has been done specifically in accumbens or the other limbic DA targets, but in view of the discrete localization of DA in the brain it does not seem too presumptuous, on some points, to extrapolate from results obtained in the caudate. a) DA is present in cell bodies in the ventral tegmental region and in axonal terminals in accumbens, the olfactory tubercle, bed nucleus of the stria terminalis, and central amygdaloid nucleus. (Ungerstedt, 1971; Dahlström and Fuxe, 1962). The axons of these

pathways can be traced by both histochemical fluorescence (Ungerstedt, 1971) or anterograde axon-degeneration (Fink-Heimer) techniques (Hedreen, 1971). b) DA is released at synapses in the caudate during nigral stimulation (Von Voigtländer and Moore, 1971). c) In cats decerebrated at midpontine levels, iontophoretically-applied DA has precisely the same effect on caudate neurons as nigral stimulation (Connor, 1970). d) Chlorpromazine (CPZ, a phenothiazine) blocks the effect on caudate neurons of both nigral stimulation and directly-applied DA(York, 1972).

2. Psychoactive phenothiazines block synaptic transmission mediated by DA. L-dopa increases DA synthesis and thereby dopaminergic transmission to neurons receiving DA inputs. The responsiveness of neurons of the caudate, as estimated by the neurons' responses to iontophoretically-applied amino acids, is identically altered by nigral stimulation and direct application of DA (Connor, 1970). The effects of both nigral stimulation and application of DA can be reversibly blocked by CPZ administered iontophoretically (York, 1972). The dose-response relationship between CPZ and the DA antagonist effect suggests a process of competitive inhibition, presumably at the postsynaptic membrane.

X-ray crystallographic analysis of phenothiazines shows that their effectiveness as anti-psychotic agents is affected by their three-dimensional conformation, and that the most effective conformations are those in which a portion of the molecule is able to adopt a structure similar to that of DA (Horn and Snyder, 1971). These studies, likewise, suggest a competitive action of phenothiazines.

The psychoactive phenothiazines increase striatal DA turnover. The most probable explanation for this phenomenon is that the DA receptor blockade causes a compensatory increase in DA release and a concommitant increase in synthesis. The following observations on the results of phenothiazine administration to rats support this explanation. Such administration causes an increase in the rate of incorporation of H^3-tyrosine in the corpus striatum; this effect is dependent on the integrity of the nigrostriatal pathway (Nyback, 1972). Furthermore, under conditions of MAO inhibition, phenothiazines cause an increase of DA synthesis beyond normal (Carlsson and Lindquist, 1963), while the striatal content of 3-methoxytyramine, the product of enzymatic extracellular degradation of DA, likewise increases (Anden, Roos, Weidinius,1964). And finally, phenothiazines accelerate the disappearance of H^3-DA formed prior to drug administration (Nybäck and Sedvall, 1970); this effect, also, has been shown to be dependent on the integrity of the nigrostriatal pathway (Nybäck, 1972).

Precedent for such a mechanism of increased synthesis of a neurotransmitter when that transmitter's action is blocked has been established by observations in the peripheral nervous system. Treatment of an animal with phenoxybenzamine (an α - blocking agent) blocks the peripheral actions of norepinephrine (NE) and causes a reflex increase in presynaptic input to, and catecholamine synthesis by, postganglionic sympathetic neurons (Dairman and Udenfriend, 1970).

It should be noted that these studies of turnover show only that more DA is being synthesized and degraded; it has not been proven that more DA is being released. The argument for a compensatory increase

in the firing rate of nigral neurons would be greatly strengthened by the addition of two items of evidence: increased tyrosine hydroxylase activity[4] in the striatum, and increased electrical activity of the nigral cells.

Chlorpromazine also influences the hypothalamic regulation of growth hormone (Kolodny, et al., 1971) and prolactin. The observation that CPZ and L-dopa act as mutual antagonists in their effects upon these regulatory mechanism (Boyd, Lebovitz, Pfeifer, 1970) suggests that CPZ affects not only the mesencephalic DA neurons projecting forward to the striatum and to certain limbic structures, but also the DA neurons of the median eminence.

Finally, the tendency of phenothiazines to induce Parkinsonian motor disorder, which is thought to result from depletion of dopamine, suggests that the phenothiazines are reducing the effectiveness of DA synapses.

In summary, it is probable that phenothiazine tranquilizers mimic the conformation of DA and thereby competitively block DA receptor sites. This blockade causes a compensatory increase in activity of the nigral neurons, which, in turn, leads to increased DA synthesis.

The effects of L-dopa on brain DA metabolism are not as well studied as the effects of phenothiazines. L-dopa is taken up and converted to DA by nearly every cell of the body, including cells of every chemical nature in the brain (Romero, et al., 1972). Although there are

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[4]In the peripheral system previously described, the enzymatic activity of tyrosine hydroxylase has been shown to be increased by greater neural activity and decreased by less neural activity (Thoenen et al., 1969).

profound effects on brain norepinephrine metabolism, these effects are quite transient with respect to the duration of the increased level of striatal DA (Chalmers, Romero, Cottman, Lytle, and Wurtman, 1972), suggesting that the anti-Parkinsonian effect may be exerted through nigrostriatal DA neurons. Dopa's site of action in altering hypothalamic GRF (growth hormone releasing factor) and PIF (prolactin inhibiting factor) release is unknown, but the minimal effects of L-dopa on hypothalamic norepinephrine (Chalmers, et al., 1972) suggest that L-dopa acts directly on the DA neurons of the median eminence. Thus, although the effects of L-dopa on Parkinsonian disorder, hypothalamic functions, and especially on behavior, may be mediated through non-dopaminergic mechanisms, much circumstantial evidence suggests that at least the first two effects are exerted directly through DA neurons. However, aside from the clinical observations that Parkinsonian patients improve and that pituitary secretions are altered, there is no proof that neurons which normally receive dopaminergic inputs are subject to greater dopaminergic stimulation after treatment with L-dopa.

3. The anti-schizophrenic effects of phenothiazines are produced by a decrease in dopaminergic transmission to postsynaptic neurons (more specifically, by a receptor blockade at dopaminergic synapses), and not by any other effect of the drug.

The most devastating criticism of this or any other hypothesis which is based on pharmacological data concerns the specificity of drug effects. It is here that the transient nature of neuropharmacological beliefs is most evident; previously unknown effects of drugs are rapidly being discovered, and hypotheses about biochemistry and behavior

which are based only on pharmacology have a high probability of soon being proven invalid. The best one can do is to formulate a theory based on a convergence of different lines of circumstantial evidence.

The class of compounds call phenothiazines is characterized by the basic ring structure illustrated in Figure 15. The specific natures of the substituents on the rings separate the class into many different compounds with powerful and widespread biological activities. For example, members of the class have been reported to be strong chemical reducing agents, to inhibit oxidative phosphorylation, to stabilize biological membranes, to block α- adrenergic receptors and inhibit catecholamine re-uptake in the periphery, and, finally, to produce the previously discussed changes in brain DA metabolism. The only reported effect which is produced by all of the psychoactive compounds and by none of the non-psychoactive compounds is the alteration of DA turnover rates. Because all other effects enumerated above are produced by non-psychoactive compounds, they can be eliminated as the basis of the psychotherapeutic action of some members of the class. In general, only those phenothiazines that are psychoactive accelerate DA turnover, and only those that accelerate DA turnover are psychoactive[5]. Furthermore, two distinctly different chemical classes of anti-schizophrenic drugs -- butyrophenones and diphenylbutylpiperidines also increase DA turnover.

Because of the known effects of NE on behavior (Cooper, et al., 1970) it is important to note that the efficacy of phenothiazines does not

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[5]The double dissociation is not quite complete. Trimeprazine accelerates DA turnover but is not psychoactive, and thioridazine is psychoactive but does not accelerate turnover.

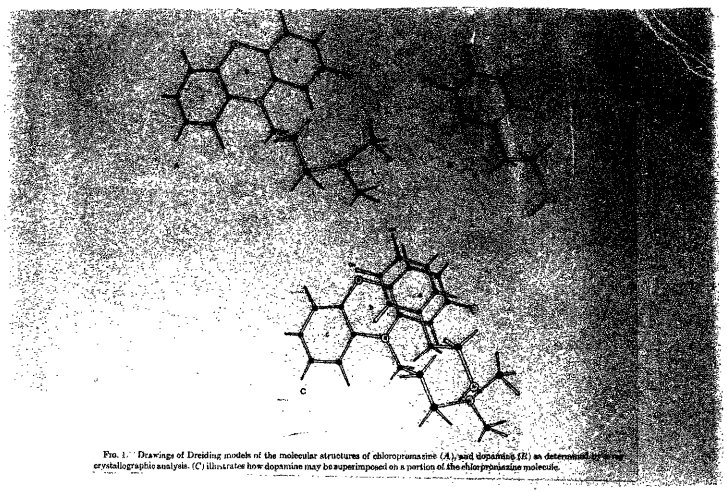

Figure 15. Drawings of Dreiding models of the molecular structures of chloropromazine (A), and dopamine (B) as determined by x-ray crystallographic analysis. (C) illustrates how dopamine may be superimposed on a portion of the chlorpromazine molecule.

(From Horn and Snyder, 1971)

depend on alteration of NE metabolism: many of the psychoactive phenothiazines do not alter NE metabolism at all. No definitive studies of the effects of phenothiazines on serotonin turnover have been reported, but it has been observed that CPZ does not change striatal 5-hydroxyindoleacetic acid (a serotonin metabolite) levels (Andén, et al., 1964).

The effect of phenothiazines on DA metabolism can be observed in the human clinic. When Delay and Deniker (1952) introduced CPZ (trade name thorazine) in 1952, they noted that it often produced Parkinsonian disorders of movement. All of the related psychoactive phenothiazines which have subsequently come into use likewise produce motor side effects. In one study it was found that Parkinsonian side effects occur in from 3% to 36% of the patients treated with various phenothiazines. The exact frequency depends on the specific drug (Cole and Clyde, 1961).

The effectiveness of phenothiazines as psychotherapeutic agents correlates well with the ability of the particular side chain to adopt a conformation similar to that of DA. A portion of the haloperidol (a butyrophenone) molecule can probably also mimic the DA conformation (Horn and Snyder, 1971). This observation reveals a possible basis for the correlation between the efficacy of a drug as an antischizophrenic agent and its efficacy as a modifier of DA metabolism: molecules with 3-D structures closer to that of DA are more effective in synaptic blockade, thereby producing greater concommitant motor effects and inducing greater compensatory activity and synthesis in DA neurons.

Several studies have shown that L-dopa treatment of Parkinsonian disorder can precipitate schizophreniform changes in behavior. One study reports the frequency as 7.7% (Celesia and Barr, 1970).

Exacerbation of the schizophrenic state is often a side effect of L-dopa treatment of schizophrenic Parkinsonian patients. One study reports 80% (Yaryura-Tobias, et al., 1970); another reports 40% (Bunney, et al., 1970).

No direct evidence can be adduced to assess which effect of L-dopa is important behaviorally. However, the previously reported circumstantial evidence which suggest that L-dopa has major effects on DA neurons, combined with the existence of limbic dopaminergic pathways, suggest a tentative explanation for the drug's psychotogenic action.

4. It is the limbic dopaminergic pathways rather than the nigrostriatal or infundibular pathways which are principally involved in the modification of affective behavior. A variety of changes in affect can be produced by stimulation or lesions of the components of the limbic system, including the hypothalamus and preoptic area. Since these effects are relatively specific to the limbic system, and no comparable changes of affect and mood have been consistently produced by stimulation or lesions of other parts of the brain, it would not seem far-fetched to assume that affective disorders have their primary functional localization in the limbic system. If changes in the effectiveness of DA synaptic transmission can influence these spheres of behavior, then one is led to assume that DA should be able to influence neural activity in limbic structures. Despite its widespread cortical afferents, the caudoputamen seems to be a structure principally involved in somatic motor mechanisms. The infundibular pathways seem to have the capability of influencing behavior only indirectly, via hormonal mechanisms. The remaining dopaminergic

pathways, however, arising in the ventral tegmental region, project to cell groups in the prosencephalon which, by virtue of the connections, must be considered components of the limbic system.

The bed nucleus of the stria terminalis is a major target of efferent fibers from the amygdala. Valverde (1965) illustrates some efferent fibers from the bed nucleus which travel back through the stria to distribute widely in the amygdala, and others which terminate in the preoptic region. The activity of these cells undoubtedly affects the functional state of the amydaloid complex.

Nucleus accumbens and the olfactory tubercle receive afferent fibers from the hippocampus, piriform cortex, and -- in the case of the tubercle -- the olfactory bulb. The tubercle also has efferent connections to the medio-dorsal nucleus of the thalamus and the nuclei gemini of the hypothalamus.

Thus, any or all of these cell groups could potentially mediate changes in patterns of neural activity in the limbic system, and it is not stretching the imagination to speculate that a substantial alteration of related dopaminergic pathways would produce just such a result.

BIBLIOGRAPHY

Andén, N. E., B. E. Roos, and B. Werdinius, 1964. Effects of chlopromazine, haloperidol, and reserpine on the levels of phenolic acids in rabbit corpus striatum. Life Sciences 3:149.

Ariëns Kappers, C. U. and W. F. Theunissen, 1908. Die Phylogenese des Rhinencephalons, des Corpus striatum und der Vorderhirncommissuren. Folia neuro-biol., Bd.1,S.173.

Boyd, A. E. III, H. E. Lebovitz, and J. B. Pfeifer, 1970. Stimulation of growth hormone secretion by L-Dopa. N. Engl. J. Med. 283: 1425-1429.

Bunney, W. E., D. L. Murphy, H. Keith, H. Brodie, and F. K. Goodwin, 1970. L-dopa in depressed patients. Lancet 1:352.

Cajal, S. Ramon y, 1911. Histologie du Système Nerveux de l'Homme et des Vertébrés, Tome II. Maloines, Paris.

Carlsson, A. and M. Lindquist, 1963. Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta pharmacol. et Toxicol. 20:140

Carman, J. B., W. M. Cowan, T. P. S. Powell, 1963. The organization of the cortico-striate connexions in the rabbit, Brain 86:525-562.

Celesia, G. G., and A. N. Barr, 1970. Psychosis and other psychiatric manifestations of levodopa therapy. Arch. Neurol. 23:193-200.

Cole, J. O. and D. J. Clyde, 1961. Extrapyramidal side effects and clinical response to the phenothiazines. Rev. Canad. Biol. 20:565.

Connor, J. D., 1970. Caudate nucleus neurones: correlation of the effects of substantia nigral stimulation. J. Physiol. (Lond.) 208:691-703.

Cooper, J. R., F. E. Bloom, and R. H. Roth, 1970. The Biochemical Basis of Neuropharmacology. Oxford University Press, New York, N. Y.

Crosby, E. C., 1917. The forebrain of Alligator mississippiensis. J. Comp. Neurol. 27:325.

Dahlström, A. and K. Fuxe, 1965. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons, Acta physiol. scand. 65. Suppl. 232:1-55.

Dairman, W., and S. Udenfriend, 1970. Effect of ganglionic blocking agents in the increased synthesis of catecholamines resulting from α-adrenergic blockade or exposure to cold, Biochem. Phamacol. 19:979-984.

Delay, J., and P. Deniker, 1952. Trente-huit cas de psychoses traitées par la cure prolongée de 4560 RP. Le Congrès des Al. et Neurol. de Langue Fr. In Compte rendu du Congrès. Masson et Cis, Paris.

De Long, M. R., 1971. Activity of pallidal neurons during movement, J. Neurophysiol. 34:414-427.

De Olmos, J. S., 1972. Some connexions of the stria terminalis in the guinea pig brain. (Abs.) Anat. Rec. 172:301.

Domesick, V. B., 1970. The fasciculus cinguli in the rat. Brain Res. 20:19-32.

Foix, C.,and J. Nicolesco, 1925. Anatomie Cérébrale. Les Noyaux Gris Centraux et la Région Mesencephalo-Sous-Optique, Masson, Paris.

Fox, C. A., 1940. Certain basal telencephalic centers in the cat. J. Comp. Neurol. 72:1-62.

Fox, C. A., D. E. Hillman, K. A. Siegsmund, and L. A. Sether, 1966. The primate globus pallidus and its feline and avian homologues, a Golgi and electron microscopic study. In: Evolution of the Forebrain, Hassler and Stephan (eds.), Plenum, New York.

Guillery, R. W., 1957. Degeneration in the hypothalamic connexions of the albino rat. J. Anat. 91:91-115.

Guillery, R. W., 1959. Afferent fibers to the dorso-medial thalamic nucleus in the cat. J. Anat. 93:403-419.

Gurdjian, E. S., 1928. The corpus striatum of the rat. J. Comp. Neurol. 45:249-281.

Hedreen, J. C., 1971. Separate demonstration of dopaminergic and non-dopaminergic projections of substantia nigra in the rat. (Abs.) Anat. Rec. 169:338.

Heimer, L., 1972. The olfactory connections of the diencephalon in the rat. In: Basic Thalamic Structure and Function, W. Riss (ed.) In press.

Heimer, L., and W. J. H. Nauta, 1969. The hypothalamic distribution of the stria terminalis in the rat. Brain Res. 13:284-297.

Herrick, C. J., 1926. Brains of Rats and Men. University of Chicago Press. Reprinted in 1963 by Hafner, New York, N. Y.

Herrick, C. J., 1927. The amphibian forebrain. IV. The cerebral hemispheres of Amblystoma. J. Comp. Neurol. 43:231-325.

Hines, M., 1923. The development of the telencephalon in Sphenodon punctatum. J. Comp. Neurol. 35:483.

Horn, A. S., and S. H. Snyder, 1971. Chlorpromazine and dopamine: conformational similarities that correlate with the antischizophrenic activity of phenothiazine drugs. Proc. Nat. Acad. Sci. USA 68:2325-2328.

Hull, C. D., G. Bernardi, and N. A. Buchwald, 1970. Intracellular responses of caudate neurons to brain stem stimulation. Brain Res. 22:163-179.

Jacobs, M. S., P. J. Morgane, W. L. McFarland, 1971. The anatomy of the brain of the bottlenose dolphin (Turcitops Truncatus). Rhinic lobe (Rhinencephalon). I. The paleocortex. J. Comp. Neurol. 141:205-272.

Jenkins, R. B., and R. H. Groh, 1970. Mental symptoms in Parkinsonian patients treated with L-Dopa. Lancet 2:177.

Johnston, J.B., 1913. The morphology of the septum, hippocampus, and pallial commissures in reptiles and mammals. J. Comp. Neurol. 23:371.

Johnston, J. B., 1923. Further contributions to the study of the evolution of the forebrain. J. Comp. Neurol. 35:337.

Källén, B., 1956. Notes on the mode of formation of brain nuclei during ontogenesis. Comptes Rendus de L'association des Anatomistes 42:747-756.

Kemp, J., 1968. Observations on the caudate nucleus of the cat impregnated with the Golgi method. Brain Res. 11:467-470.

Kemp, J. M., and T. P. S. Powell, 1971. The structure of the caudate nucleus of the cat: light and electron microscopy. The termination of fibres from the cerebral cortex and thalamus upon dendritic spines in the caudate nucleus: a study with the Golgi method. Phil. Trans. R. Soc. Lond. B. 262:383-401, 429-439.

Koikegami, H., Y. Hirata, and J. Oguma, 1967. Studies on the paralimbic brain structures. I. Definition and elimination of the paralimbic brain structures and some experiments on the nucleus accumbens. Folia Psychiat. and Neurol. Jap. 21:151-180.

Kolodny, H. D., L. Sherman, and A. Singh, 1971. Acromegaly treated with chlorpromazine. N. Engl. J. Med. 284:819-822.

König, J. F. R., and R. A. Klippel, 1967. The Rat Brain. A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem. Krieger Pub. Co., Inc., Huntington, N. Y.

Leonard, C. M., and J. W. Scott, 1971. Origin and distribution of the amygdalofugal pathways in the rat. J. Comp. Neurol. 141: 313-330.

Lorens, S. A., J. P. Sorensen, and J. A. Harvey, 1970. Lesions in the nuclei accumbens septi of the rat: Behavioral and neurochemical effects. J. Comp. and Physiol. Psychol. 73:284-290.

Nauta, W. J. H., 1958. Hippocampal projections and related neural pathways to the midbrain in the cat. Brain 81:319-340.

Nauta, W. J. H., and S. O. E. Ebbesson, 1970. Contemporary Research Methods in Neuroanatomy. Springer-Verlag, New York.

Nauta, W. J. H., and W. Haymaker, 1969. Hypothalamic nuclei and fiber connections. In: The Hypothalamus, W. Haymaker, E. Anderson and W. J. H. Nauta (eds.) C. C. Thomas, Springfield.

Nauta, W. J. H. and W. R. Mehler, 1966. Projections of the lentiform nucleus in the monkey. Brain Res. 1:3-42.

Nybäck, H., and G. Sedvall, 1970. Further studies on the accumulation and disappearance of catecholamines formed from tyrosine-^{14}C in mouse brain, effect of some pharmacological analogues. Europ. J. Pharmacol. 10:193-205.

Nybäck, H., 1972. Effect of brain lesions and chlorpromazine on accumulation and disappearance of catecholamines formed in vivo from ^{14}C-tyrosine. Acta physiol. scand. 84:54-64.

Papez, J. W., 1932. The nucleus of the mammillary peduncle. Anat. Rec. 52:72.

Powell, E. W., 1963. Septal efferents revealed by axonal degeneration in the rat. Exper. Neurol. 8:406-422.

Raisman, G., W. M. Cowan, T. P. S. Powell, 1966. An experimental analysis of the efferent projection of the hippocampus. Brain 89:83-108.

Rioch, D. McK., and C. Brenner, 1938. Experiments on the corpus striatum and rhinencephalon. J. Comp. Neurol. 68:491-507

Rolls, E. T., 1971. Contrasting effects of hypothalamic and nucleus accumbens septi self-stimulation on brainstem single unit activity and cortical arousal. Brain Res. 31:275-285.

Romero, J. A., Chalmers, J. P., Cottman, K., Lytle, L. D. and Wurtman, R. J., 1972. Regional effects of L-dehydroxyphenylalanine (L-Dopa) on norepinephrine metabolism in rat brain. J. Pharmacol. Exptl. Therap. 180:277-285.

Romero, J. A., L. D. Lytle, L. A. Ordonez, and R. J. Wurtman, (in press). Effects of L-dopa administration on the concentrations of L-dopa, dopamine, and norepinephrine in various rat tissues. J. Pharmacol. Exptl. Therap.

Rose, M., 1929. Cytoarchitektonischer Atlas der Grosshirnrinde der Maus. J. Psychol. Neurol. (Lpz.) 40:1-51.

Scheibel, M. E. and A. B. Scheibel, 1967. Structural organization of non-specific thalamic nuclei and their projection toward cortex. Brain Res. 6:60-94.

Scott, J. W. and C. M. Leonard, 1971. The olfactory connections of the lateral hypothalamus in the rat, mouse and hamster. J. Comp. Neurol. 141:331-344.

Siegel, A. and J. P. Tassoni, 1971. Differential efferent projections of the lateral and medial septal nuclei to the hippocampus in the cat. Brain, Behavior, and Evolution 4:201.

Thoenen, H., R. A. Mueller, and J. Axelrod, 1969. Transsynaptic induction of adrenal tyrosine hydroxylase. J. Pharmacol. Exp. Ther. 169:249.

Ungerstedt, U., 1971. Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol. scand. Suppl. 367.

Valverde, F.,1965. Studies on the Piriform Lobe. Harvard University Press, Cambridge, Mass.

Von Voigtlander, P. F., and K. E. Moore, 1971. The release of H^3-dopamine from cat brain following electrical stimulation of the substantia nigra and caudate nucleus. Neuropharmacology 10:733-741.

Yaryura-Tobias, J.A., B. Diamond, and S. Merlis, 1970. The action of L-dopa on schizophrenic patients (a preliminary report). Curr. Ther. Res. 12:528.

York, D. H., 1972. Dopamine receptor blockade -- a central action of chlorpromazine on striatal neurons. Brain Res. 37:91.

Ziehen, T., 1904-1908. Das Zentralnervensystem der Monotremen and Marsupialier. Jena.