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THE ORGANIZATION OF THE CEREBRAL CORTEX OF THE POND TURTLE, *PSEUDEMYS SCRIPTA ELEGANS*

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The Organization of the Cerebral Cortex of the Pond Turtle,

Pseudemys scripta elegans

A thesis presented by

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ABSTRACT

The cerebral cortex of the turtle *Pseudemys scripta elegans* consists of five areas, L, D2, D1, DM and M, forming narrow strips curving in parallel around the hemisphere.

The cell types of the cortex were studied with the Golgi method in areas D1 and D2. The principle cell type possesses a soma in the cellular layer, spiny apical and basal dendrites, and a local axon aborization as well as long-projecting collaterals. Cells with few or no spines are rarer, have local axon arborizations only and vary greatly is morphology of dendritic tree and location of somas at least one specific subtype exists.

The connections of the cortex were analyzed with retrograde and anterograde tracers. The olfactory bulb projects to the superficial third of the molecular layer of area L, the thalamus to the superficial third of the molecular layer of areas D2 and D1, and to a deeper zone in areas DM and M. Nucleus lgn of the thalamus projects to the rostral part of area D2, nucleus na to the caudal part of area D2, and nucleus dla to areas D1, DM and M. Nucleus dma and scattered perirotundal cells project densely to the striatum and diffusely to the cortex. The cortical areas also receive a topographic projection from basal forebrain structures shown to contain cholinergic neurons by immunohistochemical methods.

Cortical areas L and D1 project to the superficial and middle third of the molecular layer of areas DM and M; these areas in turn project back to the deep third of the molecular layer of L and D1. Area D2 projects to D1. Each cortical area is interconnected with itself by a plexus of axons, which run in the middle third of the molecular layer in areas L, D1 and L and in the deep third in areas DM and M.

Some other afferent and efferent connections of the cortex are described, and a model of the homology of turtle and mammalian cortex is suggested.

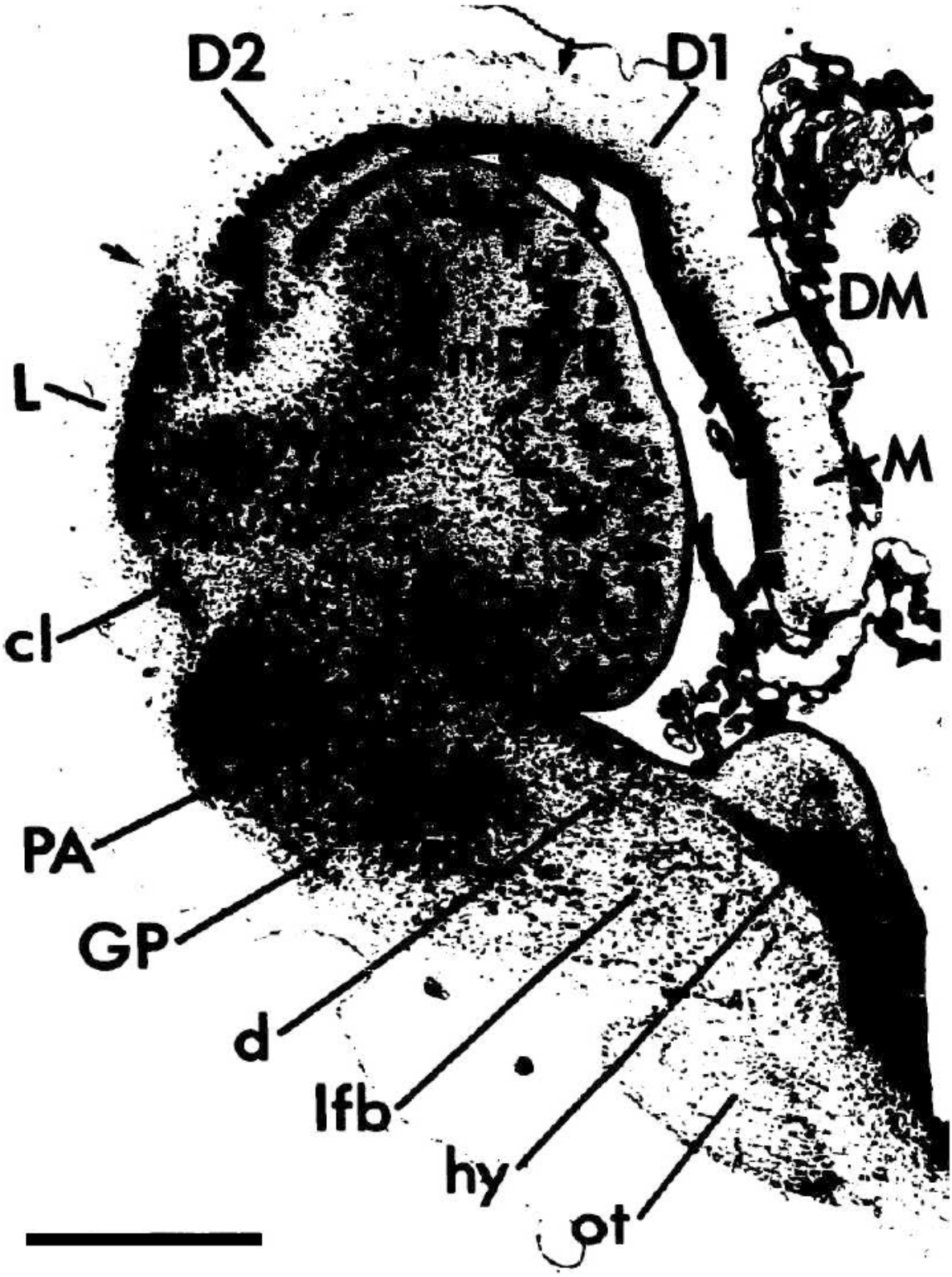
D2
D1
DM
L
M
cl
PA
GP
d
lfb
hy
ot

Figure 1. Major structures of the forebrain.

This is a coronal section, stained with cresyl violet, through the middle of the left cerebral hemisphere. The right border of the photograph is on the plane of the midline. Lateral is to the left, dorsal is up, ventral is down. Unless noted otherwise, all of the figures in the thesis are of the left hemisphere in this orientation.

Most of the large cell groups of the telencephalon are seen at this level.

The areas of the cerebral cortex form the surface of the dorsal telencephalon: from lateral to medial these are L, D2, D1, DM and M. Small arrows mark the boundaries between these areas.

The dorsal ventricular ridge bulges into the lateral ventricle and has been divided into dorsal (dDVR), medial (mDVR) and ventral (vDVR) sectors.

The basal teleacephalon at this level contains three areas which are parts of the striatum in turtles: the paleostriatum (PA), the globus pallidus (GP) and area d.

The central lateral nucleus (cl) is a. small nucleus between the cortex and the striatum.

The lateral forebrain bundle (1fb) is the major com- ponent of the cerebral peduncle. At this level it is seen crossing from the diecephalon to the teleacephalon. Medial to it is the cell plate of the hypothalamus (hy). The optic tract (ot) is also shown.

The scale bar is one millimeter long.

INTRODUCTION

This thesis is a study of the structure, afferents and efferents of the cerebral cortex of the turtle. Such a project is of interest for two reasons. First, the cortex of the turtle is one of the most primitive versions of the cortex that can be recognized. An analysis of the turtle cortex allows us to see the cortex near the beginning of its evolution. Second, the cortex of the turtle may be a useful model for the mammalian cortex. To understand mammalian cortex is to understand its synaptic connections. Understanding the synapses of all of the classes of cells in mammalian cortex seems an insuperable task. There are two reasons why the one layer cortex of the turtle might be more accessible.

First, the cerebral cortex of turtles is simpler than that of mammals. It contains five separate areas, far fewer than the most primitive mammal. The cortex consists of one main cell layer, like the mammalian hippocampus. If there are only a few types of neurons, perhaps the nature of all the synaptic connections can be determined to give a complete picture of the interactions required for a functional cortex.

Second, the turtle cortex seems ideal as an in vitro slice preparation for electrophysiological analysis of synaptic connections. The cortex is a sheet approximately 700 microns thick which is attached mainly at its lateral edge. Most of the cortex could be obtained as an intact slice by cutting along its edges. Furthermore, the turtle is very resistant to anoxia. It can survive one half to several days without oxygen, while other reptiles survive about one half hour (Belkin, 1963). Such survival probably reflects an ability to convert to anaerobic glycolysis: turtles poisoned with iodoacetate, an inhibitor of glycolysis, are healthy if breathing air but die in about one half hour if deprived of oxygen (Belkim, 1962). The retina, cochlea,. and olfactory bulb of the turtle have served successfully as *in vitro* preparations. Indeed, the cortical sheet is currently being studied as an *in vitro* slice by Connors, Kriegstein and Ransom (1983).

This thesis is an anatomical study of the cerebral cortex of the turtle. It consists of three broad chapters. The first describes the cytoarchitecture of the cortical areas, and the cell types present in the "neocortical" areas. The second chapter deals with the nature of the afferent inputs to the different areas, a topic with implications about the homology of these areas with mammalian cortical areas. The last deals with the interconnections of these areas, and their descending projections to the rest of the brain. This introduction summarizes previous work on each of these three areas in turtles and other reptiles.

A. STRUCTURE OF TURTLE CEREBRAL CORTEX

In the decades around the turn of the century neuroanatomists compared and described in exacting detail the brains of a wide variety of species. The forebrain of the turtle, like that of all reptiles, consists of pallial and basal sectors surrounding a lateral ventricle. Figure 1 illustrates these sectors. The pallium consists of the cerebral cortex, an arc of tissue which forms the roof of the hemisphere, and the dorsal ventricular ridge (DVR), a cell mass bulging from the lateral portion of the arc of cerebral cortex into the lateral ventricle. The DVR has no obvious analog in the brain of mammals. The largest component of the basal telencephalon is a heterogeneous cell mass, analogous in several ways to the mammalian striatum. The fiber tracts of the cerebral peduncle traverse this structure to each the pallium. Caudally this structure is replaced by a different cell group, roughly similar in topological location to the mammalian amygdala.

The cerebral cortex possesses generally similar subdivisions in all reptiles. The cortex is divided into medial, dorsal and lateral plates separated by discontinuities: the medial and lateral plates overlap the dorsal one medially and laterally (the medial superposition is more subtle in turtles than in other reptiles). The medial cortex can be divided into a dorsal and ventral portion, DM and M. The cells are tightly packed in Mv and loosely scattered in Md. The dorsal cortex possesses medial and lateral subdivisions, D1 and D2. The lateral cortex covers much of the lateral surface of the forebrain. The turtle cerebral cortex is one of the least differentiated among reptiles and its areas are similar in structure. Other species may have further subdivisions of the dorsal cortex.

The comparative neuroanatomy of vertebrates was the focus of intense interest in the decades about the turn of the century. The basic morphology of the reptilian forebrain was described in great detail by multiple authors (review: Ariens Kappers, Huber and Crosby, 1936). These workers especially hoped to establish homologies between brain structures in different species. Since no experimental means of determining connections were available, homologies were based on topological position within the brain and relationship to fiber tracts observed in silver-stained sections.

The cortical areas were of particular interest. The medial cortex was recognized by Spitzka (1880) as homologous to the hippocampus because it is the medialmost cortical area and because it is connected to the septum by a prominent tract resembling the mammalian fornix. This identification was accepted without question by every later worker. The lateral cortex was easily related to the pyriform, or olfactory, cortex of mammals by its lateral location and by the fibers of the olfactory tract spread over its surface. The dorsal cortex was more difficult. It was variously considered part of the hippocampus, part of the olfactory cortex, a striatal structure, a neocortex, a primordial neocortex, or something unrelated to any mammalian structure; some of these arguments are reviewed in Ariens Rappers, Huber and Crosby

(1936). (In retrospect, the confusion about the dorsal cortex was due in part to a failure of the silver stain method. Fibers from the thalamus could be traced to the striatum and DVR but only a few were seen entering dorsal cortex: in fact, these fibers do continue into dorsal cortex but presumably are not stained because they immediately form fine preterminal branches).

There have been only a few studies of the reptilian cerebral cortex with the Golgi method. These studies agree on several basic findings. The neurons of the cellular layer, the principal cells of the cortex, have dendritic trees which are densely spiny and ramify in the molecular and subcellular layers. The sparse cells of the molecular or subcellular layers may have either spiny and spineless dendrites.

Perhaps the most complete examination was by Pedro Ramon, with the assistance of his brother, Santiago Ramon y Cajal (Ramon, 1896, 1916, 1917; Ramon y Cajal, 1911; the papers of Pedro Ramon were kindly translated from the original Spanish by Silvio Glusman). In the medial cortex he described a range of principal cell types. Part of the variability was related to cell location in the cellular layer: more superfidial cells had apical arborizations which were better developed than their basilar arborizations, and deeper cells had relatively more extensive basilar arborizations. Cells located superficially in the cellular layer or just above it had an inverted triangular shape and had well developed apical dendrites, but no basilar dendrites or else a thin process which descended through the cellular layer and then divided into a few branches. Cells located more deeply had more often a pyramidal or pyriform shape, and emitted one apical and one basal process which reached the borders of the cellular layer and divided into profuse apical and basilar dendritic trees. Some cells had a more fusiform shape, others a very small round shape. All of these cells had a similar axon, which issued from the bottom of the cell body and descended directly to the ventricular surface, where it divided into two branches. The medial branch proceeded towards the septum, gave rise to infrequent collaterals ascending into the cortex and in some cases a branch into the anterior commissure, and finally ended in a widespread arborization in the septum. The lateral branch proceeded along the ventricular surface and ended in an arborization in dorsal or lateral cortex. The descending part of the axon gave rise to multiple axon collaterale which formed arborizations in the subeellular and molecular layers.

The principal cells of the dorsomedial and dorsal cortex are larger and more often pyramidal than those of the medial area. They have several protrusions from which the apical and basal dendrites issue, unlike the more strictly fusiform medial cortex cells. Their axon descends directly to the ventricular surface and proceeds medially; at least Ramon does not describe a lateral branch. The axon also gives off collaterals which ramify within the cortical area of origin.

In the molecular layer of the medial cortex Ramon described three types of cells. The first was a small stellate cell with straight radiate dendrites and a very short, varicose axon. The second had a round or elongate cell body, smooth dendrites confined to the molecular layer and an axon also confined to that layer. These cells reminded him of Cajal-Retzius cells. The third type appeared to be a typical principal cell displaced into the molecular layer.

In the subcellular layer he found small stellate cells as described above and principal cells displaced from the cellular layer. In addition he described two cell types. The first was very large and erected one or several apical dendrites into the molecular layer and one or two basal dendrites ramifying in the deepest parts of the subcellular layer. The second was very distinctive: the cell body was horizontally elongate and bore dendrites with extraordinary long, even bifurcating spines. The axon ascended to the molecular layer arid formed descending branches which ended in the cellular layer. This cell reminded him of a cell type in the mammalian fascia dentata. Cajal (1911) likened these to Martinotti cells.

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No author has described the axonal arborizations of reptile cortex in such richness as Ramon. There are three specific studies of the medial cortex. In the snake Ulinski (1977) described principal cells, "candelabra cells", which seem somewhat more homogeneous but generally similar to Ramon's description of principal cells in the lizard. These cells had axons which descended to the ventricle and bifurcated, but did not have recurrent branches which arborized in the cortex as Ramon described. Ulinski also described stellate cells with smooth dendrites in the molelcular layer and periventricular cells with spiny dendrites in the subeellular layer not unlike those seen by Ramon. In different species of lizards, Lacey (1978) and Wouterlood (1981) described a range of principal cell types which appear similar to the cells in Ramon's description, as well as smooth stellate cells in the molecular layer and spiny periventricular cells in the subeellular layer.

Several other studies offer comparisons of the different cortical areas. Ebbesson and Voneida (1969) describe systematic differences in the dendritic arborizations of cells, mainly principal cells, in the different cortical areas using

the Golgi-Cox method. Some cells seem to lack spines, which could be an artifact of this method. Axonal arborizations are not well impregnated with the Golgi-Cox method.

A study by Balaban (1977) described some cells of the lateralmost part of dorsal cortex, where it bends into the DVR. The cells of the cellular layer have a variety of soma shapes but dendritic trees which extend radially into the molecular and subcellular layers, thus resembling the usual reptilian principal cell. Cells with stellate dendritic arborizations occur in all layers, but mainly in the molecular layer. The dendrites of these cells may have no spines, sparse spines or long thin appendages. Axons of these cells were not studied.

In summary, the classification of cells in the reptile cortex with the Golgi method has not been completed. Whether there are specific classes of principal cell types or rather a continuum of possible morphologies is unclear. The arborizations of principal cells within the cortex have not been described in detail, except for Ramon"s work on the medial wall neurons in lizard. While cells of very different morphologies have been seen outside the principal cell layer, the systematic comparison necessary to classify them has not been done. The axonal arborizations of such cells, again apart from the cells studied by Ramon on the medial wall, are basically undescribed. In fact, most of these studies were done in lizard and the organization of the relatively undifferentiated cortex of the turtle is unknown.

A first goal of the thesis was to classify and describe the cell types of the turtle cortex, particularly the dorsal cortex, the presumed homolog of the mammalian neocortex.

B. INPUTS TO THE CORTEX IN TURTLE

The first experimental attempt to trace projections into the forebrain in reptiles was made by Goldby in 1937 using the Marchi method. He lesioned the olfactory bulb of the lizard *Lacerta viridis*, and traced disintegrating myelin sheaths to the anterior olfactory nucleus and amygdala. He was unsure whether any axons ended in the lateral cortex. Gamble (1952) studied degenerating fibers in the olfactory system of that species with a non-suppressive silver stain. Within the pallium, only the rostral one-half to two-thirds of the ipsilateral lateral cortex contained degenerating axons. He reached a similar conclusion in the turtle Testudo graeca, except that projections were bilateral (Gamble, 1956).

Studies with more recent suppressive silver stains agree that the olfactory bulb projects bilaterally to the anterior extent of lateral cortex. Some find that the projection extends to caudal lateral cortex. In the lizard *Tupinambis teguixin*, Heimer (1969) did find a projection to caudal lateral cortex; in the lizard *Dipsosaurus dorsalis*, Ulinski and Peterson (1981) did not. In the alligator *Caiman sklerops*, Scalfa, Halpern and Riss (1969) found bilateral projections to the entire rostrocaudal extent of lateral cortex. In snakes, Ulinski and Rainy (1980) and Halpern (1974) described projections to rostral lateral cortex on both sides of the brain; Kovell (1974) described projections to the full thickness of the molecular layer in rostral and part of caudal lateral cortex, which is an unprecedented observation.

No study of olfactory system in turtles with modern methods exists. An early experiment of this thesis was to identify the projections of the olfactory system in the turtle.

Reptiles have a large and obvious thalamus. It consists of a central ovoid nucleus, the nucleus rotundus, surrounded by cell groups whose number and differentiation vary among the reptiles. The turtle thalamus is relatively undifferentiated. It consists of a large, cell sparse nucleus rotundus, surrounded by a thin, cell dense shell which can be divided into several more or less distinct cell groups. The medial wall of the shell has been termed the nucleus dorsomedialis anterior or dma (Papez, 1935). Rostrally, the dorsolateral wall is the nucleus dorsolateralis anterior or dla, and the ventrolateral wall the nucleus anterior. More caudally the ventral part of the lateral wall is termed the lateral geniculate nucleus or lgn. At these levels the shell is opened ventrally: the ventral edge of the lateral wall swings laterally so that the lgn lies flat against the optic tract. The ventromedial part of the shell becomes the nucleus reuniens. The nucleus caudalis is located along the midline behind the nucleus rotundus.

Initial attempts to detect a thalamic input to the cortex were unsuccessful. Powell and Kruger (1960) used the retrograde cell degeneration method to study the projection of the thalamus on the cortex in the lizard *Laceria viridis*. Following near total removal of the forebrain hemisphere only two thalamic nuclei degenerated, the nucleus rotundus and the nucleus dcrsomedialis anterior. The rest of the thalamus was unaffected. Removal of the entire cortex and DVR led to no retrograde cell loss in the thalamus. However, lesion of the lateral part of the striatum gave complete loss of cells in the aforementioned thalamic nuclei. These authors concluded that the thalamus does not project to the dorsal pallium at all. Kruger and Berkowitz (1960) observed similar results in the alligator *Alligator mississippiensis.*

However, the latter paper also provided evidence that sensory information does reach the cerebral cortex. Evoked potentials in the cortex were mapped after electrical stimulation of the olfactory bulb or peripheral nerves and after photic stimulation of the eye. After shock of the olfactory bulb potentials were observed over the lateral surface of the hemisphere, roughly corresponding to the lateral cortex. After shock of any of several different peripheral nerves,

potentials were obtained over the dorsal surface of the hemisphere, approximately over the dorsal cortex. Both of these stimuli generated smaller, longer latency potentials on the medial wall. After light flashes delivered to the contralateral eye, potentials were recorded in the dorsal cortex, perhaps in a subset of the region activated by stimulation by peripheral nerves. These results confirm that olfactory fibers innervate the lateral cortex and that visual and somatosensory information is conveyed directly or indirectly to the dorsal cortex. Moreover, there appears to be a zone of weak, overlapping olfactory and somatosensory input on the medial wall.

Similar experiments in the turtle *Pseudemys scripta elegans* by Orrego (1961) suggested that visual and somatosensory input might be further segregated in dorsal cortex. Following olfactory stimulation he found activity in the lateral cortex and posterior part of dorsal cortex. Following visual stimulation he found responses in the anteriormost part of the dorsal surface. Stimulation of the dorsal columns of the spinal cord elicited responses in approximately a middle region of the dorsal surface.

Hall and Ebner (1970) were the first to trace sensory pathways to the forebrain in reptiles. Following lesions of the optic tectum, degenerating fibers were stained with the Fink-Heimer method into the nucleus rotundus. Following eye enucleation, degeneration was present over the lateral geniculate nucleus.

Following large lesions of the thalamus, degenerating fibers could be traced through the lateral forebrain bundle and into extensive areas of the striatum and the DVR. Furthermore, some axons passed around the lateral edge of the dorsal cortex and ended in terminals in the outer half of the molecular layer of the dorsal cortex. Following a lesion mainly restricted to the nucleus rotundus, dense degeneration was seen in the DVR but no degeneration was seen in the dorsal cortex. A small lesion dorsally located in the perirotundal shell of nuclei, between the dla and lgn, yielded degenerating fibers in a zone within medial dorsal cortex. A lesion limited to a more ventral part of the lateral perirotundal shell, in the lgn, produced degeneration in a more lateral zone of dorsal cortex. While a complete map of thalamic projections could not be determined from their experiments, the thalamus apparently projects with some organization upon the cortex.

The thalamocortical projection in turtle has been subsequently confirmed and analyzed with electron microscopy of degenerating terminals (Smith, Ebner and Colonnier, 1980). Thalamocortical terminals are found mainly on spines but also on smooth dendrites. Since the spines probably belong to the dendrites of the principal cells, most terminals seem to end on principal cells but many end on cells with smooth dendrites. Indeed, because principal cells must vastly outnumber any other cell type, an individual smooth dendrite cell might receive many more terminals than a principal cell.

Additional thalamic projections to the turtle forebrain have been described by Balaban (1981). First, using anterograde degeneration and retrograde transport methods, Balaban (1981) showed that three thalamic nuclei project to the DVR. The anterior DVR consists of three cytoarchitectonically distinct sectors. The nucleus rotundus projects to the dorsal sector, as noted by Hall and Ebner (1970). The nucleus reuniens projects to a ventral sector, and the nucleus caudalis projects to a medial sector; work described below in other species of reptile indicates that these two nuclei are auditory and somatosensory relay nuclei, respectively. Thus, the DVR receives ascending projections from three distinct thalamic nuclei.

Second, an additional thalamic nucleus projects to widespread areas of the forebrain. Large lesions of the thalamus produce two systems of degenerating fibers. A set of large caliber axons runs in the lateral forebrain bundle and corresponds to the system described by Hall and Ebner (1969). A set of fine caliber axons run in both the lateral and medial forebrain bundles. The small caliber system of axons is labelled by small lesions or tritiated proline injections of the nucleus dma of the thalamus. An injection of HRP in any of the three sectors of the DVR or in the underlying striatum labelled a few cells in the nucleus. The nucleus dla seemed a part of this system. Such a projection might correspond to the intralaminar system in mammals, which projects diffusely to the cortex and densely to the striatum (Jones and Leavitt, 1974).

Concurrent experiments on the thalamic projections to the forebrain in alligator and lizard indicates that the pattern of afferents to the DVR in alligator and lizard is similar to that in turtle. The thalamic projection to the cortex is somewhat different.

Pritz (1975) demonstrated a projection of the nucleus rotundus in crocodilians to the DVR analogous to that in turtle. Both Foster and Peele (1975) and Pritz (1974) have described a projection from the nucleus reuniens to the ventral DVR, in lizards and crocodilians, respectively. The nucleus reuniens receives an input from the torus semicircularis, an equivalent of the inferior colliculus, and presumably relays auditory information (Foster, 1974; Pritz, 1974a). The nucleus caudalis (or its equivalent) projects to medial DVR in crocodilians; this nucleus receives spinal input (Northcutt and Pritz, 1978). Thus, crocodilians and lizards, like turtles, have three channels from the thalamus to the DVR; in all of these orders these channels carry visual, auditory and somatosensory information.

Distel and Ebbesson (1975) made stereotaxic lesions in the thalamus of the Monitor lizard *Varanus benegalensis* and analyzed the consequent degeneration in the forebrain with Nauta and Fink-Heimer techniques. As might be expected, the nucleus rotundus projects to a lateral part of the DVR and the nucleus reuniens to a more medial part. Only the nucleus dla, and not the lgn, projected to the dorsal cortex.

Lohman and van Woerden-Verkley (1978) made electrolytic lesions of the thalamus as well as small HRP injections in the DVR and cortex. The nucleus rotundus projects to a distinct nucleus in the DVR. The nucleus reuniens projects to a mediorostral area of the DVR. Although lesion of the lgn produced no degenerating axons in the forebrain, the caudal part of the lgn was labelled by HRP injections in the rostrolateral DVR. Only the nucleus dla projected to cortex: after injections in the dorsal cortex labelled cells were found mainly laterally in the dla and after injections in medial cortex mainly medially in the dla. Both of these connections were bilateral. All of the HRP injections in the DVR or cortex produced scattered cell labelling in the nucleus dma, implying that it projects diffusely as in turtle. Lesions of the thalamus produce degeneration in similar patterns in the forebrain as seen in turtles, except that degeneration in the medial cortex is confined to a narrow lamina in the molecular layer rather than filling the depth of the cortex.

A magnificent study of afferents to the forebrain in two lizards, *Gecko gecko* and *Iguana iguana*, has recently been completed by Bruce (1982). Small deposits of wheat germ agglutinin-horseradish peroxidase conjugate dried onto the tip of a pin were made throughout the cortex and DVR. The DVR receives projections from three thalamic nuclei such as described above. The "pallial thickening", the region between the cortex and the DVR receives input from the lgn. A11 four of these areas receive a projection from the nucleus dma. The dorsal cortex receives only a projection from the more medial division of the dla and the medial cortex from the more lateral division. Each of these nuclei projects bilaterally to its cortical target.

In summary, the reptile thalamus does project to both the cortex and DVR. The nucleus rotundus, nucleus reuniens and nucleus caudalis seem to receive tectal, auditory and somatosensory information and project to specific zones of the DVR. In turtles the lgn does project to cortex along with other parts of the perirotundal shell, although the organization of this projection is unknown. In lizards the lgn does not project to cortex but to the DVR, and two divisions of the dla project to the dorsal and medial cortex. The nucleus dma appears to project diffusely in both reptiles.

Analogous experiments on the bird brain have found a similar set of sensory pathways through the thalamus. The optic tectum projects to the nucleus rotundas in the thalamus (Karten and Revzin, 1966), and this nucleus projects to the core region of the ectostriatum in the forebrain (Revzin and Karten, 1966/7; Karten and Hodos, 1970). The apparent homolog of the mammalian inferior colliculus projects to the nucleus ovoidalis in the thalamus (Karten, 1967), which projects to Field L in the forebrain (Karten, 1968). The ectostriatum and Field L are components of the greatly thickened dorsolateral wall of the bird telencephalon, a region probably equivalent to the dorsal ventricular ridge in reptiles. The retina projects to a set of thalamic nuclei, together termed the nucleus opticus principalis thalamus, which project to the Wulst, the dorsalmost structure of the forebrain (Karten and Nauta, 1968; Karten, Hodos, Nauta and Revzin, 1973). The Wulst is not separated from the underlying "dorsal ventricular ridge" by any ventricular space, and might be considered equivalent to either cortex or dorsal ventricular ridge in reptiles. In some birds it is elaborately laminated. These results led to the suggestion that these various areas of the forebrain of birds and reptiles are in some way homologous to the mammalian cortex, particularly its sensory areas (Nauta and Karten, 1970).

In mammals, the hippocampus is the major target of axons from medial septum. Reptiles possess an area which might be considered on topological grounds homologous to the septum. An area of reptilian cortex receiving projections from the septum might be considered homologous to the mammalian hippocampus. The projection of the septum on the cortex in reptiles is unclear. Hoogland, ten Donkelaar and Cruce (1978) lesioned the septum in the lizard *Tupinambis nigropunctatus*. Degenerating axons were present in the intermediate layer of the medial cortex and in a superficial layer in medial dorsal cortex. However, these axons resemble the pattern of thalamic afferents, and perhaps reflect interruption of thalamocortical afferents passing up the medial wall. These authors did find labelled cells in the lateral septum after HRP injections in dorsal cortex. (The results of lesioning the septum thus suggest that septal afferents terminate in the same layers as thalamic afferents). Reperant (1976) also noted labelled cells in the septum after HRP injections in dorsal cortex in snakes. The recent study by Bruce (1982) is the most complete analysis. Labelled cells were present in the septum after WGA-HRP injections in the ventral division of the medial cortex. Labelled cells were present only after very large injections of dorsal cortex: she interpreted such labelling as resulting from spread of the tracer into medial cortex.

A second chapter of the thesis deals with the afferents to the cerebral cortex in the turtle. An important aim was to determine the organization of the projection of the thalamus upon the cortex in turtles, especially the projection of the lateral geniculate nucleus which is seemingly different from that in other reptiles. A second aim was to examine the projection of the septum, which might identify an area homologous to the mammalian hippocampus.

C. PROJECTIONS OF THE CORTICAL AREAS

An intriguing pattern of intercortical connections is indicated by experiments using anterograde degeneration methods in lizards and snakes. Lohman and Mentiek (1972) made small lesions in the cortex of the lizard, *Tupinambis teguixin*. After a lesion in lateral cortex degenerating axons stream medially just under the pia to end in the superficial lamina of the molecular layer of the medial cortex. A lesion in dorsal cortex interrupts these axons and produces additional degenerating axons which end in a second lamina just below the axons from lateral cortex. A lesion in the dorsal, magnocellular, part of medial cortex transects all these connections and also yields degenerating fibers in the lowest lamina of the molecular layer and the upper lamina of the subcellular layer of the ventral, parvocellular, part of the medial cortex on both sides of the brain. These results suggest that the lateral, dorsal and large-celled part of the medial cortex project to successively deeper parts of the neuropil of the small-celled part of the medial cortex. After lesions in either the small- or large-celled medial cortex degenerating axons are present in the deeper two thirds the cortex. This suggests that medial cortex in turn projects back to the dorsal cortex, but not to the lateral cortex.

Ulinski (1975) performed analogous experiments in the snakes *Natrix sipedon* and *Thamnophis sirtalis*. The convergence of afferents from the lateral, dorsal and dorsomedial areas onto the medial cortex is similarly laminated. Dorsomedial cortex projects to contralateral medial and dorsomedial cortex, as in the Tegu lizard. The medial cortex projects not only to the deep portions of dorsomedial and dorsal cortex, but also to the lateral cortex, a projection not seen by Lohman and Mentink (1972). Voneida and Ebbesson (1969) noted essentially these contralateral projections after large ablations of one hemisphere in the Tegu lizard. However, Butler (1976) detected additional commissural projections in Gecko. Large lesions of dorsal wall cortex result in degeneration in contralateral dorsal cortex as well as medial and dorsomedial cortex. Lesions of lateral cortex result in degeneration in contralateral lateral cortex. In the study by Bruce (1983) using transport of WGA-HRP, injections in each sector of lizard cortex labelled cells in the homotopic contralateral area.

Experiments relying on anterograde degeneration methods also detect organized projections from the cortex to the septum. Lohman and Mentink (1972) and Lohman and van Woerden-Verkley (1976) illustrate projections to the septum from medial and dorsal cortex in the Tegu lizard. The terminal fields of the two areas are discrete and non-overlapping. Ulinski (1975) found in snake that medial and dorsal cortex each project to a long column in the septum. The medial cortex projects to a dorsal region running the length of the precommissural septum. The dorsal cortex projects to an underlying region in the precommissural septum, which shifts dorsally in the postcommissural septum. Halpern (1974) confirmed this pattern. (Other authors have illustrated projections to the septum without analysis: Butler, 1975; Bruce, 1983).

Some axons continue in the fornix system. Lohman and Mentink (1972) and Lehman and van Woerden-Verkley (1976) describe two paths. The precommissural fornix runs forward, down through the septum and caudally into the lateral hypothalamus. The postcommissural fornix drop behind the commissure and enters the periventricular nucleus of the hypothalamus, a cell group at the medial and dorsal limit of the hypothalamus, adjacent to the thalamus. The medial cortex, rostral and caudal, contributes to the precommissural fornix. Rostral dorsal cortex contributes to the postcommissuial fornix, and caudal dorsal cortex to the precommissural fornix. Intervening regions of dorsal cortex send axons into both paths. Unfortunately, the terminal arborizations of these axons did not stain adequately and could not be determined.

A study by Hall, Foster, Ebner and Hall (1977) using anterograde degeneration and anterograde transport of proline reported much more extensive projections of the cortex. Fibers leaving the cortex travel either medially into the fornix system or laterally around the hemisphere into the lateral forebrain bundle. Descending fibers travel lateral to the hypothalamic cell plate. Some turn dorsally to enter the thalamus. After large lesions of cortex, the lgn, dla, dma and the nucleus rotundus contain degenerating axons. Afferents to the 1gn terminate over the cell plate, immediately below the zone in which retinal afferents terminate The remaining axons continue caudally into the tegmentum, where some terminate and others run dorsally into the tectum. With very long survival times, a projection from the cortex to the tectum has been demonstrated with anterograde degeneration in the lizard *Agama agama* (Elprana, Wouterlood and Alones, 1980).

This thesis attempts a basic characterization of the structure and connections of the cerebral cortex of the turtle. The first chapter is a study of the cytoarchitecture of the cortex, as seen in Nissl-stained sections and in material impregnated with the Golgi method. An atlas of the remainder of the forebrain is given to allow description of the connections of the cortex with the subcortical hemisphere. The second chapter is a study of the afferent connections of the cortex, using both anterograde and retrograde tracing methods. The basal forebrain proved to be one source of afferents and was examined with immunohistochemical methods for the localization of cholinergic cells to identify cell groups which might be homologous with mammalian cholinergic cell groups. The third chapter describes the interconnections and descending projections of the cortical areas. Perhaps the single simplest conclusion is the great similarity in the organization of the forebrains of turtles and mammals.

METHODS

The subject of these investigations was the red-eared pond slider, *Pseudemys scripta elegans*. This species is plentiful in the ponds and swamps of the American South, and is available readily and inexpensively. Juveniles were once sold in great numbers as pets for children. The specimens used in these experiments were adult turtles measuring approximately 17 cm in carapice length. They were purchased from Kone Scientific Company (Germantown, WI). The turtles were kept in a large tank of slowly changing, filtered water at room temperature. Animals used promptly did not require feeding, animals used in experiments with long survival times were fed worms and lettuce.

Most of the experimental procedures in the thesis were adapted from those used for mammals, and are described in the section immediately below. However, the use of methods for tracing connections based on axonal transport gave results differing from those seen in mammals. These differences and their implications are discussed in a second section.

1. SURGICAL AND HISTOLOGICAL PROCEDURES

Turtles were anesthetized for surgery with halothane. The jaws of the turtle were held open with a small surgical retractor. The opening of the trachea is visible in the middle of the tongue and is easily cannulated with a piece of polyethylene tubing. The turtle was respired with moist air with a small animal respirator (Harvard Apparatus, model 665) at a stroke volume of 10 cc and a frequency of 8 strokes per minute. The air to the pump was passed through a halothane vaporizer (Fluotec, Fraser-Sweatman, Buffalo, NY), set for a 4% concentration of halothane for inducing anesthesia, and for a 1-2% concentration for maintaining anesthesia. These figures may not be accurate at the low flow rates used. During recovery the animal was respired with room air.

For surgery the head of the turtle was held in an improvised stereotaxic instrument. The head was secured by two ear bars of a diameter and taper appropriate to the shallow external ears of the turtle, a bar underneath the upper jaw and a bar across the top of the snout.

Methods for surgery were largely conventional. The skin was incised medial, anterior and posterior to the desired site of craniotomy and carefully separated from the underlying bone, reflected laterally and kept moist. The bone was opened with a Dremeltool and dental bit. The dura was torn with forceps. After completion of the operation the opening was plugged with gelfoam and the skin flap glued back in place with cyanoacrylate adhesive (Quick Set 404, Loctite).

Injections of anatomical tracer substances were made with conventional glass micropipettes, broken off at a tip diameter of 10-30 microns. The pipette was held in a micromanipulator and inserted into the brain with reference to recognizable landmarks. The depth of insertion was gauged with respect to a mark made on the side of the pipette. Thin structures, such as the cortex, were difficult to inject precisely and were injected obliquely. The injections were made in small aliquots separated by 1-2 minutes. After the injection the pipette was left in place several minutes. After removing the pipette the surface of the brain was rinsed with saline, which seemed to be very helpful for reducing external spread of the injection.

For perfusion the turtle was anesthetized with 50-100 mg sodium pentobarbital (Nembutal, Abbott). The bottom half of the shell was clipped free of its bony attachment along its lateral margins with bone cutters, and dissected away. The pericardium was cut open and two sutures passed around the aorta. The ventricle of the heart was cut and a stubby Pasteur pipette passed through it and into the aorta. The aorta divides immediately into three branches: the leftmost, as seen in this procedure, gives rise to the carotid arteries. The cannula was inserted into this branch of the aorta and secured with the sutures. The left strum (as exposed here) was slit for the efflux of perfusate.

The circulation was first rinsed with approximately 100 cc of 0.1 M phosphate buffer. The fixatives used were either 4% paraformalãehyde or 2% glutaraldehyde in 0.1 M phosphate buffer. A volume of 1-1.5 1 of fixative was required for satisfactory fixation. The second half of that volume was run through at a slow rate.

Because the turtle brain consists of several small and delicate parts, it must be embedded before cutting. For frozen sections, used in most of the experiments, the brain was sunk in 30% sucrose in fixative or buffer, and the membranes adherent to the surface of the brain as well as the choroid ependyma in the ventricles removed with fine forceps under the operating microscope. The brain was embedded with a rapid method. Four ml of a solution of 30% albumen, 0.5% gelatin, and 25% sucrose in 0.1 M phosphate buffer is rapidly mixed with 0.2 cc 50% glutaraldehyde in an embedding mold, and the brain added. The mixture hardened within an hour. The block could then be cut on a freezing microtome in the usual fashion.

In some experiments other methods of embedding were used. For the analysis of cytoarchitectonics, some brains were embedded in celloidin and cut on a sliding microtome. For clearer autoradiography, some brains were embedded in paraffin and cut at 10 microns on a rotary microtome. Protocols for dehydrating and infiltrating with celloidin and

paraffin were copied from protocols for rodent brain. For immunohistochemical experiments brains were cut unembedded to avoid additional exposure to fixative.

Brains of 82 turtles were processed according to different variants of the Golgi method. There are three stages to most such procedures. The first stage is optional and consists of fixation of the brain by perfusion, perhaps with the addition of some mordant to improve staining. The second stage is impregnation in a solution of fixative and potassium dichromate, again with the possible addition of mordants. The final stage is development in dilute silver nitrate. The fixative and mordants used, the times for each stage can be varied endlessly. Furthermore, some brains were embedded in agar or egg yolk in efforts to reduce the accumulation of artifact on the brain surface. Forty two brains were reacted with the Rapid Golgi method, the original method of Cajal and Golgi with the use of osmium tetroxide (Scheibel and Scheibel, 1978): most of these brains were perfused with 4% paraformaldehyde as this increased the quality of impregnation. Nineteen brains were processed with the Colonnier method, a Golgi-Kopsch variant using glutaraldehyde as fixative during impregnation (Colonnier, 1964): these brains were not perfused. Fourteen brains were processed with the Adams protocol, a glutaraldehyde Golgi-Kopsch method in which the brain is perfused with the impregnating solution (Adams, 1979). Nine brains were reacted with the Golgi-Cox method, which uses a mercuric chloride-dichromate impregnating solution (Ramon-Moliner, 1970).

After staining, the brains uere embedded in epon and sectioned according to the protocol of Nevin, Tanaka and Cruce (1978).

2. TRANSPORT METHODS IN TURTLE

The injection of anatomical tracers yielded different results from those which might be expected in mammals. These differences generated both problems and opportunities.

The first difference was that the injected tracer, either HRP or a radioactive amino acid, seemed to spread extensively and thus form a large diffuse injection site. Thus, a small injection of HRP might yield an injection site covering a good portion of the dorsal forebrain and lightly labelled cells, presumably labelled by diffusion, in much of the rest of the forebrain. In such a case it was difficult to distinguish any cells in the forebrain labelled by specific transport of HRP. Moreover, all of the thalamus was labelled, certainly by specific transport: it would be hard to analyze any topographic projection in this way. Very Small injections of HRP gave more reasonable injection sites but failed to label cells reliably elsewhere in the brain. The problem was not to obtain small injections but rath small injections which would yield good transport. Precise injection sites important because the various nuclei of the turtle brain are small. Small sites are crucial also because almost every region is adjacent to a ventricle, and any injection site encroaching on the ventricle labels periventricular structures at great distances. Several techniques were useful in reducing the non-specific labelling.

The first method was to add approximately 2% lysolecithin to the injected HRP solution (Kennedy, Harris and Frank, 1978; ll-HRP). While this increases the injection size considerably it increases the retrograde transport of the HRP even more. In general the smallest volume of HRP which could be injected sufficed, less than 5 nanoliters.

The second method employed HRP coupled to wheat germ agglutinin (HRP Type VI coupled to wheatgerm agglutinin,Sigma; WGA-HRP) at a concentration of about 2.5 %. This tracer did not appear to diffuse as much as ordinary HRP. The most compact injection sites were obtained by electrophoretic injections of 5% WGA-HRP (reagent and protocol from Russ Carey, Harrow Neurological Institute, Phoenix, AZ).

Finally, iontophoresis of HRP provided much smaller injection sites than pressure injection. However, the method was highly variable and usually yielded many fewer labelled cells than either 11-HRP or WGA-HRP methods. Many of the retrogradely labelled cells were bulk filled, with partial or complete filling of the dendritic tree.

The anterograde tracer tritiated proline also diffused over large areas, even injected in small volumes. Iontophoretic delivery was not much better. No method for restricting this spread was found. Consequently, tritiated proline could only be used to trace projections between distant parts of the brain.

The second striking difference between turtles and mammals was that HRP, particularly in conjunction with the lysolecithin, filled axons for considerable distances. Much of this filling seemed to be in the anterograde direction. For example, injections in the dorsal cortex labelled its axonal projection to the medial cortex, a projection which was confirmed with the anterograde transport of proline: however, only a very few of the axons to the dorsal cortex from the medial wall were labelled, and none of the collaterals of these axons in the medial cortex were labelled. Moreover, the labelling of fibers of passage occurred only weakly. A powerful projection from the lateral cortex passes through the injection site just described, *yet* few of its axons fi11 in either the anterograde or retrograde direction. If fiber of passage labelling is minimal, then this method can be used as a means of tracing anterograde projections.

Such a method requires that axons forming terminal arborizations can be distinguished from axons en route to their synaptic targets. In general, two patterns of labelling were seen in sections reacted with the DAB chromogen, which shows labelled fibers in fine detail. Regions containing fine axons with multiple varicosities, tortuous courses and collateral branches seemed to be typical of terminal regions. Single dots of reaction product were often observed in such regions, as if the enzyme were concentrated in terminal boutons and varicosities. On the other hand, regions containing thicker fibers which ran straight continuous courses were considered axons en route. Such axons usually bore at least some varicosities, even in regions without apparent neuropil such as the anterior commissure. Perhaps these varicosities are artifactual dilatations, generated during fixation. Consequently, in certain cases it was impossible to determine whether some axons with straight courses and multiple varicosities were making synaptic contacts. These distinctions could not be made in any case on sections reacted with the chromogen TMB, which yields a reaction product of large granules obscuring fine details.

Injections or iontophoresis of WGA-HRP did not yield much bulk filling of axons, but gave a dust-like label over the terminal regions of the axons of cells near the injection or iontophoresis site. Certain batches of WGA-HRP gave terminal labelling with almost no labelling of the intervening axon, while other batches were less specific. As with 11-HRP, there did not seem to be as much labelling of fibers of passage and of retrograde collaterals as of labelling of axons of cells in the injection zone.

Iontophoretic injections of HRP, on the other hand, did not show such selectivity. Retrogradely filled axons were prominent and could sometimes be followed to a bulk-filled neuron. Anterogradely filled axons were generally fewer in number than after 11-HRP injections, but each individual fiber was more densely labelled.

The use of the anterograde transport of any of these reagents to trace neural connections requires reliable filling of axonal projections. For example, small injections or injections with short survival times do not entirely fill known axonal projections. A particular connection cannot be assayed in an experimental case unless transport of the anterograde label in that case is adequately vigorous to label the connection. Thus, all the cases analyzed for the study of connections within the forebrain contained axons labelled as far as the tegmentum. Usually, even long connections seemed to fill readily. However, there may be axonal projections, perhaps distinctive by size or myelination, which do not fill well and which were missed in the present study.

In summary, the transport of HRP or HRP conjugates identifies both anterograde and retrograde connections in the turtle.

In these experiments, alternate sections were reacted with the chromogens DAB and TMB. The TMB method was more sensitive for detecting retrogradely labelled cells. In some cases the TMB reaction product labelled many cells in nuclei in which the DAB product labelled only a few or no cells. The TMB method is not usually more sensitive in detecting axonal labelling, but generates a heavier and more obvious product. The DAB method gives a finer picture of the morphology of axons, which is necessary for distinguishing terminal regions and branching patterns. The DAB sections were also counterstained with cresyl violet to identify cytoarchitectonic boundaries.

CHAPTER ONE

The first chapter is concerned with some basic observations on the cytoarchitecture of the turtle forebrain. First, the cytoarchitectonics of the cerebral cortex are described. In lizards and snakes the cortex is divided by two discontinuities into three divisions, termed medial, dorsal and lateral cortex. The cell plate of the lateral cortex extends over the cell plate of the dorsal cortex; the cell plate of the medial cortex overlies the cell plate of the dorsal cortex similarly. The medial cortex can be subdivided into a dorsal magnocellular region and a ventral parvocellular region. The cell plate of the magnocellular region is loosely packed, while the cell plate of the parvocellular region is packed soma to soma. The dorsal cortex possesses a variable number of subregions in different species.

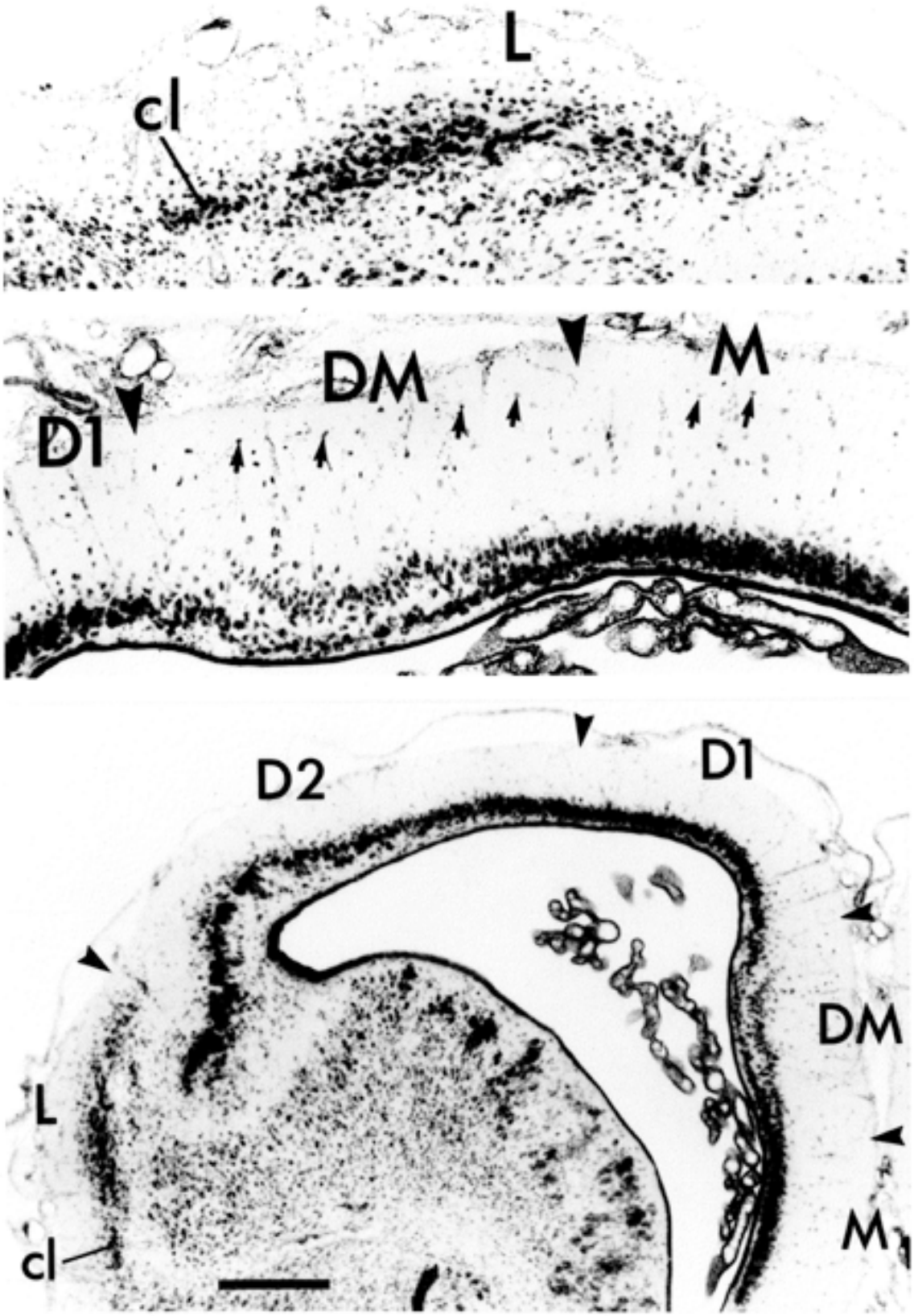
The present analysis suggests that the turtle cortex can be divided into similar areas in turtle. The dorsal cortex possesses two subregions. When all these areas are reconstructed in depth, they form long narrow strips curving around the hemisphere. This pattern is important in the arrangement of corticocortical connections.

Second, the structure of the cortex was investigated with the Golgi method. Only a few brief studies of the cortex of the turtle exist. The present study hoped to distinguish fundamental cell types and describe intra-cortical axonal patterns, which have hardly been seen since the time of Ramon (1896, 1917). Only the dorsal cortical areas were studied in detail. The principal cell type of the cortex is a cell with long spiny apical and basal dendrites. Although such cells differ in morphology, all follow similar fundamental patterns and represent one basic type. These cells give rise to recurrent axon collaterals ascending through the molecular layer. The cortex also contains cells with no, few or irregular dendritic spines. Most of these cells vary widely in terms of location of the cell body, morphology of dendritic shafts and size and density of dendritic arborization. Their axons, to the extent impregnated, arborize randomly in the molecular layer. Such cells could not be divided into specific classes. Some of these cells, however, have dendritic and axonal arborizations concentrated just underneath the pial surface. These cells probably represent one or two specific classes.

Figure 2. Cytoarchitecture of cortical areas.

This legend applies to the following two pages. The entire cortex is shown at low power in the bottom panel on the first page. The indicated cortical areas are shown in the surrounding panels. Arrowheads mark the borders between cortical areas. Arrows indicate flat, periventricular cells in D1 and D2.

Scale bar, 500 microns.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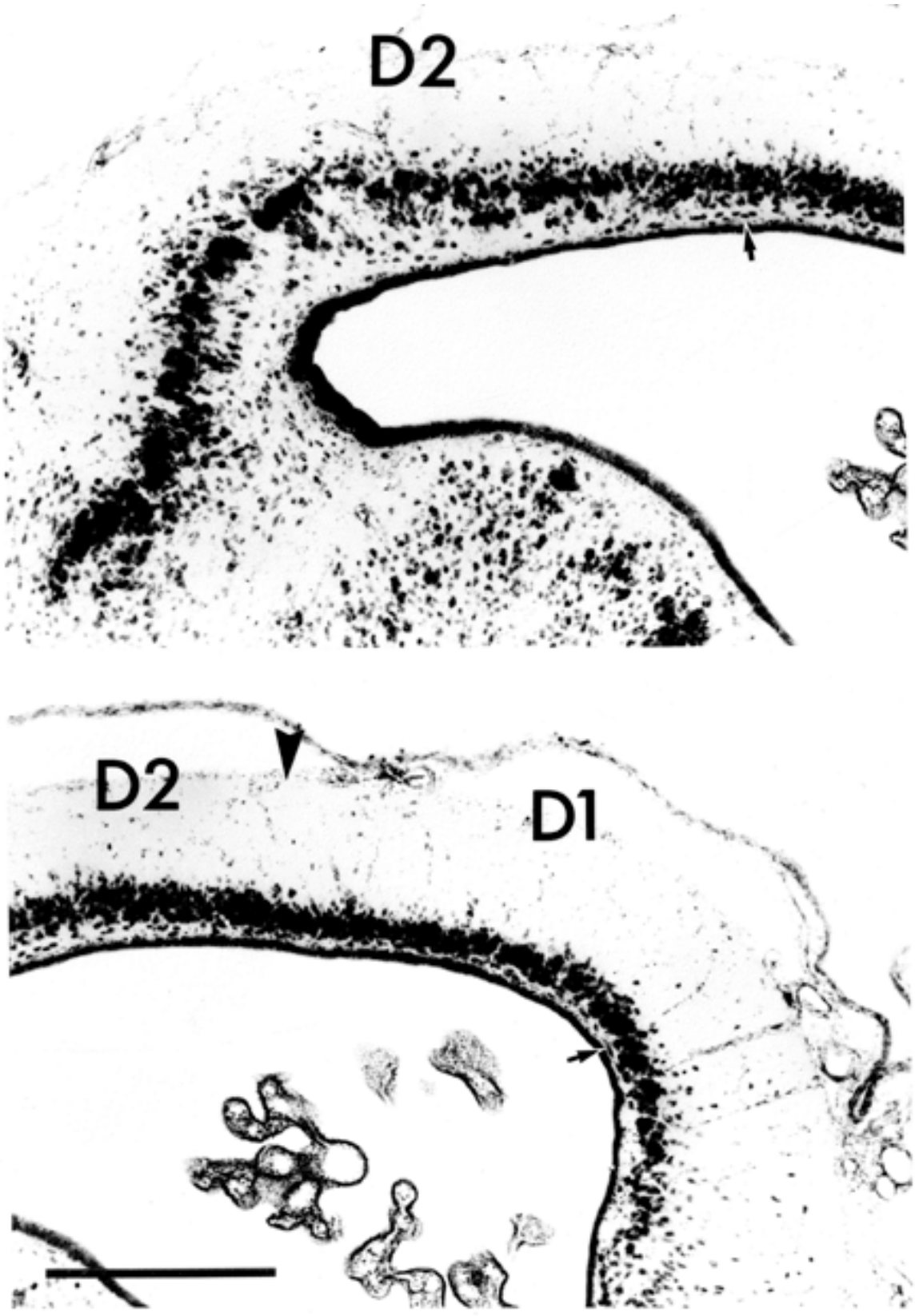

D2
D2
D1

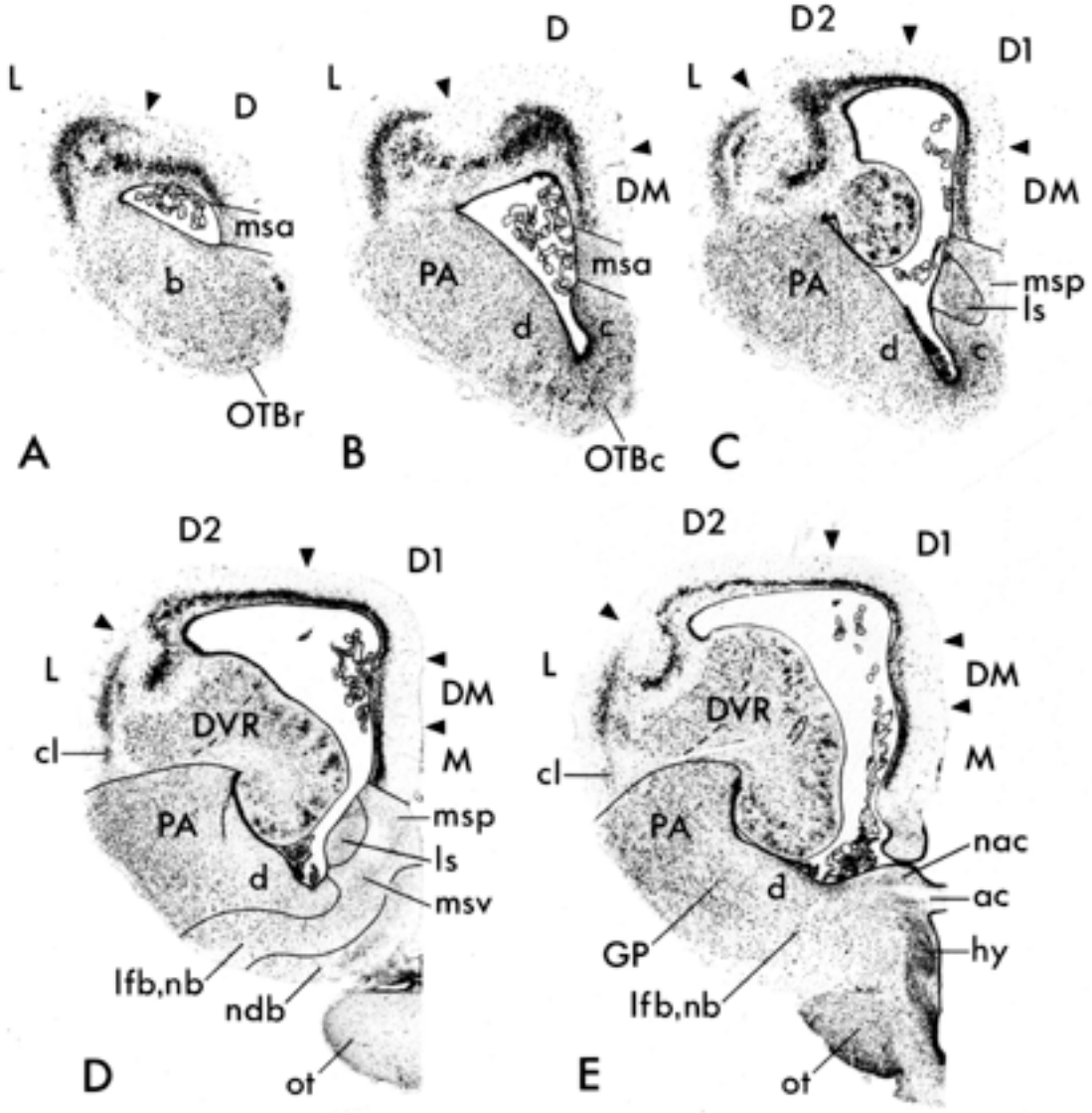
Figure 3. Atlas sections.

These are sections of celloidin-embedded brain, with cresyl violet.

The scale bar is one millimeter.

ABBREVIATIONS USED

ac	anterior commissure
at	area triangularis
b	area b
BA	nucleus basalis amygdalae
c	area c
CA	nucleus centralis amygdalae
cl	central lateral nucleus
d	area d
D	dorsal cortex
D1	first dorsal cortical area
D2	second dorsal cortical area
dla	nucleus dorsolateralis anterior
DM	dorsomedial cortical area
dma	nucleus dorsomedlalis anterior
DVR	dorsal ventricular ridge
GP	globus pallidus
hy	hypothalamus
L	lateral cortical area
lfb	lateral forebrain bundle
lgn	lateral geniculate nucleus
ls	lateral septal nucleus
M	medial cortical area
MA	nucleus medialis amygdalae
mb	mammillary body
msa	medial septal nucleus, anterior division
msp	medial septal nucleus, posterior division
msv	medial septal nucleus, ventral division
na	nucleus anterior
nao	nucleus of the anterior commissure
nb	nucleus basalis
ndb	nucleus of the diagonal band
NLOT	nucleus of the lateral olfactory tract
nr	nucleus rotundus
ot	optic tract
OTBc	olfactory tubercle, caudal part
OTBr	olfactory tubercle, rostral part
PA	paleostriatum augmentatum
reu	nucleus reuniens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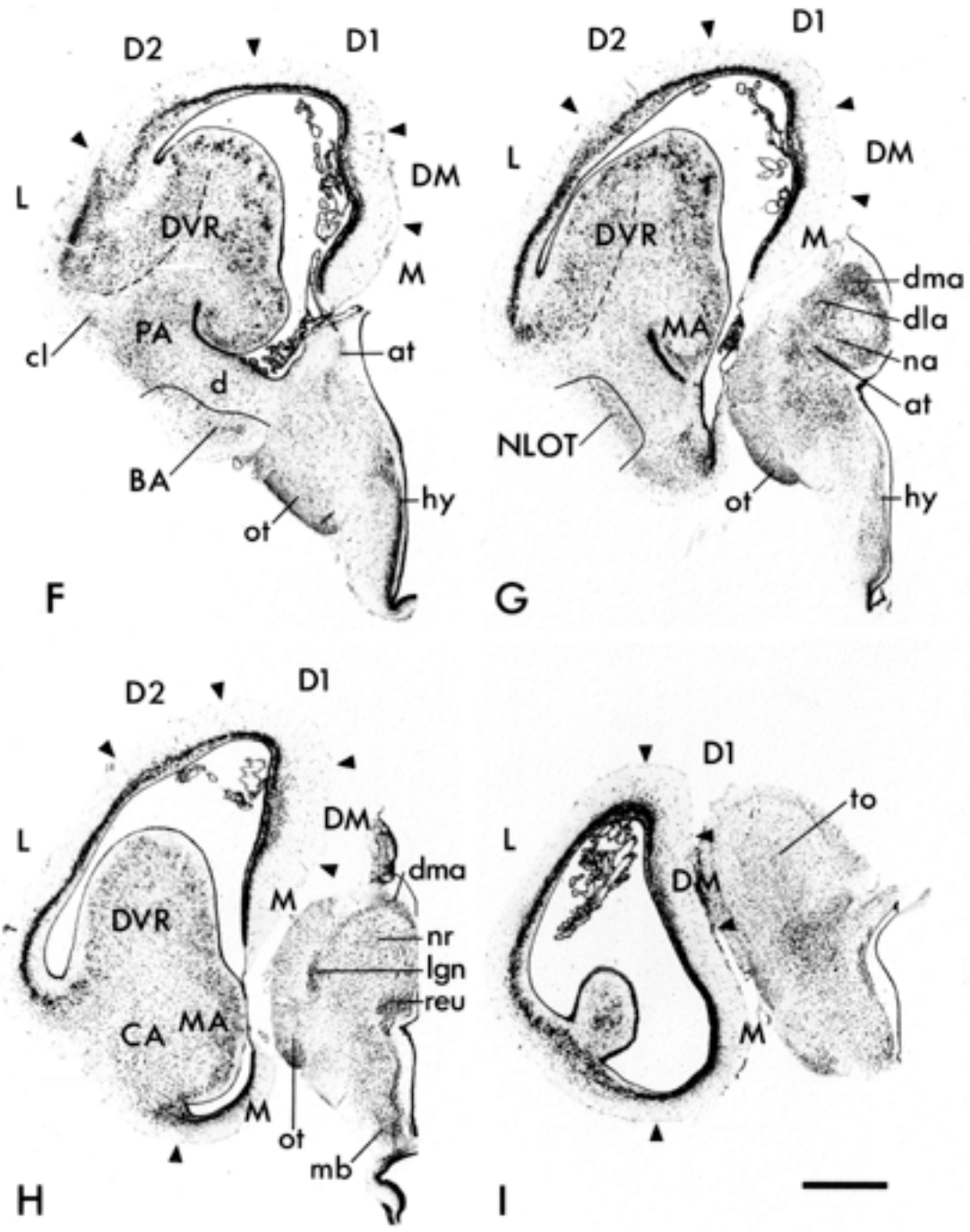
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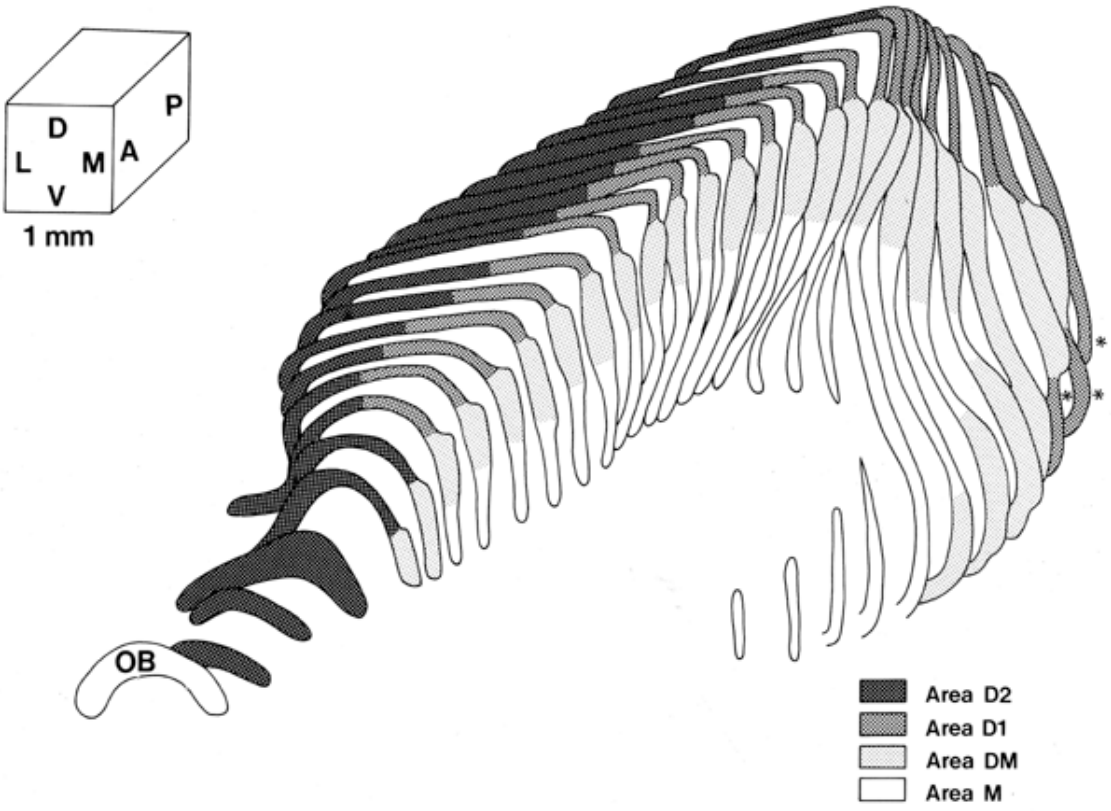
Figure 4. Reconstruction of the cortical areas.

The cell plates of areas D2, D1, DM and M were traced from successive sections through the hemisphere. Each tracing was displaced progressively dorsally and medially, creating a false perspective view of the hemisphere from its medial side. The cortical areas are distinguished by different shading. The plane of section is tilted approximately 30 degrees from coronal, in order to cut the hemisphere perpendicular to its long axis, which runs rostromedially to caudolaterally.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Area D2
Area D1
Area DM
Area M

Finally, a brief description and atlas of the cytoarchiteeture of the remainder of the telencephalon is presented. While several detailed studies of the forebrain as well as a comprehensive atlas exist (Johnston, 1915; Northcutt, 1967; Reiner, 1983), this attempt is different in several ways. First, it was constructed in hindsight, with some information about the connections of the regions involved. Second, each region was examined both in sections stained with cresyl violet and in sections stained for the activity of the enzyme acetylcholinesterase (AChase). Some boundaries which are obscure in the Nissl section are distinct in the histochemical section, while some areas which are diverse in cellular structure are associated by a common distinctive level of enzyme activity. Third, celloidin-embedded material was used for Nissl sections. Some faint cytoarchiteetonic boundaries are almost invisible in frozen sections or thin paraffin sections but apparent in thicker celloidin sections.

A. CYTOARCHITECTONICS OF THE CORTEX

The cortex is composed of four layers. From the pial surface inwards, these are the molecular layer, the cellular layer, the subcellular layer, and the ependymal layer. The molecular and subcellular layers are cell-sparse. The cellular layer is relatively cell-dense. The ependymal layer borders the ventricle and contains the tightly packed cell bodies of ependymal cells whose processes extend through the overlying layers.

The cortex can be divided into five areas. From medial to lateral these are the medial area, or M, the dorsomedial area, or DM, the first dorsal area, or D1, the second dorsal area, or D2, and the lateral area, or L. These areas are illustrated in Figure 2.

The medial area, or M, has a narrow subcellular layer and a tightly packed cellular layer (Figure 2). It is not present at the rostral levels of the cortex (Figure 3A-C). Dorsally it merges without a sharp boundary with the dorsomedial area, or DM (Figure 2). Area DM is distinguished by the scattered arrangement of the cells in the cellular layer. The cells are spread further from the ventricular surface than in the adjacent areas. Frequently an incomplete cell plate underlies the scattered cells, as in Figure 2. This cell plate may or may not be in continuity with area M on one side or area D1 on the other. The molecular layer of areas M and DM contains cells which are often smaller and more lightly staining than the cells of the cellular layer. Few cells are found in the outer most zone of the molecular layer. Cells seem to be particularly concentrated along the inner border of this zone: a number of such cells are visible in figure 2. Areas M and DM seem to be analogous to the two divisions of the medial cortex in other reptiles, although there is no difference in the size of their cell bodies.

The first dorsal area, area D1, possesses a narrow subcellular layer and a dense, homogeneous cellular layer (Figure 2). Usually the cell plate of this area seems to extend slightly underneath the scattered cells of DM: this may represent the medial superposition which is more apparent in other reptiles. Cells located below the cell plate frequently have an elongate, horizontal cell body (arrow, panel E, Figure 2). Cells seem to extend from the cellular layer of area DM into the molecular layer of the neighboring part of area D1, giving this region a slightly different structure. There are fewer cells in the molecular layer of the remainder of area D1 than in the medial areas. Such cells occur throughout the thickness of the molecular layer, even against its outer edge. A somewhat disproportionate number are located about 50 to 100 microns below the pia.

The second dorsal area, area D2, is identified by the packing of its cellular layer (Figure 2). Gaps divide the cell plate into clusters of cells. The coarseness of the clusters increases progressively in more lateral parts of D2. The width of the subcellular layer also increases, beginning at the boundary with area D1. As in area D1, there are horizontally elongated cells near the ventricular surface (arrow, panel D, Figure 2). The molecular layer of area D2 contains a few cells, which are distributed as in the molecular layer of area D1. Laterally the cell plate dips away from the surface of the brain and extends to the dorsal ventricular ridge. This region was termed pallial thickening by Johnston (1915). The cellular layer in this region is broken up into large clusters of cells. The cellular layer is not distinctly separated from the molecular and subcellular layers.

The lateral area, L, is separated by an obvious discontinuity, the lateral superposition (Figure 2). The molecular layer of area L contains scattered cells which are more frequent in the inner half of the molecular layer. The cellular layer is loosely packed with modest clustering. There is no subcellular layer as such in the rostral part of area L where it is bordered internally by area D2 and the dorsal ventricular ridge. Cell clusters or a cell plate are found below the main cellular layer. In the caudal hemisphere area L abuts on the lateral ventricle. A subcellular layer is present and the cell packing density increases, particularly ventrally.

The cell plate of area L is in tenuous continuity with a small cell group, the central lateral nucleus (cl), at its ventral limit through the middle levels of the hemisphere (Figure 2).

A reconstruction of these areas in depth was prepared. One hemisphere cut on a freezing microtome in a plane perpendicular to its long axis, which runs rostromedially to caudolaterally about 30 degrees off the midline. The brain

was embedded and frozen and marks made along the sides of the block parallel to the axis of advance to permit accurate alignment of sections. The cell plates of the cortical areas in spaced sections were traced, each outline displaced successively medially and dorsally. The resulting false perspective view of the turtle hemisphere seen from medially and above is seen in Figure 3. The lateral cortex is not included in this version. The cytoarchitectonic areas described above are shown by density of shading.

The cortical areas D2, D1, DM and M form parallel strips of even width curving around the hemisphere. Areas D2 and D1 are present only on the dorsal surface of the hemisphere, but areas DM and M continue down the medial wall and curve rostrally again. In the caudal and ventral parts of this curve area DM is bordered by area L, except at the rostral end of its ventral section, where it is bordered by amygdalar regions. (Unlike the other cortical areas, area L is not a strip. It is narrow rostrally, becomes wider and covers the posterior pole of the hemisphere).

Figure 5. (upper panel) Principal cells of the dorsal cortical areas.

The pial surface of the cortex is near the top of the photograph, the ventricular surface near the bottom. The cell on the left half figure represents a typical principal cell of area D2. The apical dendrites are covered with compact spines through most of the molecular layer, but in the outer molecular layer the dendrites become thinner and have fewer, longer and more irregular spines. The basal dendrites are more erratic in course and have fewer spines which may also be long and irregular. The cell in the right half of the figure is a typical cell for lateral area 1. The apical dendrites resemble those of the preceding cell, but the basal dendrites are much less well developed. The axon hillock emerges from the stalk below the cell body and the axon can be seen branching in the region below the cell body. One collateral is visible rising obliquely through the molecular layer.

The scale bar represents 100 microns.

Figure 6. (lower panel) Horizontal axons in the molecular layer.

The pial surface is near the top of the photograph. The cellular layer is marked by impregnated cell bodies and a finely granular precipitate. Multiple horizontally running axons are visible in the molecular layer. (large arrow).

The four principal cells marked by small arrows illustrate a common pattern of dendritic branching. Each of these has a cell body near the top of the cellular layer, and directs a thick process throughout the cellular layer. This process divides and gives rise to the basal dendritic tree below the cellular layer. Conversely, the cell which is out of focus to the right of these four has a cell body below the cellular layer and extends an apical process which crosses the cellular layer and arborizes.

The scale is the same as in Figure 5.

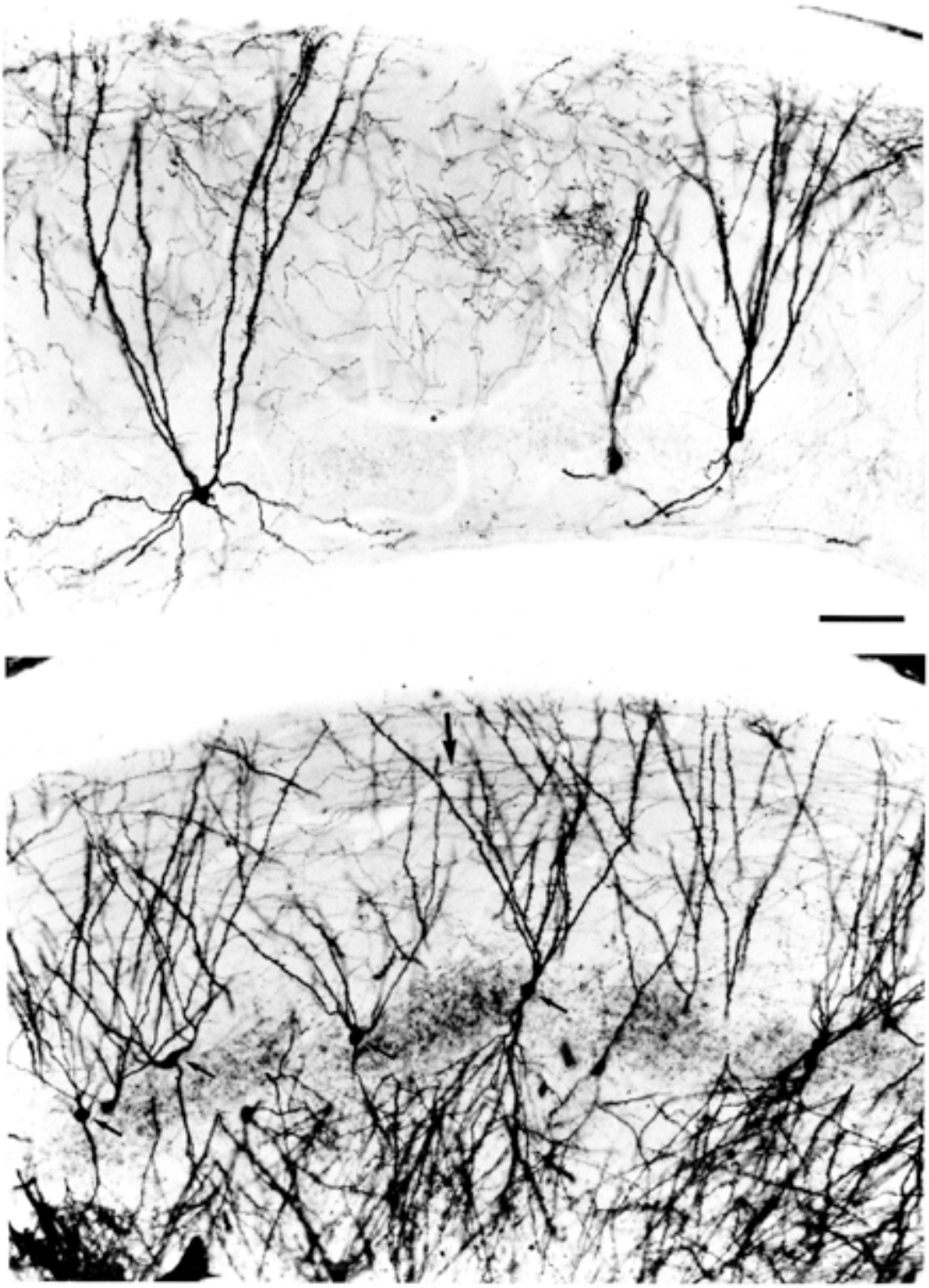


Figure 7. Non-spiny cells of the dorsal cortex.

a. (upper left) This cell with radiate dendritlc arborization is located in the cellular layer in area D2. Much of the axon visible around the cell originates from it.

b. (upper right) The dendritic tree of this cell in the molecular layer of area D2 is somewhat bipolar. A subpial cell is located above it.

c. (lower left) This cell is located in the cellular layer of area Dl. Note rising axon hillock.

d. (lower right) This cell lies immediately above the cellular layer of area Dl. Its dendritlc arborization is very large and sparse. The axons in the field derive from this cell.

The scale bar represents 100 microns.

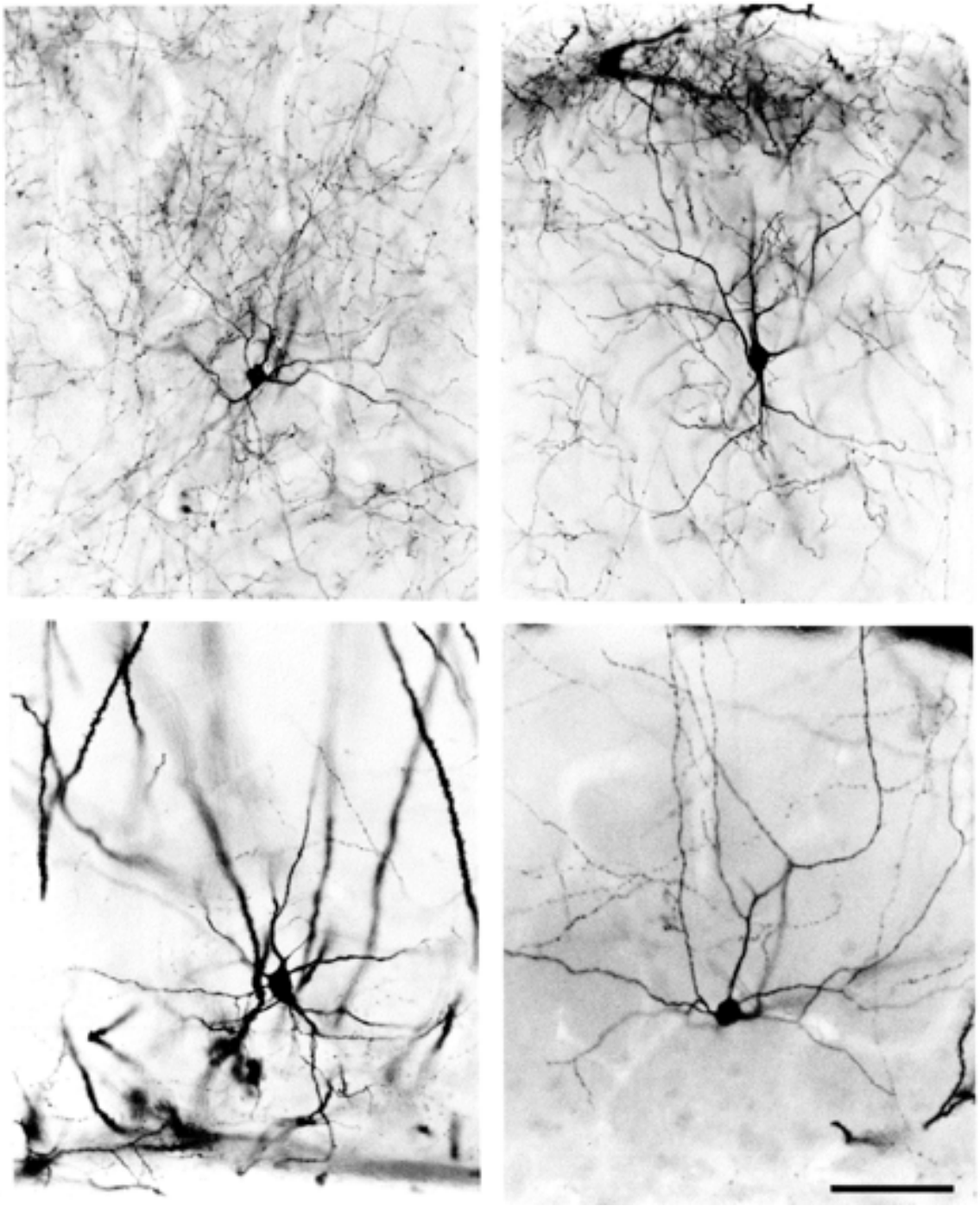
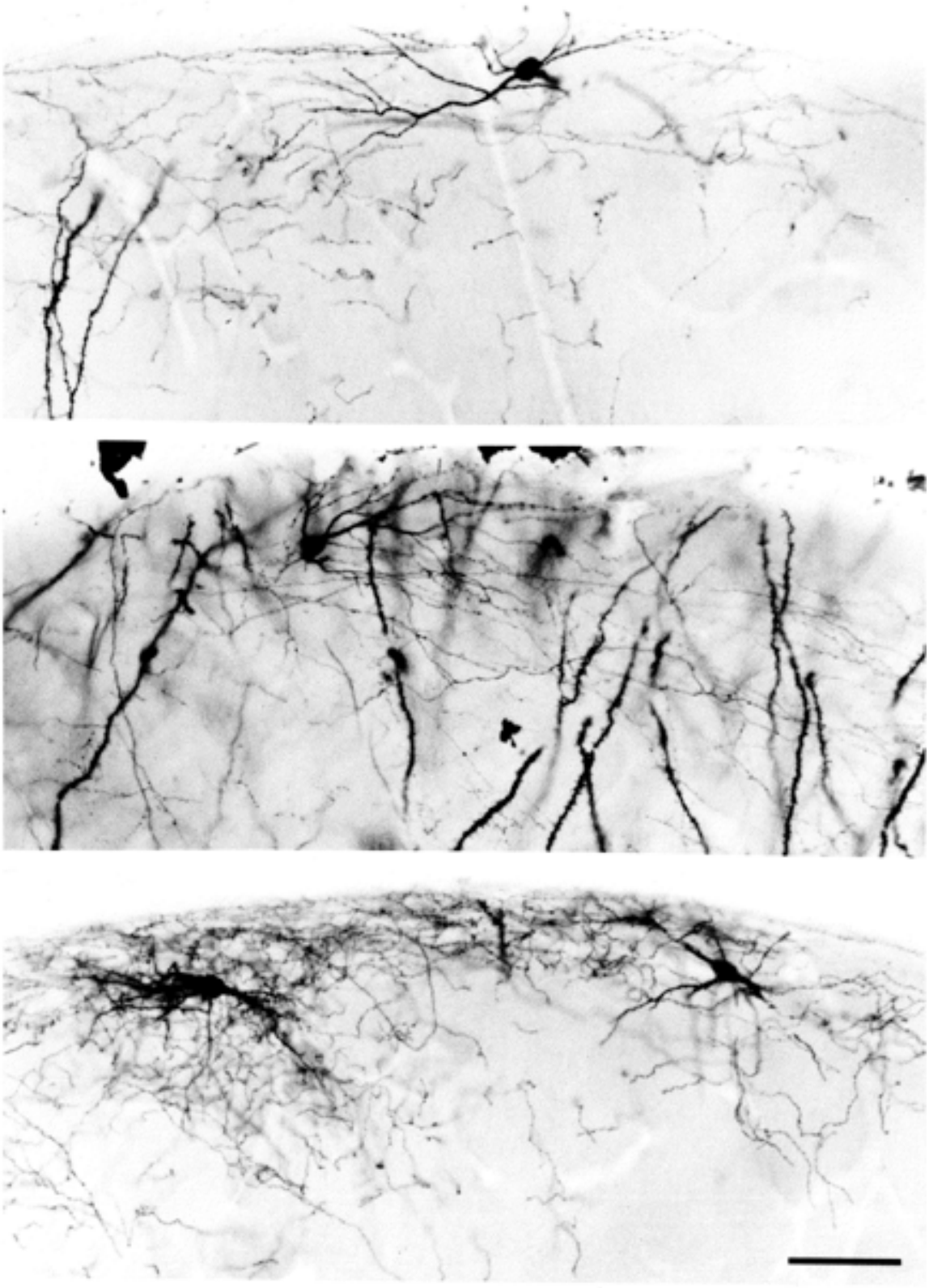


Figure 8. Sub-pial cells in the dorsal cortex.

The pial surface is adjacent to the top edge of each photograph. The dendrites of the sub-pial cells in the top two panels are beaded. There are two sub-pial cells in the bottom panel. Most of the axon visible in the photograph comes from the cell on the left. Most of the arborization is in a band immediately below the pia, with frequent exceptions.

The scale bar represents 100 microns.



B. GOLLI STUDY OF THE DORSAL CORTICAL AREAS

Eighty two brains were impregnated with variants of the Golgi method. The methods used are listed in Table 1. The traditional Rapid Golgi method applied to tissue briefly perfused with 4% paraformaldehyde yielded the clearest and most complete impregnation of cortical neurons. While other methods gave satisfactory staining of the dendritic arborizations of spiny neurons, only the Rapid method stained the dendritic arborizations of non-spiny neurons adequately. The Rapid method also impregnated axonal arborizations of all cell types with greater completeness.

The most frequently stained cell type in the cortical areas of the turtle is a neuron with long apical and basal dendrites covered with profuse spines, termed a principal cell or P-cell. Some examples are seen in Figure 5. The cell body is usually located in the cellular layer and does not bear spines. The apical dendrites are straight and ascend vertically or obliquely through the molecular layer to the pia, branching occasionally at acute angles. No branch stops short of the pial surface. At the pial surface many apical dendrites turn to run horizontally, often emitting short curvilinear collaterals. The initial portions of the apical dendritic shafts in the molecular layer do not bear spines. More distally, the shafts become densely covered with short pedunculated spines. The final portions of the dendritic shafts in the outer third of the molecular layer have fewer spines, which are longer and more variable. Some P-cells were partially reconstructed from serial sections. Although in individual sections the dendritic arbors may appear quite narrow, the total extent of the apical dendritic field forms a cone encompassing approximately a right angle, at least in the mediolateral plane of these sections. The basal dendrites follow tortuous courses in the subcellular layer, branch frequently and end randomly. The basal dendrites have sparse, irregular, long spines resembling those of the distal apical dendrites.

The configuration of the dendritic arborization varies in the different cortical areas. In the middle of area D2, which has a large subcellular layer, the basal dendrites are well developed and roughly equal in number to the apical dendrites. Medially, the width of the subcellular layer decreases and the number and complexity of basal dendrites decreases also. In area D1, where the subcellular layer is almost obliterated, many cells lack basal dendrites or have only one, which is often directed towards the side where the subcellular layer is thicker. This difference can be seen in the cells in Figure 5. Conversely, in more lateral D2 (or in caudal D2) the subcellular layer becomes thicker. The basal dendrites become heavily spined, long and straight and form a pyramidal arborization like the arborization of the apical dendrites. Cells of the double pyramidal type, actually from caudal area L, are illustrated in Figure 5. Finally, in the pallial thickening, the buried lateral portion of area D2, the dendrites of P-cells radiate symmetrically in all directions, and apical and basal dendrites cannot be distinguished. Some cells in the pallial thickening and adjacent part of D2 on the dorsal surface lack basal dendrites. Commonly a basal process leaves the cell, turns around and branches in the molecular layer.

Within each area the morphology of individual P-cells differs. The configuration of the soma and proximal dendrites varies dramatically. Some cells have a pyramidal shape with a apical tuft and radiate basal dendrites, others an inverted pyramidal shape exactly opposite. Some cells are stellate, others double pyramidal. A few cells have horizontal somata. The majority of cells bear apical and basal dendrites on tufts which emerge at irregular angles from the soma. Such tufts allow the proximal dendrites to avoid branching in the cellular layer. For examle, in figure 5, four cells at the superficial edge of the cell plate have radiate apical dendrites, but one thick basal process. This process extends through the plate into the subcellular layer and branches into the basal dendritic arborization. One cell, which is out of focus, is at the bottom edge of the cell plate and has the opposite configration. Although P-cells differ radically in the shape of the soma and proximal dendrites, the shape of the overall dendritic arborization is constant.

Some cells with spiny dendrites have their cell bodies outside the cellular layer. Some of these cells are identical to P-cells except for the location of their somata. Others possess unusually shaped somata. In the molecular layer the somata of some cells are extended by several thick proximal dendritic shafts with caliber nearly equal to that of the 7 soma itself. These processes branch into dendritic arborizations resembling those of typical P-cells. Both the soma and proximal dendrites may bear spines typical of the level of the molecular layer in which they are found. In the subeellular layer some neurons have a tuberous, elongate horizontal soma bearing tufts of dendrites at its ends or along its length. These dendrites form typical P-cell arborizations, the apical dendrites tending to branch only in the molecular layer.

The axon hillock of P-cells emerges in a downward direction from the soma or a proximal dendrite. The axon runs toward the ependymal surface, giving off collaterals which branch repeatedly below the cell body. Unfortunately, the axonal arborizations of P-cells impregnated only in regions of dense axonal staining. Consequently it is difficult to follow the paths of these collaterals. Some branches are directed into the subcellular and cellular layer in the vicinity of the cell. Other branches ascend into the molecular layer some running straight to the pial surface at vertical or oblique angles, others pursuing an erratic course and emitting collaterals. A few branches ascend obliquely away from the cell body and continue roughly horizontally in the middle molecular layer as far as could be followed. The original axon is usually lost near the ependyma, a region which impregnates poorly, but sometimes are seen to turn and run parallel to

the ependymal surface, perhaps emitting other collaterals with similar courses.

Occasionally an apparent P-cell axonal arborization was impregnated spontaneously in a relatively clear area of neuropil. Such arborizations derive from a downwardly directed axon hillock, but no cell body is stained. The arborization resembles the arborization of P-cells described above, but could be followed in much greater detail. Such arborizations give rise to a palisade of axons ascending through the molecular layer, over a radius of several hundred microns from the location of the cell body.

A smaller number of cells are stained whose dendrites are sparsely spined or smooth. The cell bodies of such cells are located in the molecular layer or cellular layer. None are stained in the broad subcellular layer of D2. A wide variety of such cells are observed. They can be divided into two general classes.

The first class has dendrites which arborize in all directions around the cell body. The dendritic arborization can be small or cover the thickness of the cortex, and be sparse or dense. Some cells have completely smooth dendrites, some have scattered thorns or spines. A few cells have relatively many spines, and a very few have multiple long excrescences along their dendrites. Many cells have dendrites that are beaded or of uneven caliber. The axon hillock may be directed in any direction. In many cases a small number of axon branches can be traced. These arborize randomly around the cell, bearing frequent varicosities, and often extend through the whole thickness of the molecular layer. In a few cases a complete axonal arborization could be traced: such arborizations are much denser than the arborizations of P-cell axons, and cover most, if not all, of the molecular layer. Four examples of non-spiny or sparsely spiny cells are shown in Figure 7. (Very similar types of cells seem to be present in areas DM and M).

The second class have dendrites which are mainly limited to a zone immediately underneath the pial surface. Most cells in this class have dendrites which taper rapidly to narrow beaded processes, which could be thin enough to resemble varicose axons. Other cells have spineless dendrites which are very uneven in contour but not regularly beaded. A distinct axon could be followed in some examples of either type. Such axons distribute generally within the same zone as the dendrites, but pieces of the arborization may extend to underlying levels of the molecular layer. Finally, a few examples of cells with very long horizontal dendrites which bore frequent long spines were found just below the pia. None of these cells had a well-impregnated axon that could be traced. Several examples of subpial cells are shown in Figure 8. No subpial cells of any type were found in cortical areas DM and M.

Finally, in some regions the neuropil of particular levels within the molecular layer contains multiple axons running horizontally with little branching. An example is shown in Figure 6.

COMMENT: CYTOARCHITECTURE OF TURTLE CEREBRAL CORTEX

The division of the cortical areas described here is straightforward and reflects a basic reptilian pattern. Our system is similar to that proposed by Riss, Halpern and Scalia (1969) for the closely related turtle *Chrysemys picta*.

The pallial thickening has been considered either part of the cortex or part of the DVR. It is labelled part of D2 in this account for two reasons. Its cytoarchitecture continues the trend towards clustering of the cellular layer which begins in the main, cortical part of D2 and because later results of this thesis show that it has connections similar to those of the cortical part of D2.

Several authors have noted that the cortical areas of reptiles are long and narrow. Surface view reconstruction have been presented for the lizard *Scincus* (Platel, 1968); for the turtle *Testudo* and the crocodile *Caiman* (Platel, Beckers and Nieuwenhuys (1973); also for the crocodile *Caiman*, and for the turtle *Podocnemis*. (Riss, Halpere and Scalca, 1969). These reconstructions show the parallel, concentric course of the cortical areas. The lizard and turtle have areas which run the long axis of the hemisphere, while the crocodile has areas running more rostromedially to caudolaterally, concentric about a point near the caudal. pole of the hemisphere. Our reconstruction emphasizes the evenness of width of the medialmost three areas. Such careful design must have important functional implications.

The curve of the areas of the medial wall caudally, ventrally and then rostrally again perhaps represents the beginning of the rotation of the temporal pole present in the mammalian brain. The transition from the tubular amphibian brain to the pallial reptilian or mammalian brain is accomplished by a rotation of its hind end, preserving the parallel arrangement of the cortical areas.

The significance of the cytoarchitectural features of the cortical areas is unknown. The distribution of the cells in the molecular layer may relate to the lamination of the connections of that region. The zone containing few cells in the molecular layer of areas DM and M corresponds to the zone of termination of a particular intra-cortical connection described in Chapter 3. The cells in the cellular layer may be distributed loosely or tightly, evenly or in clusters. In lateral D2 such clustering leaves open spaces for the passage of thalamocortical axons.

The survey of cell types with the Golgi method agrees with every other study of the reptile cortex in finding that the main cell type of the cortex is a cell situated in the cellular layer with long spiny dendrites and descending axon hillock.

Although the shape of the soma and initial dendritic arborization of P-cells is highly variable, there seems to be one fundamental type. The variability in these cells is largely related to the level the cell body. Cells within the cellular layer have a tufted appearance because there is an apparent constraint against dendritic branching within that layer. The development of apical processes by deep cells and basal processes by superficial cells also reflects this constraint. P-cells in the molecular layer with spines on the cell body or proximal dendrites are following laminar patterns of spine development. Cells below the cellular layer with apical dendrites which ascend to the cellular layer and then branch are responding to the usual laminar rules of branching. These cells presumably correspond to the horizontal cells seen at the ventricular surface in Nissl-stained material. Such "periventricular" cells have been described in the medial wall in other reptiles (Ramon, 1917; Ulinski, 1977; Wouterlood, 1961).

Another part of this variability relates to the width of the subcellular layer. Neurons in the more lateral part of D2 have basal dendrites with relatively more "apical" character: the dendrites are straighter, branch less and bear more, shorter spines. In the pallial thickening, where the lamination pattern of the cortex described in subsequent parts of the thesis breaks down, the dendritic arborization of P-cells cannot be divided into apical and basal: most P-cells have similar processes directed into both the molecular and subcellular layer. A few have only apical processes, and are the only distinct example of P-cells differing fundamentally from their neighbors.

Ebner and Colonnier (1975) observed in the electron microscope that P-cells have sparser, longer spines in the outer part of the molecular layer. As noted by these authors, the same is true of mammalian dentate gyrus cells (Laatsch and Cowan, 1966) and somatosensory cortex pyramidal cells (Jones and Powell, 1969). The increase in length is probably somewhat greater in turtle than in mammal. Such specializatlons might relate to a particular type of afferent in the outer molecular layer, or might be a necessary device for the terminal regions of long dendrites in general. The presence of such spines in very different cortical areas favors the more general alternative.

In some P-cells a small number of collaterals ascending through the molecular layer could be traced. Such collaterals resemble the sparse collaterals illustrated by Ramon (1917) for neurons of the dorsal and medial cortex. If the isolated axonal arborizations impregnated in this study are indeed P-cell axons, then each individual P-cell influences a substantial zone of the neuropil around itself.

Neurons with no, few or irregular spines have been described in most studies of reptile cortical areas. The non-spiny or sparsely spiny cells in this study varied continuously in several features, differing in location within, the cortex, morphology of dendritic shafts and spines, and size and density of dendritic arborization. The axons which impregnated arborized randomly in the vicinity of the cell and did not distinguish the cells. Whether these neurons represent examples of a number of very specific classes or samples from a continuum of properties cannot be determined from our sample. A larger sample or a sample with more extensive axonal filling might disclose more clustering of properties. Methods for identifying neurochemical differences might identify separate classes.

The subpial cells may form a distinct type. Alternatively, these cells may be conventional stellate cells located under the pia converting the upper half of their dendritic arborization into a subpial arborization. In that case they represent only one end of a continuum of properties.

Many of these cells have beaded dendrites. Beading is a common artifact of the Golgi method, and the impregnation of the subpial area was usually not optimal. If the beading in these dendrites is real, it argues that these cells are a distinct type. Such beading may affect the electrical properties of the dendrites or serve some other purpose. The dendrites and axons of these cells are limited approximately to the zone of thalamocortical termination within D1 and D2. These cells may have some relationship to the thalamic afferents, receiving an input from thalamic axons, ending on P-cell dendrites near thalamic synapses or both. Indeed, no such neurons were impregnated in areas DM and M, which do not receive a subpial thalamic input; there are few neurons in this zone in the medial areas at all.

The subpial cells with long horizontal dendrites bearing frequent long thin spines may represent another type, but were not impregnated in adequate number for analysis.

Most of the neurons in the molecular layer stain for GAD, and presumably use GABA as a transmitter (F. F. Ebner, personal communication). The distribution of the axons of these cells throughout the layers of the cortex seen with the Golgi method is consistent with the presence of synapses with flat vesicles and symmetric specializations throughout the thickness of the cortex (Ebner and Colonnier, 1975).

Cells with smooth, sparse or unusual spines are present with a wide range of morphologies in mammalian cortex (e.g.,

Jones 1975). While a detailed comparison of types requires a better description of the neurons of the turtle, the cell type with the most prominent dendritic beading in that study is the Type 5 cell found in layer IV, a thalamorecipient layer. Most non-pyramidal neurons in the mammalian cortex also stain for GAD (Ribak, 1978). The presence of a diverse population of such cells begins early in the evolution of the cortex.

The different laminar distribution of neurons in the molecular layer in different cortical areas suggests that certain groups of these neurons may relate to the laminated axon systems described elsewhere in this thesis. Perhaps in the simple cortex of the turtle such relationships can be worked out.

There have been several descriptions of the dorsal cortex of the turtle with the Golgi method. Most of the cells seen by other workers are equivalent to types observed here.

Balaban (1981) illustrates radiate spiny cells and smaller stellate cells with no, few or irregular spines in the pallial thickening. Northcutt (1970) has described pyramidal, double pyramidal, polygonal and pyriform neurons within dorsal cortex. He also described large stellate cells in all layers with highly branched axons (unfortunately not illustrated). Davydova and Goncharova (1979) report seven types of neurons in the dorsal cortex of the turtle *Testudo horsfieldi*. In pallial thickening they find P-cells with radiate dendrites like those described here. Within the cortex on the surface, in D2 and D1, they identify P-cells with wide, medium and narrow apical arborizations as well as P-cells with a single apical process. They find stellate. cells with local axons in the cellular layer, but not molecular layer. All of these types of neurons fall within the range of cell types observed in this study.

Finally, they also describe cells with narrow horizontal dendritic arborizations within the molecular layer. Such cells have been described in the cortical areas of various reptiles (Ramon, 1917; Minelli, 1966) but have not been found in this study.

Figure 9. Acetylcholinesterase staining in the basal forebrain.

The upper panel illustrates a section through the olfactory tubercle and anterior septum, the lower panel a more caudal section through the posterior septum and nucleus of the diagonal band.

The striatal areas PA, d and c stain intensely: note that the olfactory tubercle (otb) does also. The septal area is (erroneously labelled msl in the figure) also stains relatively darkly. The septal area msv stains moderately. Both areas msa and msp are conspicuous by absence of staining. The horizontal limb of the diagonal band (ndbh) stains intensely, the vertical limb (ndbv) lightly. The DVR stains moderately. No staining is present in the regions of cortex in this photograph.

Scale bar 500 microns.

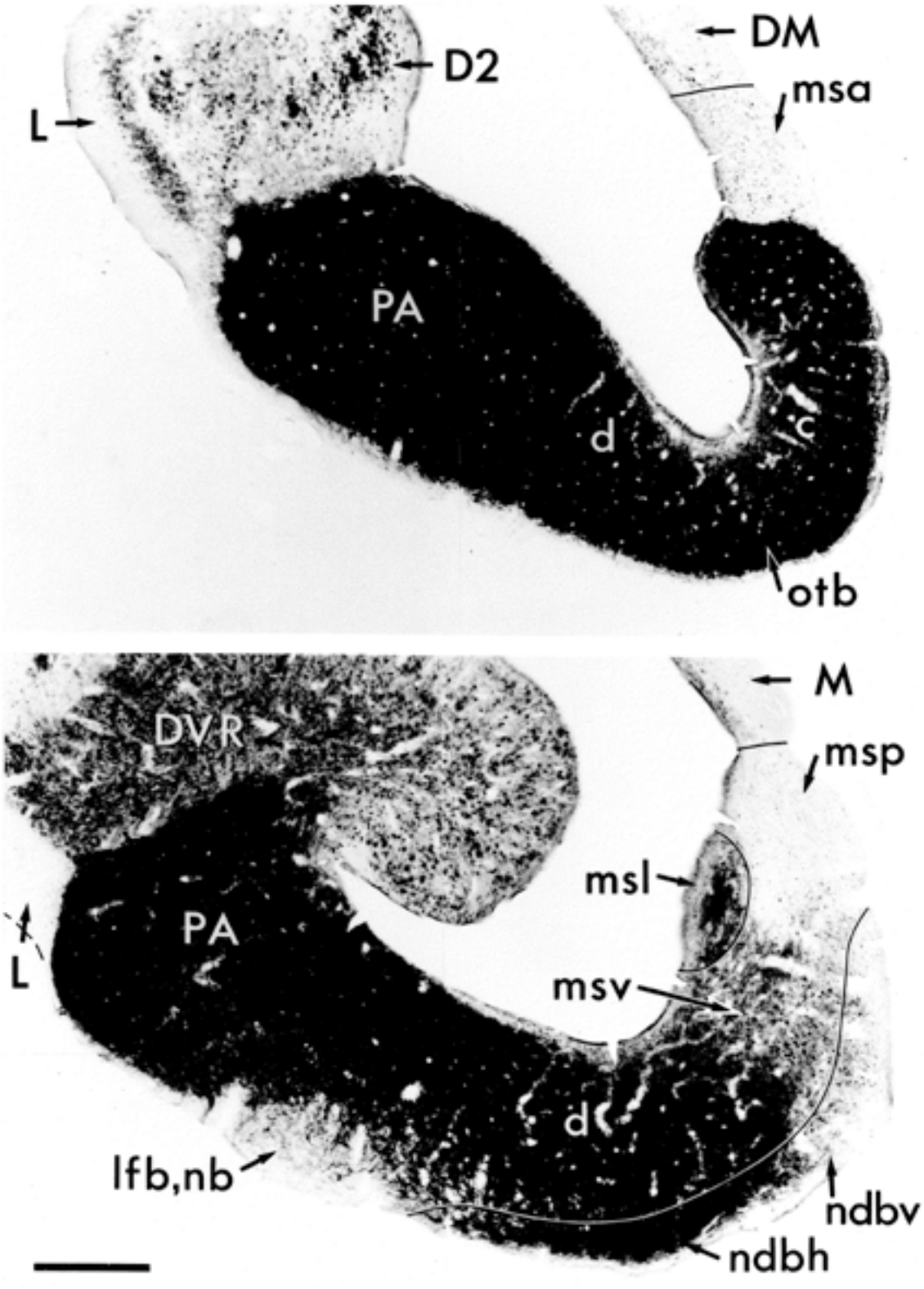
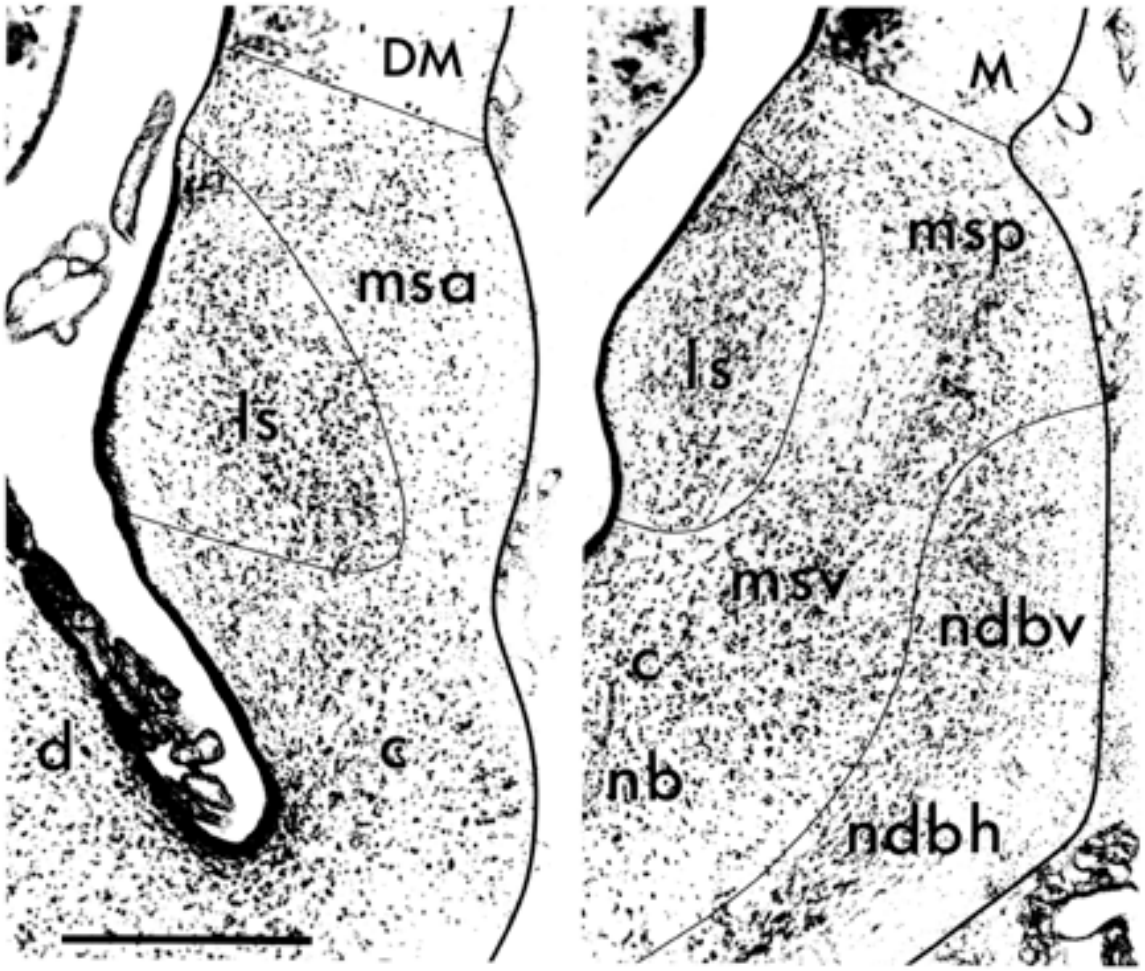

DM
D2
msa
L
PA
d
c
otb
M
DVR
msp
msl
PA
L
msv
d
lfb,nb
ndbv
ndbh

Figure 10. Cytoarchitecture of the septal region.

The left and right panels of this figure correspond to sections C and D of the atlas plates of Figure 3, respectively. In the rostral parts of the hemisphere the medial wall is occupied by the area msa and the striatal area c. As the striatal area, or nucleus accumbens, decreases in size more posteriorly, a new group of cells, area ls, appears in the ventrolateral corner of area msa (left panel). This group continues further posteriorly, occupying a bulge in the ventricular surface (right panel). Area msa is continuous caudally with a slightly more cell-dense region, msp. The area msv gradually separates from this region, as seen in the right panel.

Scale bar 500 microns.


DM
msa
ls
d
c
M
msp
ls
msv
ndbv
c
nb
ndbh

C. CYTOARCHITECTURE OF HEMISPHERE

The telencephalon can be divided into pa11ia1 and basal divisions. The pallial division consists of the cerebral cortex, which has just been described, and the dorsal ventricular ridge (DVR).

The DVR is a large bulge protruding into the lateral ventricle for much of the length of the hemisphere (Figure 3). Balaban and Ulinski (1981) have described four sectors of the anterior DVR in the turtle. The dorsal sector, unlike the other sectors, lacks cell clusters near the ventricular surface. The dorsal sector is the only relevant area in these experiments and is separated by a dashed line in the atlas sections of Figure 3.

The basal telencephalon consists of the striatum, the septum and associated nuclei, and the amygdalar areas. Sections stained for AChase were useful in identifying the components of this region. The staining pattern of the basal forebrain regions at the level of the anterior and posterior septum are shown in Figure 9.

The striatum is defined here as the area of intense acetylcholinesterase activity in the basal forebrain of the turtle, described originally by Baker-Cohen (1969) and Parent and Olivier (1970). This structure occupies most of the basal forebrain. It corresponds approximately to areas b, c, d, and 9 of Riss, Halpern and Scalia (1969); the term paleostriatum is substituted for area 9, and anterior olfactory nucleus for area b in the atlas by Reiner (1980).

The striatum appears at the rostral limit of the hemisphere as a compact area staining densely for AChase located below the lateral ventricle (Figure 3A). It is labelled area b in Riss et al. (1969) and anterior olfactory nucleus in Reiner (1980). It is separated by a cell sparse region from the rostral olfactory tubercle, OTBr, a group of cells continuous laterally with the lateral cortex. The cells of OTBr are clustered. This area stains moderately for AChase. It is termed area j in Riss et al. (1969) and olfactory tubercle in Reiner (1980).

Immediately caudally, the striatum expands in size. The area of intense AChase activity extends to the pial surface medially and is separated from the surface laterally by a very narrow area, the anterior lateral area, al (Figure 3B; the anterior lateral area is not labelled). A spur of the lateral ventricle extends ventrally and divides the striatum into a medial and lateral part. The medial part resembles the nucleus accumbens of mammals in location, and corresponds to area c of Riss et al. (1969) and Reiner (1982). In the ventromedial corner of the striatum there are obvious large clusters and tubes of cells, particularly noticeable in the external parts of the striatum. This area demonstrates intense AChase activity like the rest of the striatum. This region is also termed area j and olfactory tubercle by the aforementioned authors: it is marked caudal olfactory tubercle or OTBc to distinguish it from the the more rostrally situated OTBr which does not stain intensely for AChase and is not an intrinsic part of the striatal neuropil.

At more posterior levels, the striatum is displaced progressively ventrally and off the medial wall by the expanding septum (Figure 3C). The striatum is bordered ventrally by the medial and lateral forebrain bundles, the continuations of the cerebral peduncles. As these expand near the junction with the diencephalon, the striatum shifts laterally (Figure 3D,E). The striatum is abruptly replaced by the amygdalar areas NLOT, BA, MA and CA at the level of the thalamus.

The septum, as the term is used in this thesis, occupies the medial wall between the nucleus accumbens and the cerebral cortex. It can be divided into two regions, medial septum or ms and lateral septum or is, on the basis of cell density and AChase activity. The medial septum consists of several contiguous regions: an anterior divison, msa, a posterior division, msp, and a ventral division, msv.

Area msa begins at the anterior limit of the hemisphere. It is a long narrow zone along the medial edge of the cerebral cortex (Figure 3A,B). This area was originally termed primordium hippocampum by Johnston (1915) and corresponds to area a in the numerical nomenclature of Riss et al. (1969). Area msa does not stain for AChase activity.

Caudally, the septum increases in size. A relatively dense group of cells appears in the ventrolateral corner of the septum. It stains moderately for AChase. This is the anterior tip of the lateral septal nucleus, area ls (Figure 3C, detail in Figure 10A). The remaining part of the septum is cell-sparse, does not stain for AChase and appears continuous with area msa. It is termed here area msp.

Further caudally a narrow cell-free space appears between is and msp. It contains fibers descending to the anterior commissure. A group of cells separates from the ventral edge of msp: it is here labelled area msv (Figure 3D, detail in Figure 10B). At the anterior commissure msp shrinks to a small cell group attached to the ventral margin of cortex as it separates from the basal telencephalon, and msv disappears. The ls is replaced by the nucleus of the anterior commissure. The nucleus of the diagonal band, or ndb, is present at the same location as the analogous mammalian structure. It is a narrow zone just under the surface at the ventromedial corner of the forebrain at precommissural levels. The horizontal limb lies underneath the forebrain bundles. The vertical limb lies outside the nucleus acoumbens and area osv. Dorsally the vertical limb ends against area msp. The horizontal limb stains moderately for AChase, the

vertical limb stains only lightly.

CHAPTER TWO

The aim of this series of experiments is to identify the afferents of the turtle cortex and compare them with the afferents of mammalian cortex. Such a comparison may reveal regions of the turtle cortex which are homologous to regions of mammalian cortex.

First, the mammalian olfactory cortex receives projections from the olfactory bulb, while the neocortex and hippocampus receive projections from the thalamus. In this study, large injections of radioactive proline were used to label the entire projections of the olfactory bulb and the thalamus. The olfactory bulb was found to project to a superficial lamina over all of area L, and the thalamus to project to a superficial lamina of D2 and D1 as well as a narrow deeper lamina of areas DM and M. These experiments demonstrate that the turtle cortex, like mammalian cortex, is divided into a zone which is primarily olfactory and a zone which is primarily thalamic.

Secondly, the mammalian thalamus consists of separate nuclei projecting to specific parts of the cortex. In mammals, the injection of large amounts of radioactive proline in the eye results in labelling in the primary visual cortex by transneuronal transport though the lateral geniculate nucleus. Similar injections in turtles label a rostral portion of area D2, suggesting that the primary visual nucleus of the turtle projects to this portion of area D2. This conjecture was confirmed by a study of the topography of thalamocortical connections in general using small and large injections of HRP in the cortex. The rostral part of D2 receives projections from the visual nucleus lgn, and the caudal part of D2 from the anterior nucleus. Nucleus dla projects bilaterally to areas D1, DM and M. The different cortical areas also receive diffuse projections from different zones in the nucleus dma and a set of cells, the perirotundal cells, which extends through a large part of the thalamus. Cells in the dma and the perirotundal cells project densely to the striatum. Thus, turtles seem to possess both specific and diffuse thalamocortical systems, the latter also innervating the striatum.

Third, mammalian cortex receives an orderly input from a set of basal forebrain structures (Jones, Burton, Saper and Swanson, 1976; Mesulam and Van Hoesen, 1976; Lamour, Dutar and Jobert, 1982; Mesulan, Mutson, Levey and Wainer, 1983). The nucleus basalis of Meynert projects with a rough topography to the neocortex. The medial septum and vertical limb of the nucleus of the diagonal band project to the hippocampus and entorhinal cortex. The nucleus basalis of Meynert projects to the cerebral cortex. The horizontal limb of the nucleus of the diagonal band projects to the olfactory cortex. If this system were present is turtles, its relationship to the cortex might suggest homologies between the cortical areas of turtles and those of mammals. These structures contain a variable proportion of cholinergic neurons, as assayed by immunohistochemical staining for the enzyme choline acetyltransferase (Mesulam, Mufson, Levey and Wainer, 1983). In order to locate any analogous regions in the turtle, sections of the forebrain were reacted with two different antibodies to mammalian choline acetyltransferase. Cholinergic neurons are present in analogous locations in the turtle basal forebrain, although their relative distribution among these areas differ from the mammalian pattern. No other cells are stained in the forebrain.

Figure 11. Comparison of the projection of the olfactory bulb and thalamus to the forebrain.

The density of labelling at five levels of the forebrain is shown after an injection of tritiated proline into the olfactory bulb (first page) and into the thalamus (second page) {NOTE: put on one page for digital file}. The cortical cell plate and the dorsal division of the DVR are outlined. Abbreviations are the same as used in Figure 3.

The olfactory bulb projects mainly to cortical area L and the NLOT. Additional projections may include structures of the rostral ventromedial hemisphere. The largest projec- tion of the thalamus is to the striatum. The thalamus also projects to a superficial zone over most of areas D2 and D1, and to a deeper zone in rostral and caudal areas D2 and D1 as well as in areas DM and M. The diffuse labelling of the caudal parts of areas DM and M may reflect spread from the injection site.

The scale bar is 500 microns.

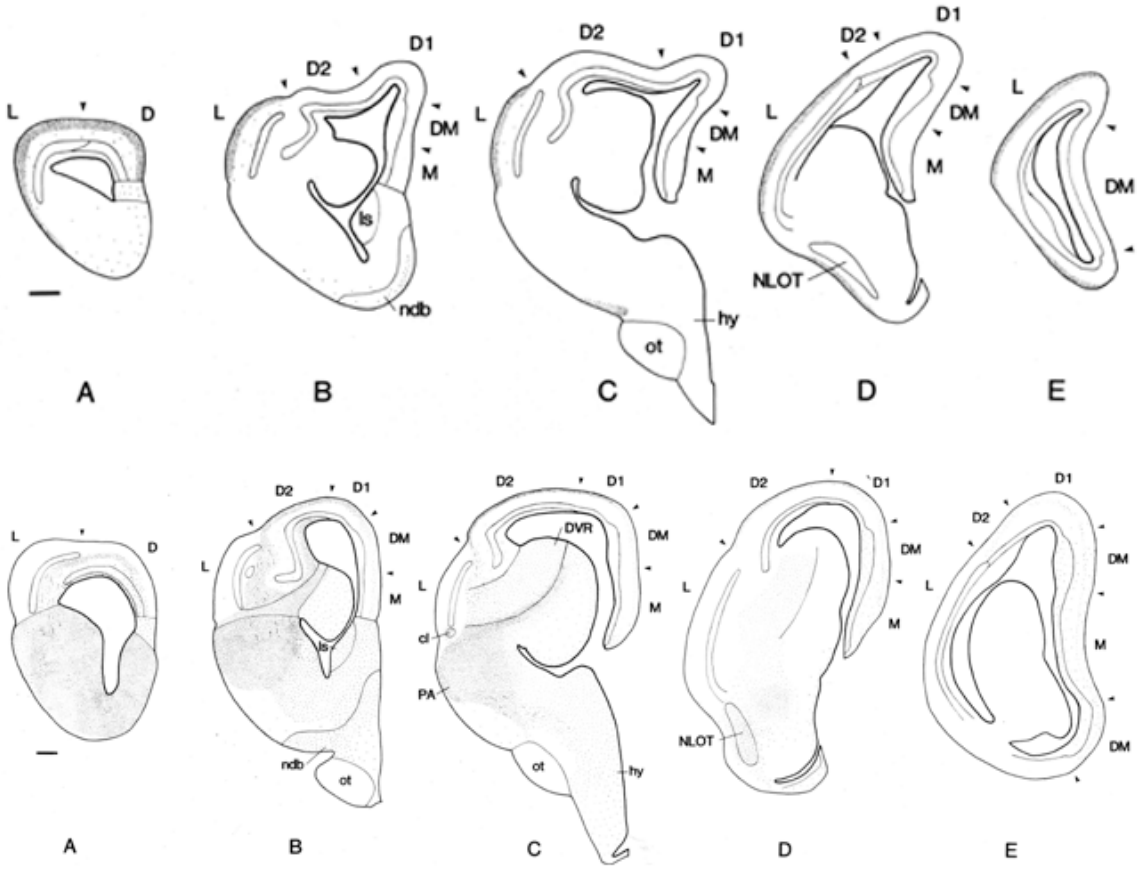

L
D
A
D2
D1
DM
M
ls
ndb
B
hy
ot
C
NLOT
D
E
DVR
cl
PA

Figure 12. (upper panel) Projection of the olfactory bulb to the cerebral cortex.

In this figure the ventricular surface of the brain has been traced with a thick white line, and the cellular layers of the cortical areas outlined with a thin white line. The pial surface has been marked with a dashed line where unclear. The borders between the cortical areas are indicated with triangles.

Following an injection of tritiated proline into the olfactory bulb, label is seen over a narrow zone just below the pia over area L (arrow).

Scale same as Figure 13 below.

Figure 13. (lower panel) Projection of the thalamus to the cerebral cortex.

The edges and cellular layers of the cortex have been marked as in the preceding figure. Following an injection of tritiated proline into the thalamus, heavy label is present in a narrow sub-pigl zone over area D2 (large arrow) and most of area D1. In the more medial parts of area D1 the label becomes lighter and comes to occupy a slightly deeper zone in the molecular layer; this pattern continues over areas DM and M (small arrow). Label is also visible over the DVR and in a small field over the nucleus cl immediately below the cell plate of area L.

The scale marker indicates 500 microns.

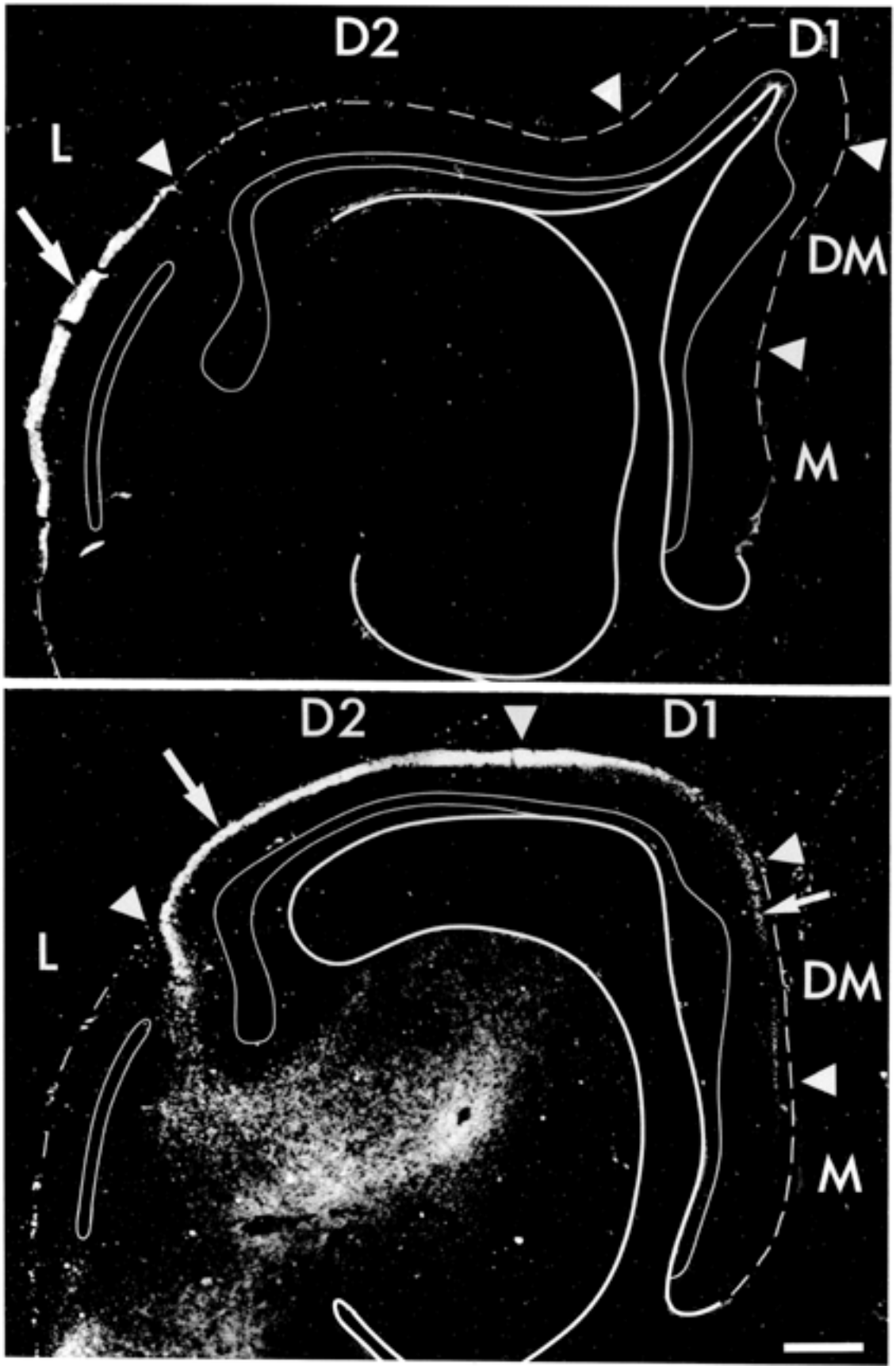

D2
D1
L
DM
M
D2
D1
L
DM
M

Figure 14. Transneuronal transport from the eye.

Stipple represents the autoradiographic label in sections through the anterior, middle and posterior levels of the area labelled in cortex following intra-ocular injection of tritiated proline. Arrowheads mark the borders between areas.

The scale marker is 500 microns.

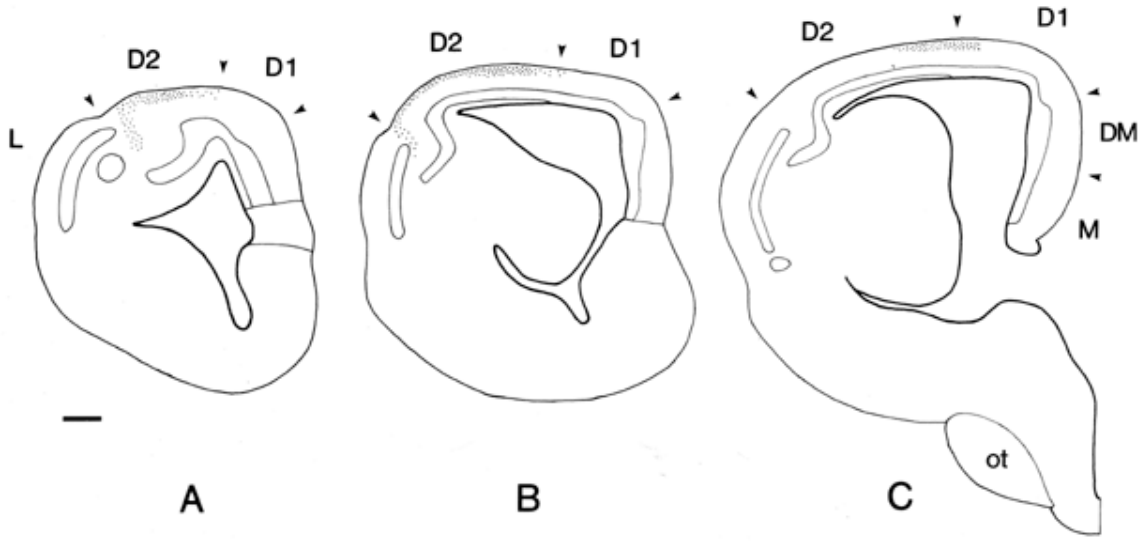

D2
D1
L
A
D2
D1
B
D2
D1
DM
M
ot
C

Figure 15. Transneuronal transport of tritiated proline to the cortex.

Following an injection of tritiated praline into the eye, label is seen in the thalamic termination zone in area D2-(arrow). Landmarks indicated as in preceding figure.

Scale marker represents 500 microns.

Plate missing

The projection of this system onto the cortex was analyzed in the series of cortical HRP injections previously mentioned. There is a topographic projection of these areas onto the areas of the cortex that is highly reminiscent of that in mammals. The pattern implies homologies of the cortical areas consistent with the other conclusions of the thesis. The projection of the basal forebrain differs from that in mammals in that a rostral area, msa, projects to all the cortical areas.

A. INJECTIONS OF THE OLFACTORY BULB

The olfactory bulb was injected with tritiated proline is four animals. The injections were made into the medial wall of the bulb at about the middle of its rostrocaudal extent, but intense labelling was present over the entire bulb. The adjacent basal forebrain and contralateral olfactory bulb were slightly labelled. Two systems of projections are labelled.

The first system consists of a layer of label over the lateral cortex and two areas which are adjacent to it, the anterior lateral nucleus (al) and the nucleus of the lateral olfactory tract (NLOT). The label occupies a zone immediately under the pial surface with a sharp internal border. The projection to the lateral cortex is shown in Figure 12. In the rostral half of the lateral cortex faint label is present in the underlying molecular layer and the cellular layer.

The distribution of this system is illustrated in Figure 11. In the rostral telencephalon label is present over area L from its border with the olfactory bulb. A small spur of labelling covers area al also. At the level of the rostral thalamus, a group of fibers extends abruptly from the ventral side of lateral cortex, across the ventral surface of the hemisphere and up the side of the thalamus by way of the stria medularis. These fibers continue caudally, decussate in the habenular commissure, and give rise to an identical labelling of the olfactory structures of the contralateral hemisphere. In transit across the ventral side of the hemispheres these fibers cross the anterior edge of the nucleus of the lateral olfactory tract. A spur of labelling extends caudally over this nucleus, ipsilaterally and contralaterally. At the caudal limit of the hemisphere the lateral cortex forms the entire lateral wall of the hemisphere and is labelled over its entire extent. These projections correspond to the projections of the lateral and intermediate olfactory tracts of previous studies described in the introduction.

A second, smaller system of projections appeared to involve the structures at the external margin of the rostral hemisphere, but could not be clearly studied due to the diffusion of label from the injection site.

B. INJECTIONS OF THE THALAMUS

The thalamus was injected with tritiated proline in four cases. Such injections label widespread projections to the striatum, the DVR and the cortex. These projections are illustrated in Figure 11, which is a reconstruction of the case with the largest injection covering all of the dorsal thalamus, nearby parts of the ventral thalamus and the rostral tectum.

There is a dense projection to the entire striatum as delimited above. Rostrally there is dense projection to the striatum and the caudal olfactory tubercle, OTBc. There is a lighter projection to the rostral olfactory tubercle, OTBr. More caudally, label is heavier over the lateral striatum, area 9, than over more medial parts c or d. The septal region is also lightly labelled. The label extends to the caudal tip of the striatum. The dorsal peduncle of the forebrain is labelled, the ventral peduncle is not.

The dorsal ventricular ridge also receives a projection which was not studied.

Finally, some fibers extend into the cortex. The dorsal, dorsomedial and medial cortical areas contain label. In the dorsal areas D2 and D1 the label is confined to a narrow zone underneath the pia; in the medial areas DM and M label is present in an imprecise zone separated from the pia by a narrow gap (Figure 12).

The precise distribution of this projection is illustrated in Figure 11. At the most rostral level of the dorsal and dorsomedial areas the label is confined to the middle third of the molecular layer. The boundaries of this zone are not precise, especially on the medial wall. Immediately caudally the zone moves outward to lie under the pial surface. Labelled fibers reach the cortex by passing through the pallial thickening, the lateral extremity of area D2. One strand of label reaches around the lateral edge of the pallial thickening into the superficial molecular layer of the cortex; a second strand runs underneath the pallial thickening and occupies a periventricular position in D2. Over the part of area D2 on the surface of the brain the label becomes concentrated in a sharp subpial layer about 100 microns thick. On the medial wall, in areas DM and M, weaker label is present in a very narrow imprecise zone separated from the pia by a narrow gap. Further caudally, the labelling in the underside of the pallial thickening and D2 disappears and the lamination of cortical afferents becomes sharper. At thalamic levels the intensity and the width of the labelled regions in the dorsal cortex decrease. In addition to the narrow zone of label in the upper molecular layer, the medial wall areas contain a diffuse faint distribution of label, which increases in intensity close to the thalamus. Such labelling

presumably represents diffusion from the thalamic injection site. In the caudal limits of the dorsal cortex the labelled zone comes to lie progressively further below the pia. It becomes faint and diffuse and disappears shortly behind the last illustrated section. The narrow zone of label in the molecular layer of the medial wall areas is visible, against a diffuse labelling of the thickness of the cortex, to the posterior end of the hemisphere.

C. EYE INJECTIONS

Large interocular injections of tritiated proline were made in 4 turtles. Six weeks after the eye injection the animal was perfused and the brain processed for autoradiography. Results were similar in all four cases with a survival time of six weeks. (In preliminary experiments, no transport to the cortex was obtained in turtles with a survival time of four weeks or shorter).

Background labelling is slightly elevated throughout the brain. The optic nerve, optic chiasm and optic tract are heavily labelled, and areas of the forebrain opposed to these structures diffusely labelled. The regions of termination of the optic nerve in the thalamus, described in greater detail below, were intensely labelled. A gradient of diffuse labelling extended from these regions medially over the lateral thalamic nuclei and the nucleus rotundus.

In the cortex label was present in a subpial zone in the molecular layer of cortical area D2 (Figure 15). The labelling is present in the rostral half of D2 (Figure 14). At more posterior levels the labelling in the lateral parts of D2 becomes weaker and disappears. Just behind the level of the anterior commissure a faint zone of label is limited to a medial part of D2. No label is found more caudally. No ipsilateral projection to the cortex was detected.

D. COMMENT: ANTEROGRADE LABELLING OF AFFERENTS

The olfactory bulb projects to a narrow superficial lamina of the molecular layer over all of area L on both sides of the brain. As noted in the Introduction, several studies in other reptiles using degeneration methods yielded a similar result, while others found only a projection to the rostral parts of L. The only study of olfactory projections in the turtle described only rostral projections (Gamble, 1957). Such results should be checked in other reptiles with the autoradiographic method: perhaps the caudal area L undergoes a radical, and interesting, evolutionary change.

In the rostral parts of area L sparse grains were observed in some experiments in the remainder of the molecular layer. These may represent label in axons passing to more caudal levels of cortex; leakage of label by axons in the superficial molecular layer and its subsequent diffusion and reuptake; labelling of the intrinsic axon system of area L (described below) by spread from the injection site; or a real, slight projection to the deep molecular layer.

A superficial lamina of label extends over much of the ventral surface of the caudal telencephalon: much of this zone is very thin, and perhaps represents fibers of the olfactory tract. The zone over the nucleus of the lateral olfactory tract (NLOT) is thicker, like the zone of termination in the cortex, and presumably represents terminal labelling. Finally, the central lateral nucleus (cl) is next to the ventral edge of the cell plate of area L, and could receive olfactory input.

Conversely, the thalamus projects to much of the forebrain. projection of the thalamus and olfactory bulb do not overlap in the cortex. The thalamic and olfactory projections may overlap in the external structures of the anterior forebrain. They may also converge in the central lateral nucleus, which receives an input from the thalamus over its cellular core and perhaps extends dendrites into overlying olfactory bulb terminals.

The projection to the cortex lies immediately below the pia in most of the dorsal areas D2 and D1, and bends slightly away from the surface of the brain in the medial areas DM and M. A gap is also present between the thalamic projection zone and the pia at the rostral and caudal limits of the dorsal cortex Experiments presented in the Chapter 3 suggest that these gaps are occupied by another axon system. The layer of terminations is widest and densest in the rostral dorsal areas, being relatively weak in the medial areas and extremely narrow in the caudal dorsal areas. An additional zone of label is present along the ventricular surface in part of rostrolateral area D2: such an area could be a bundle of axons passing to the superficial tier by an odd route, or a tier of thalamic termination present only in rostrolateral cortex.

In the present experiments only large injections of tritiated proline labelled the thalamic afferents clearly, especially in the medial wall and caudal dorsal cortex. Injection sites are not well confined in turtles and the background labelling in these experiments was high. Against this background, a sparse projection or a projection which covered the full thickness of the cortex could have been missed. Furthermore, the ventral portions of the medial areas around the level of the thalamus were diffusely labelled, presumably by diffusion from the injection site. The thalamic projection could not be studied in this region.

A similar general pattern of thalamocortical termination was described by Lohman and vanWoerden-Verkley (1978) in

the Tegu lizard using degeneration methods. Following lesions of the thalamus, degeneration is seen in a narrow zone at about the middle depth of the molecular layer of the small-celled part of the medial cortex (area M in the turtle), and in a superficial zone of the molecular layer of the dorsal cortex.

Balaban and Ulinski (1981) have identified a different pattern in turtle. Lesions or injections of tritiated proline into the nucleus dma of the thalamus label an extensive system of fine caliber axons over wide regions of the telencephalon. Within the cortex the medial areas M and DM contain fibers throughout their full thickness. Although autoradiographic methods could be confused by spread from the thalamus to the overlying medial areas, lesion methods are immune from such artifacts. These experiments suggest that the elevated labelling in the medial wall areas near the thalamus reflects a real projection.

The projection of the thalamus to the striatum is more massive than its projection to the cortex. The termination within the basal forebrain correlates with AChase activity, being highest in PA, OTBc, less in OTBr, and dropping through the areas d and c and the septal areas. The zones of thalamic termination in the cortex also display high AChase activity (F. Ebner, personal communication).

The results with intra-ocular injections of radioactive tracers suggest that a visual pathway to the cortex ends within area D2. It is lim- ited to area D2 rostral to the anterior commissure, and was entirely crossed. The labelled zone occupies the full width of area D2 rostrally. Caudally the lateral border of the labelled area moves progressively medial, so that it does not correspond to the cytoarchitectonic field.

This method risks several artifacts. First, the background labelling is high and the intensity of transported label low, so that a weak projection might evade notice. Second, all parts of the visual pathway might not transport label evenly, allowing an underestimation of the visual area. Finally, label may diffuse within the miniscule thalamus of the turtle to cells other than the actual post-synaptic ones, causing an overestimation of the visual area.

E. THALAMIC CYTOARCHITECTURE

The thalamus in turtle consists of a dorsal region which is divided into nuclei of cells of moderate size, and a ventral region, which consists of complex, amorphous zones of small cells. The nomenclature used by Balaban and Ulinski (1981) agrees with the present results and is used with some additions.

The dorsal thalamus consists of the nucleus rotundus, a large, ovoid nucleus, surrounded by an incomplete shell of densely packed cells, which can be divided into separate nuclei. The boundaries between the cell groups of the shell are not precise. The rostral tip of the shell occupies the front of the thalamus (ts, Figure 16A). No cytoarchitectonic boundaries are obvious within.

Immediately caudally the nucleus rotundus appears (Figure 16B). The shell around it is triangular in shape. The medial wall is termed the nucleus dorsomedialis anterior, or dma. The dorsolateral wall is the nucleus dorsolateralis anterior, or dla. The ventrolateral wall is the nucleus anterior, or na.

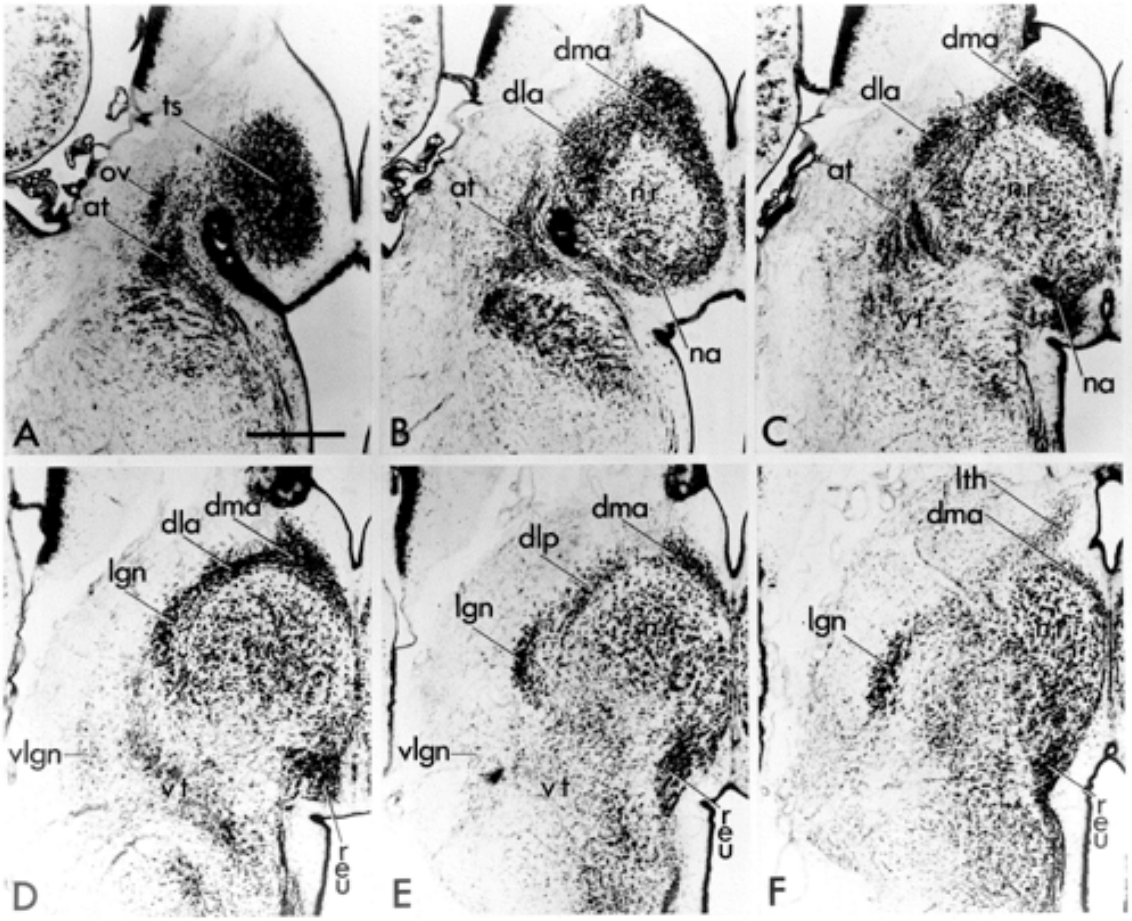
Further caudally the shell expands in size and opens ventrally (Figure 16C). As this occurs a small oval zone of dense cells separates from the underlying dla; this segment merges again just caudally. The dma occupies the crest of the shell and the very thin medial wall. The dla occupies the thick dorsolateral wall of the shell. The lgn appears as the small portion of the lateral wall of the shell ventral to dla.

In the middle of the thalamus the lateral wall of the shell is a continuous arc which cannot be divided into a dorsolateral and ventrolateral part (Figure 16D). The dla forms only a narrow sector of the shell adjacent to the dma, and disappears in more caudal sections. The part of the shell displacing the dla is a thin, densely packed plate of cells, and is termed here the dorsolateral posterior nucleus, dip. Caudally this segment becomes diffuse (Figure 16E). The major portion of the lateral wall is the of the lgn. It is composed of a thin leaflet at the border with the nucleus rotundus, and an external, larger layer separated by a cell-sparse space.

In the caudal thalamus, the external layer of the 1gn shifts ventrally away from the nucleus rotundus and continues caudally adjacent to the optic tract (Figure 16F).

Figure 16. Cytoarchitecture of the thalamus.

The main cell groups of the thalamus are visible in six evenly spaced sections: the thalamus consists of a dorsal portion, the nucleus rotundus and surrounding nuclei, and a ventral portion, composed of irregular groups of small cells (vt, vlgn, at) as well as scattered cells Celloidin- embedding, Nissl stain. Abbreviations used: at, area tri- angularis; dla, nucleus dorsolateralis anterior; dip, dorso- lateral posterior; dma, nucleus dorsomedialis anterior; lgn, lateral geniculate nucleus; na, anterior nucleus; nr, nucleus rotundus; ov, nucleus ovalis; reu, nucleus reuniens; ts, tip of thalamic shell; vlgn, ventral lateral geniculate Vt, ventral thalamus.
Scale bar, 500 microns.


ts
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dlp
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vt
reu
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dma
lgn
reu
F

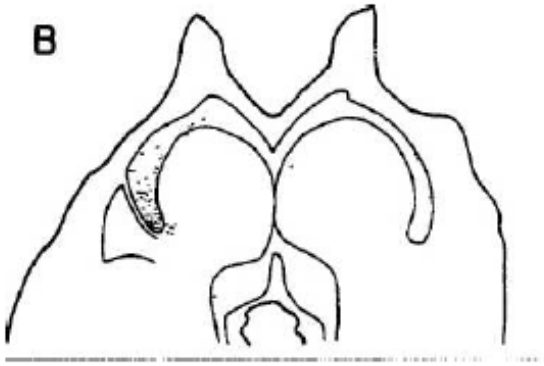
Figure 17. Retrogradely labelled cells in the thalamus following small and large injections of HRP into area D2.

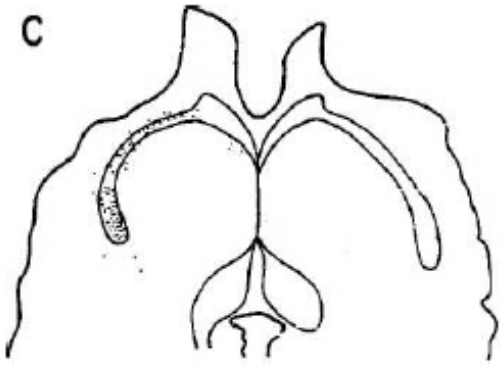
Each column illustrates four spaced coronal- sections through the thalamus of an experimental animal. The top section is through the rostral thalamus where the shell nuclei completely surround the nucleus rotundus. The bottom section is through the caudal thalamus where the nucleus lgn has separated from the other shell nuclei. The intervening two sections are evenly spaced between these extremes. The outer, darker line traces the external boundary of the thalamus; thinner lines outline the shell nuclei, and the area triangularis in the rostral thalamus. These nuclei are labelled on the right side of the figure. The left side of each section depicts the thalamus ipsilateral to the cortical injection. Each dot represents one cell, except in some areas of high labelling density where each cell could not be separately represented. Scale bar is 500 microns long.

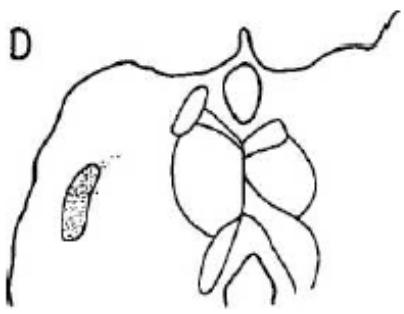
The left column shows a case of an injection of moderate size in area D2 at central levels. On the ipsilateral side, the dense accumulations of labelled cells are located in nuclei na, lgn and dlp. Scattered perirotundal cells are also labelled. On the contralateral side, a few cells are located in the nuclei dla and lgn, always in the external parts of these regions.

The right column shows a case with a much larger injection. Dense groups of labelled cells are found in the same regions of the thalamus as in the preceding case, but many more perirotundal cells were labelled throughout the thalamus. Many more cells were labelled in dlp also.

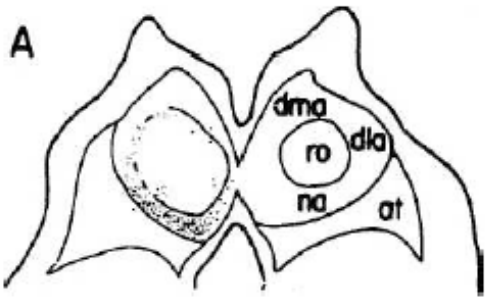
Area D2

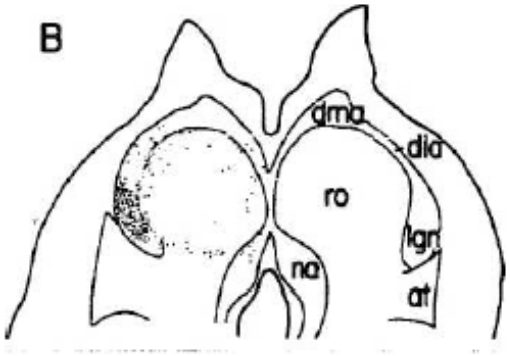


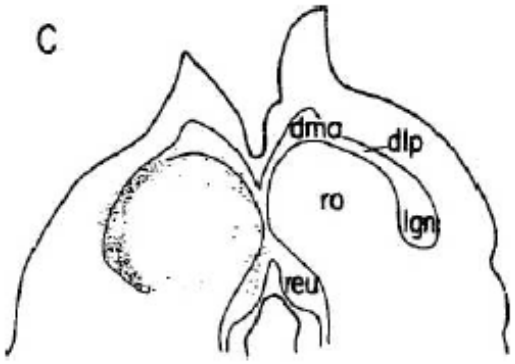




Area D2: large







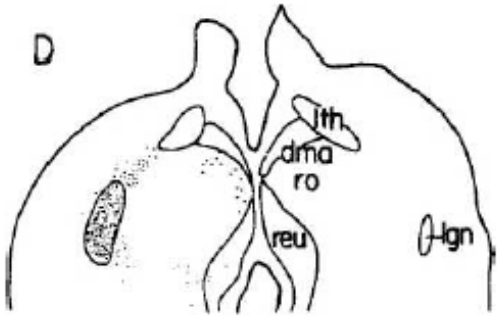


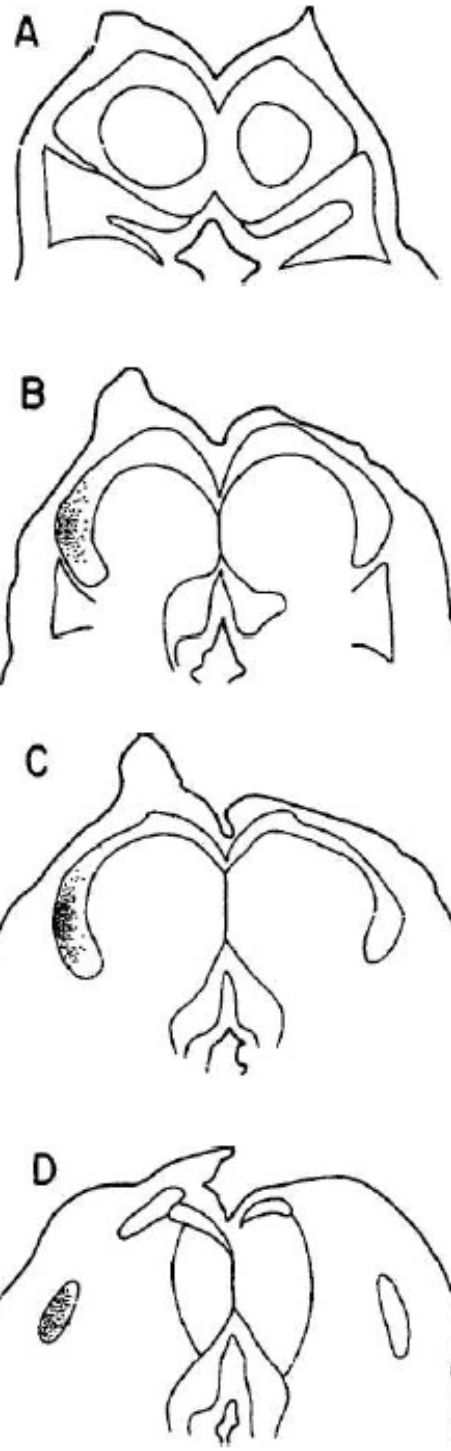
Figure 18. Retrogradely labelled cells in the thalamus after injections in rostral and caudal area D2.

The left column represents an injection limited to rostral area D2. Labelled cells are found in lgn, with some spread into adjacent parts of dla, but not in na.

The right column represents an injection limited to caudal area D2. Labelled cells are found only in na.

Notation and magnification as in preceding figure.

Area D2: rostral



Area D2: caudal

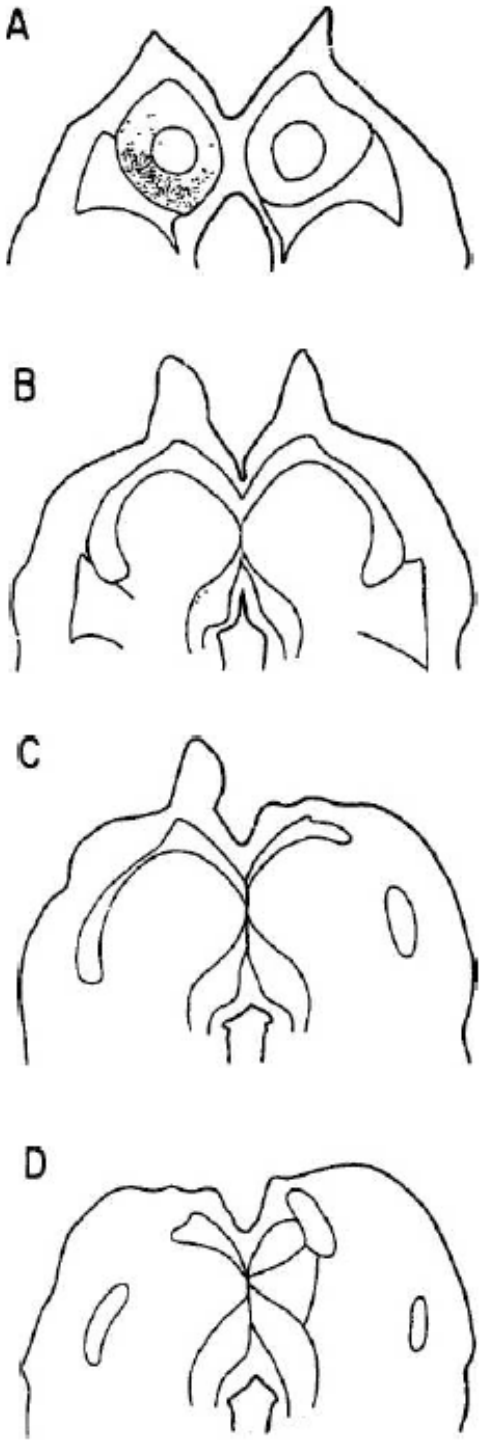


Figure 19. Retrogradely labelled cells in the thalamus after injections into areas D1 and DM-M.

The left column displays a case with an injection into area D1. On the ipsilateral side cells are present at high density in dla, both internal and external segments. Cells are present at lower density in the external half of dma. Some cells are present in the medial part of na: these cells are connected to the group in dla by a intervening group of cells in more anterior sections. On the contralateral side cells are present only in the external segment of dla.

The right column shows a case with an injection in areas DM and M. Labelled cells are located in similar locations as in the preceding case, although at lower density.

Notation and magnification as is preceding figure.

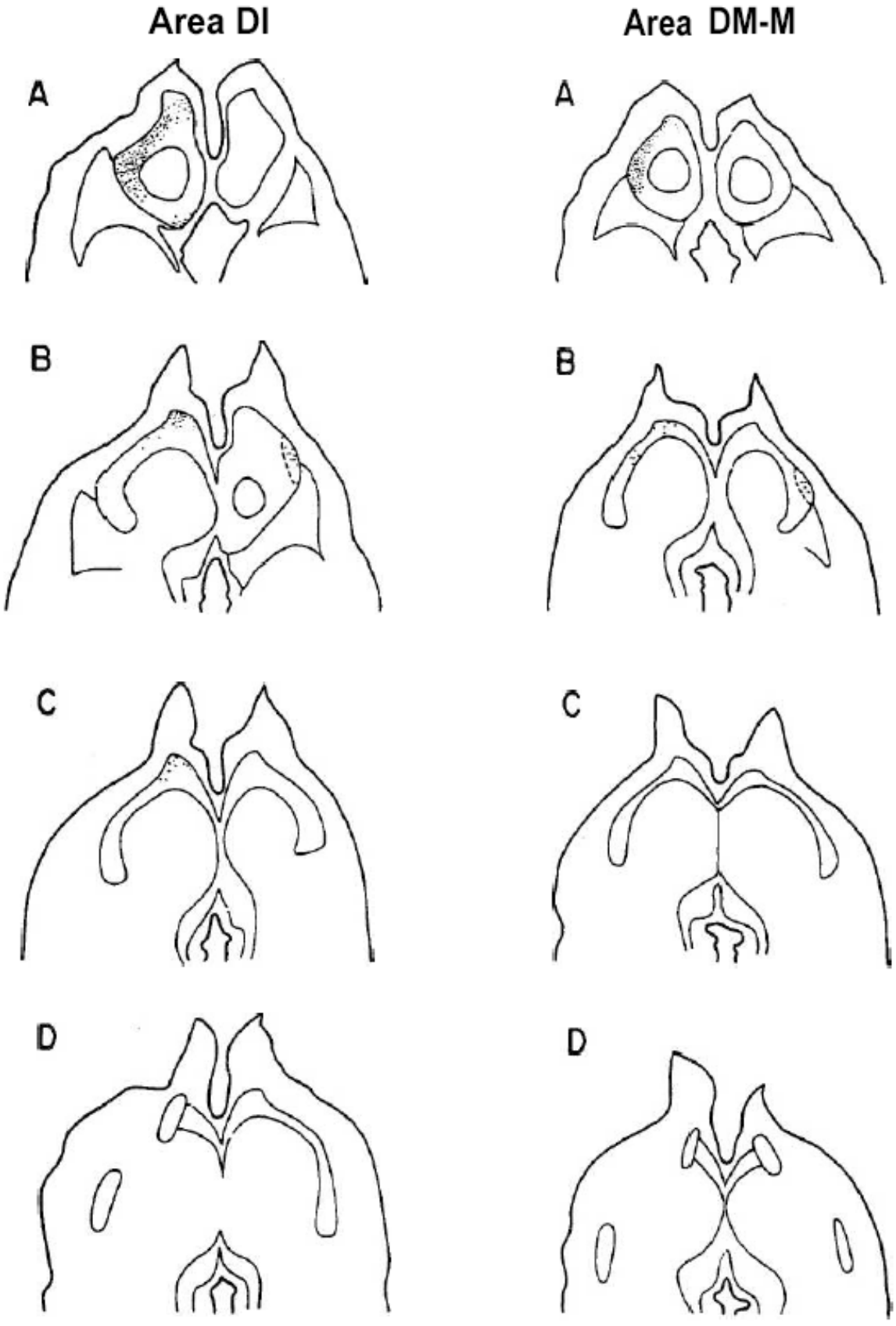

Area DI
Area DM-M
A
A
B
B
C
C
D
D

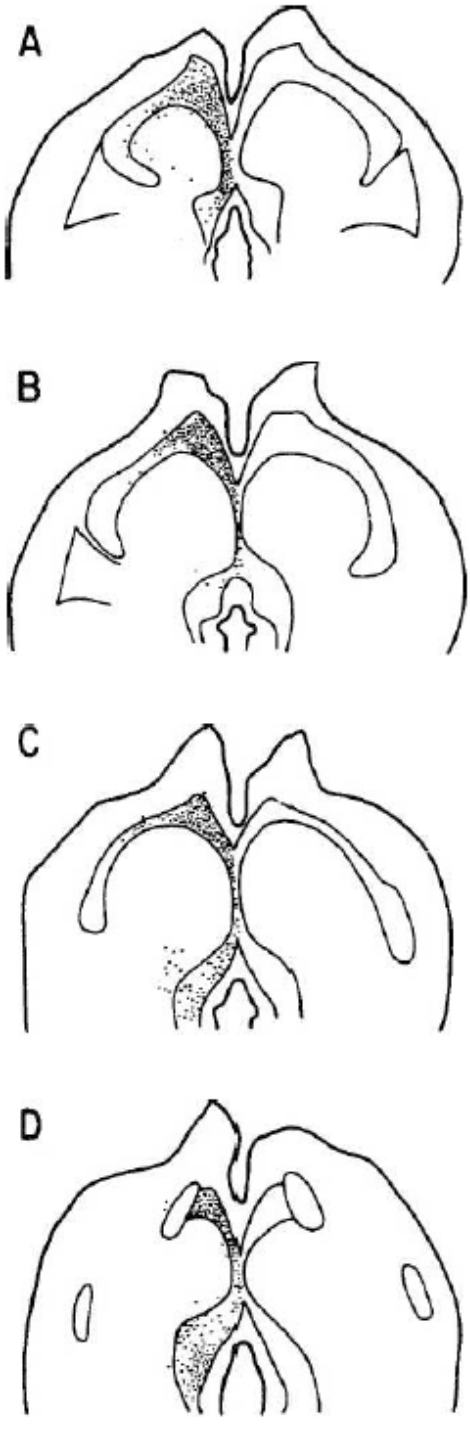
Figure 20. Retrogradely labelled cells in the thalamus after injections in the medial and lateral striatum.

The left column represents an experimental injection into area d of the striatum. The nucleus dma is filled with labelled cells. A few perirotundal cells are labelled. The cell labelling in reu may be due to spread of the tracer to the overlying DVR.

The right column shows an experimental injection of the lateral striatum, area PA. While dma contains fewer labelled cells than in the previous case, many more perirotundal cells are labelled.

Notation and magnification as in preceding figures.

Striatum: medial



Striatum: lateral

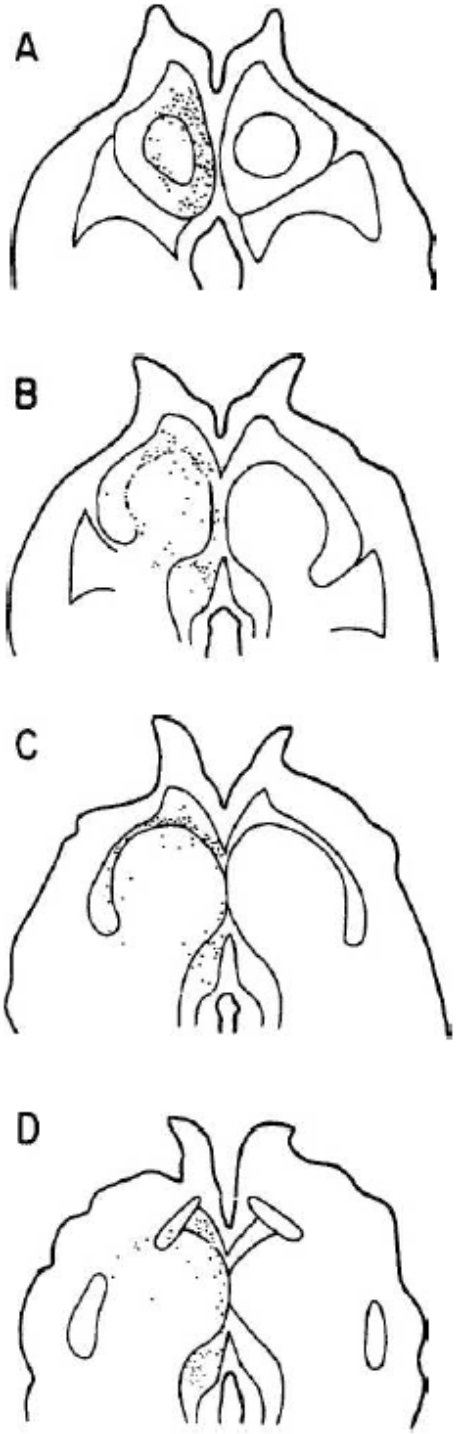


Figure 21. Comparison of cell populations projecting specifically to area D1 or D2, diffusely to area D2, and to the striatum.

This is a darkfield photograph of sections reacted with the tetramethylbenzidine method. Labelled cells are small white dots. The larger irregular patches near blood vessels are artifacts.

The outlines of the shell nuclei are traced in white lines. The central area is the nucleus rotundas (nr).

Injections into area D2, D1 or the striatum label cells in na, dla or dma, respectively. These cell groups occupy approximately complementary portions of the thalamic shell. A large injection of area D2 labels an additional set of cells located at the borders of the nucleus rotundas, immediately internal to the shell nuclei.

Scale 500 microns.

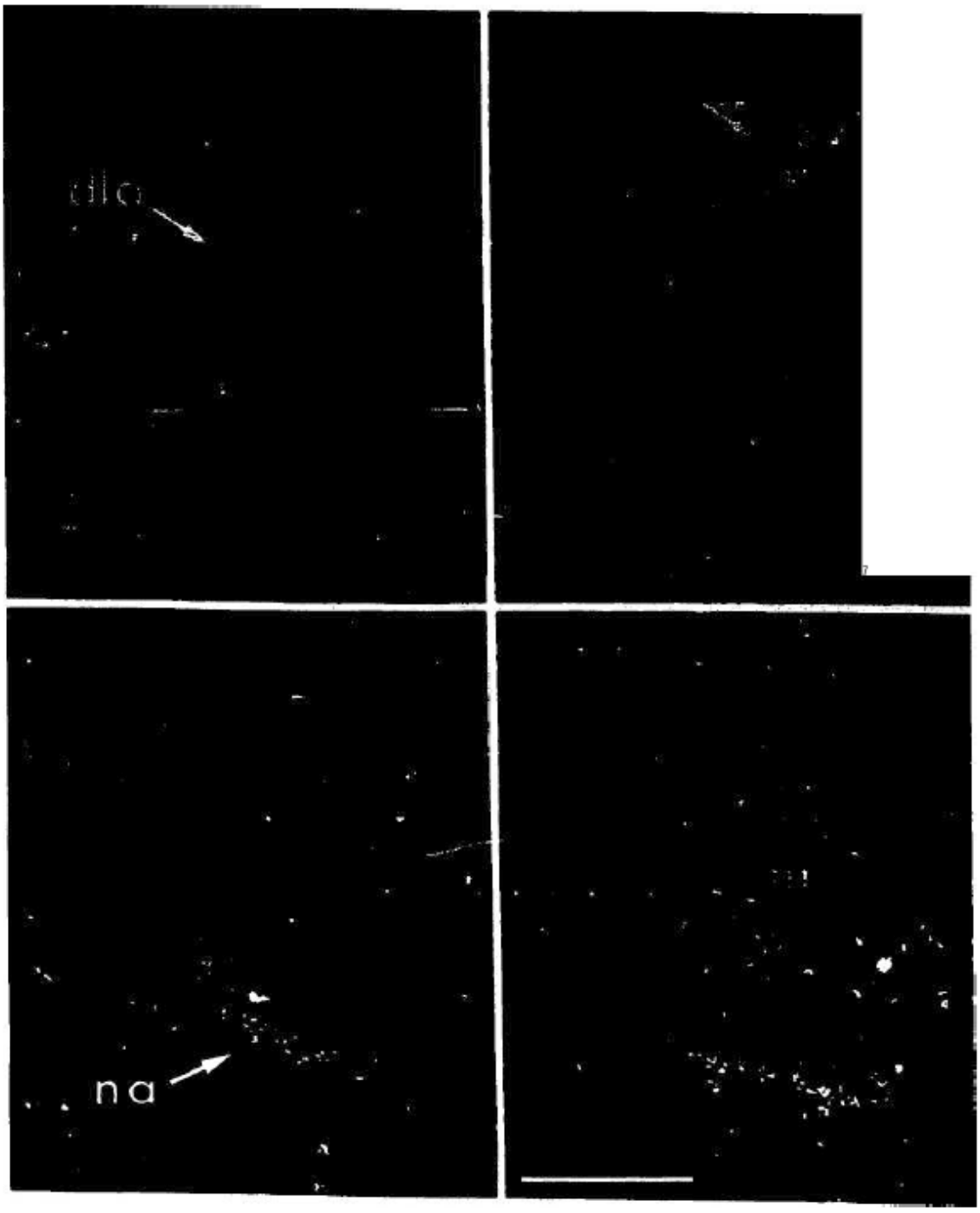
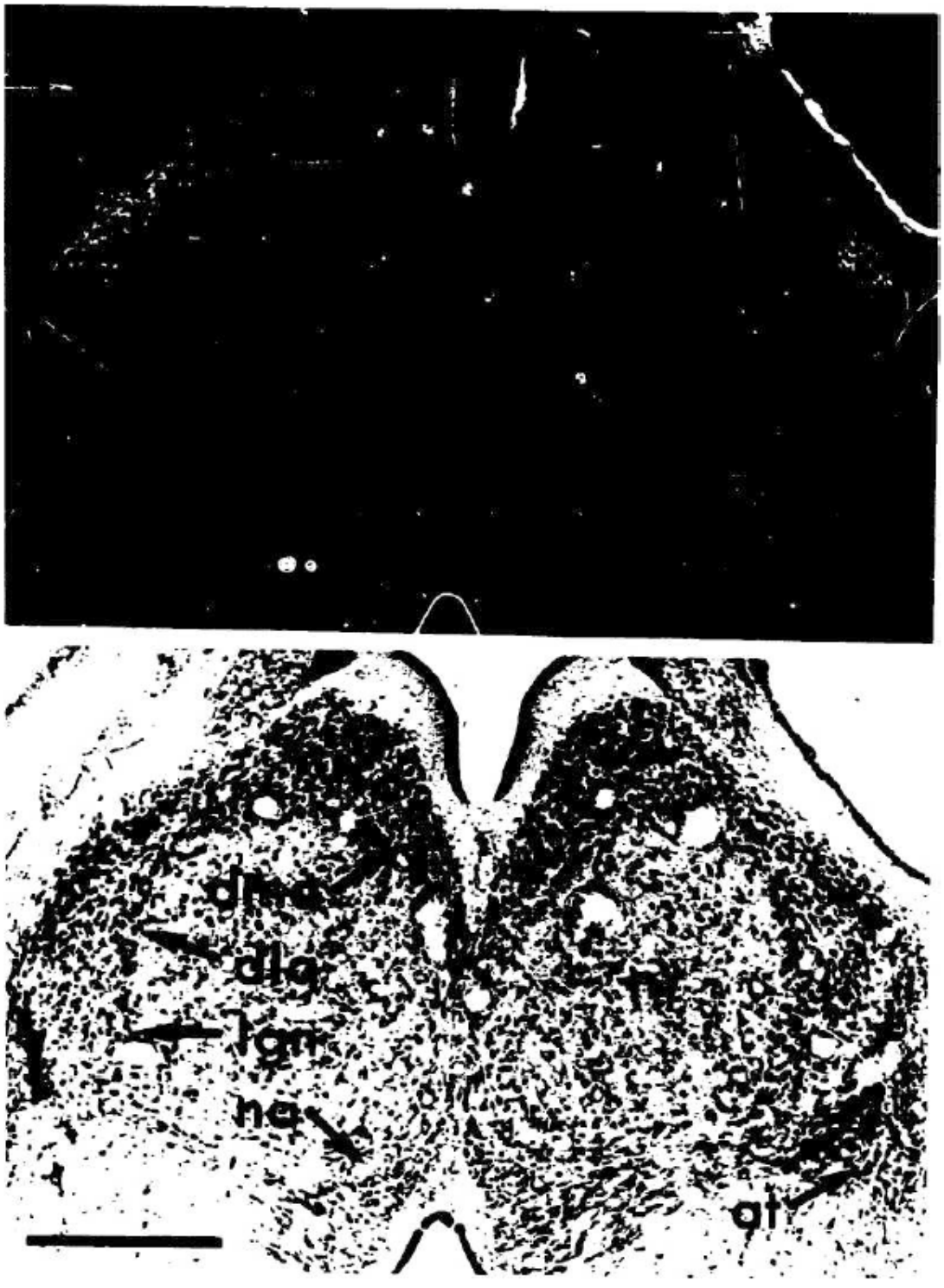

ulc
na

Figure 22. Comparison of ipsilaterally and contralaterally projecting cells in dla.

The top panel is a darkfield photograph of labelled cells in the ipsilateral and contralateral dla following an injection in D1. The lower panel is the same section stained with cresyl violet and rephotographed. The nucleus dla on each side of the brain has a partially separated external segment visible in the photograph: the contralaterally labelled cells are limited to this segment, while the ipsilaterally labelled cells extend further internally. (Note that the external segment in the left side oft the figure is somewhat narrower than the external segment on the right: the section is skewed and the left half is further caudal).

The scale bar denotes 500 microns.


dlm
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ne
or

F. TOPOGRAPHY OF THALAMOCORTICAL PROJECTION

The topography of the projection of the thalamus upon the forebrain was studied with small and large HRP injections in the cortex and striatum. A series of injections of various sizes in each cortical area at middle levels of the hemisphere was completed. Injections in area D2 at different rostral to caudal levels was also obtained. In addition, the medial and lateral portions of the striatum were injected.

Injections of cortical area D2 label two systems of cells in the thalamus. The first is a densely packed set of cells which are frequently heavily labelled, and the second a diffuse set of cells which are only lightly labelled. The results of an injection of moderate size into area D2 at about the middle of its rostrocaudal extent are illustrated in Figure 17, left column. The injection site covers both the pallial thickening and the medial part of D2, with little spread into D1.

A dense group of labelled cells occupies a long continuous zone in the ipsilateral thalamus. Labelled cells are found ventrally in the tip of the perirotundal shell at the rostral end of the thalamus. The cell group expands and fills the na. A few cells are present in the neighboring parts of dla. Further caudally this nucleus splits up. A few cells remain medially where the nucleus merges with the reu, and a larger number laterally where it merges with the dla. The lateral' group expands and continues caudally, as the rostral division of the lgn. The labelled cell group shifts away from the nucleus rotundus and extends to the caudal limit of the thalamus as the caudal division of the lgn. In short, the ventral edge of the shell of the thalamus is labelled along its rostral and lateral extent, as is the caudal extension of this zone, the caudal part of the lgn.

A few cells were labelled in the homologous regions of the contralateral thalamus. These cells were located in the dla and rostral lgn, and were found superficially in the cell plate.

A small number of cells were labelled over a widespread area of the thalamus, and are termed the diffuse system. First, scattered cells on the border between the nucleus rotundus and the lgn, dla, dma, and na were labelled: these cells have been termed perirotundal cells. Second, labelled cells were present in the dip, that portion of the shell caudal to the dla and dorsal to the lgn. They were present at much lover density than cells labelled in the lgn, and could represennt either a part of the parirotundal group or a division of the lgn.

The labelling of the diffuse system was variable in different experiments. It was best labelled with large injections of area D2, and was not usually seen at all with small injections. An experiment with extensive labelling of the diffuse group produced by an injection limited to area D2 is shown in Figure 17, right column. The injection covers rostral D2, including the pallial thickening. In the dense system cells are labelled in the rostral and caudal lgn. In the diffuse system, large numbers of perirotundal cells are labelled throughout the thalamus, as well as many cells in the inner parts of the caudal dma. Cells are also present at moderate density in the dlp.

The dense cell group projects with some topography to the cortex. Injections in caudal D2 label few or no cells in the caudal thalamus. Such a case is illustrated in Figure 18, right column. The injection site is at the caudal limit of area D2. The labelled cells in the thalamus are limited to na. Conversely, after an injection in rostral cortex few or no cells are labelled in the rostral thalamus. Such a case is shown in Figure 18, left column. The labelled cells are limited to the lgn. Note that in both these cases with smaller injections the diffuse system of projections is unlabelled. Thus, rostral parts of D2 seem to receive from more caudal portions of the thalamus, and caudal parts of D2 from more rostral thalamus.

Injections of the cortical area D1 also label both a dense and diffuse system of cells. An injection at middle levels of the area is shown in Figure 19, left column. A dense group of cells fills the external half of the nucleus dla, including the external segment, in the ipsilateral thalamus. Some cells are found in the adjoining na. A diffuse group of cells is present in the ipsilateral thalamus. Some cells are scattered in the outer half of dma adjacent to the nucleus dla. These cells continue in this location to the caudal end of dma. In addition, some cells are distributed in the deeper parts of dla below the heavily labelled zone. On the contralateral side the external segment of the dla contains labelled cells (in some cases 1abelling in the contralateral external segment was almost as heavy as in the ipsilateral). Thus, cortical area D2 receives input from a specific nucleus of the thalamus, and from a portion of that nucleus on the other side of the brain. It also receives diffuse input from a set of cells in the dma and deeper dla.

A series of injections of D1 at rostral and caudal levels was not available. However, injections involving the caudal end of D1 label a similar zone in the thalamus, suggesting either that dla projects equally to the caudal end of D1 or that the methods used cannot detect the topography of the projection of dla onto D1.

Small injections of D1 did not in general label the diffuse system in dma: that projection, like the analogous projection to D2, require heavy or widespread labelling of the cortex.

A small number of injections of the medial cortical areas were analyzed. These areas are difficult to inject without spread to the underlying thalamus. Three small injections of areas DM and M together and one injection of M alone produced retrograde labelling in the thalamus resembling that following small injections of D1. The nucleus dla was labelled ipsilaterally and its external segment contralaterally. The diffuse system was not labelled, as expected from the small size of the injections.

Injections were also made into the medial and lateral parts of the striatum. An injection of the medial striatum is shown in Figure 20, left column. The injection site corresponds to area d as identified by Riss et al. (1969), with spread into adjacent parts of the basal forebrain. There is some spread into the overlying DVR. This injection is medial to the path of the thalamocortical axons. In the thalamus the dma is densely filled with labelled cells. The dense lone of labelled cells extends into the deeper part of the adjacent dla. Scattered cells were labelled at the perirotundal surface of the remaining part of dla and na. A few cells were labelled within dla. Finally, many intensely labelled cells were present in the nucleus caudalis and nucleus reuniens, presumably because of the involvement of the ventral DVR.

The perirotundal cell group is more heavily labelled following injection of the lateral striatum. Such an injection is illustrated in Figure 20, right column. This injection occupies area 9 in the terminology of Riss et al. (1969), and lies in the region traversed by thalamic axons en route to cortex. The dma does not contain as many cells as in the previous case. There are many more perirotundal cells, chiefly in the nucleus anterior and the ventromedial border of the nucleus rotundus. The main portions of the lgn and dla are unlabelled.

Thus, the striatum receives input from a large population of cells in the thalamus, both from the nucleus dma and from cells distributed around the periphery of the nucleus rotundus. These regions are the same as those giving rise to the diffuse projection to the cortex.

G. COMMENT : RETROGRADE LABELLING OF AFFERENTS

The cortical areas of the turtle receive both a specific and a diffuse projection from the thalamus.

Within the specific system, area D2 receives input from the lgn and na nuclei of the thalamus. Nucleus lgn projects rostrally and the na caudally. Areas D1, DM and M together receive a projection from nucleus dla.

This map agrees with the results of transneuronal labelling of the visual pathway discussed above. The lateral geniculate projects to the anterior portion of area D2, of which the zone labelled by intra-ocular injections is a subset.

The map also agrees with the two experiments illustrated in the original report of Hall and Ebner (1970) on the thalamocortical projection in the turtle. Animal 598 has a lesion in the lgn and degeneration in area D2, while animal 600 has a lesion in dla and degeneration in a patch in what appears to be area D1. This experiment suggests the projection of dla may be topographic.

The zone of projection of the lgn mapped by retrograde methods matches that identified in the experiments of Heller (1983) in which HRP was injected into the lgn: anterogradely filled fibers could be traced across the pallial thickening and the cortical part of area D2, stopping at the medial border of D2.

These results agree also with experiments in lizards, provided a suitable transformation is applied. Lohman and vanWoerden-Verkley (1978) injected HRP into divisions of the DVR and cortex of the Tegu lizard. The lateral geniculate contained labelled cells after injections in the rostrolateral DVR. The nucleus dla contained labelled cells after injections in either the medial cortex or the dorsal cortex: the cells were located laterally in the nucleus after an injection of the medial cortex, medially after an injection of the dorsal cortex. Bruce (1982) performed a similar series of injections in *Gecko* and *Iguana*. The results were similar. The lateral geniculate projects to a lateral area of the DVR which she identified as pallial thickening. The nucleus dla projects to both the medial and dorsal cortex: a differentiated lateral segment, pars magnocellularis, projects to medial cortex, while a medial segment, pars parvocellularis, projects to dorsal cortex.

These results can be reconciled with those in turtle by assuming that the lgn projection area is mobile in evolution. In these lizards it is buried in the dorsal ventricular ridge; in turtles it is pulled out onto the surface of the brain. Additionally, the cells of dla have separated into two populations projecting to the dorsal and medial cortex. Presumably a stage in the process of nuclear differentiation is illustrated in these species.

The nature of the organization of the thalamic projection in the anteroposterior dimension of the cortex is not yet clear. While the lgn projects rostrally and the na caudally in area D2, this could either represent either a continuous mapping of the na-lgn band in the thalamic shell onto the cortex, or a dichotomous projection of two nuclei to two areas. Many injections of area D2 label much of the lgn, with a zone of greatest labelling at one level. This argues that the lgn is

mapped continuously onto the cortex. No injections were made in rostral or caudal area D1.

The group of labelled cells always occupied the full width of the lgn or dla following injections in the medial or lateral side of the relevant cortical area. There does not appear to be any mediolatera topography in the thalamocortical map. The observation by Heller (1983) decribed above that geniculate axons cross the entire width of area D2 makes such topography impossible.

There is an additional dimension of organization in that contralaterally projecting cells are always located at the external boundary of the thalamic shell. The cells projecting to contralateral D1 are numerous and form a partially separated segment of dla. Such cells are not segregated, however, as ipsilaterally projecting cells are also present in this segment. Only scattered cells were labelled in contralateral lgn after injections in D2. These were always located at the very rim of the lgn, or occasionally the adjoining dla.

The cortex also receives a projection. from a diffuse system. These cells are only retrogradely labelled by larger injections of HRP, and often are lightly labelled in any event. Presumably the projection of this system is formed by axonal arborizations that are lighter or more diffuse than those of the first system.

The topography of the projection of this system is not diffuse. Injections in area D1 label cells in superficial dma and deeper dla. Injections in area D2 label cells on around the border of the nucleus rotundus, the perirotundal cells. These two populations overlap minimally.

Three previous studies found that injections in each of the sectors in the DVR retrogradely label dense cells in a specific nucleus in the thalamus, and scattered cells in nucleus dma (in lizards, Lohmaa and van Woerden-Verkley, 1978 and Bruce, 1982; in turtles, Balaban and Ulinski, 1981). Widespread projections from dma seem to be a common reptilian pattern. The perirotundal cells are perhaps an extension of nucleus dma around the nucleus rotundus.

Figure 23. ChAT-positive cells in the forebrain of the turtle.

The location of ChAT-positive cells are plotted on evenly spaced coronal sections of the forebrain. Each dot represents one ChAT-positive cell. Most of the reactive cells are located immediately below the striatum among the fibers of the lateral forebrain bundle. Some are located in the nucleus of the diagonal band, and a very few within the septum.

The right hemisphere was traced in this figure.

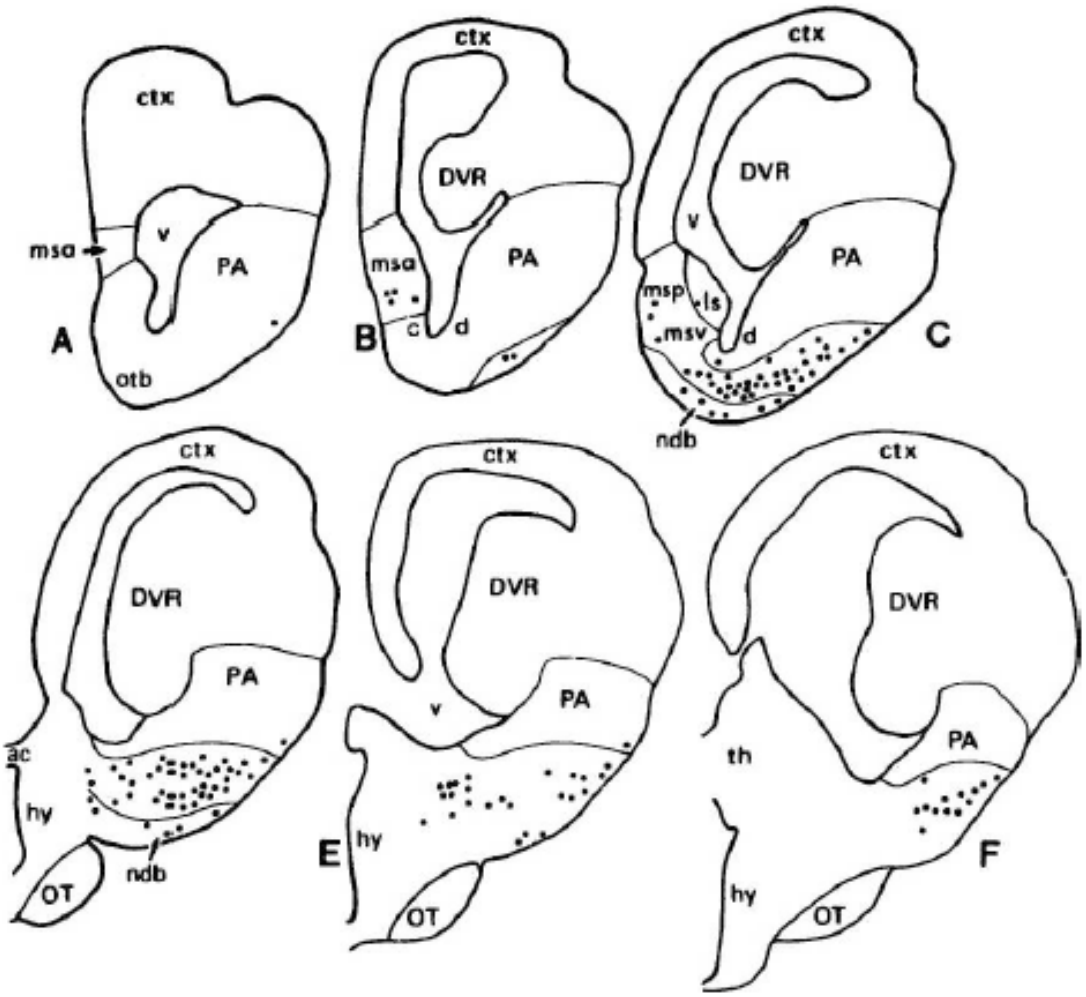
ctx
DVR
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Figure 23, continued. Cells of the nucleus basalle and the nucleus of the diagonal band.

B. Upper panel. Immunobistochemical localization of ChAT. The large arrow indicates a large cell of the nucleus basalis. The small arrows indicate cells in the horizontal limb of the nucleus of the diagonal band.

C. Lower panel. Neuron of the nucleus basalle back- filled with HRP from area D2. Note straight unbranching dendrites.

D. Inset. Neuron of the nucleus of -the diagonal band back-filled with HRP from area L. Dark field.

Both scale bars are 100 microns.

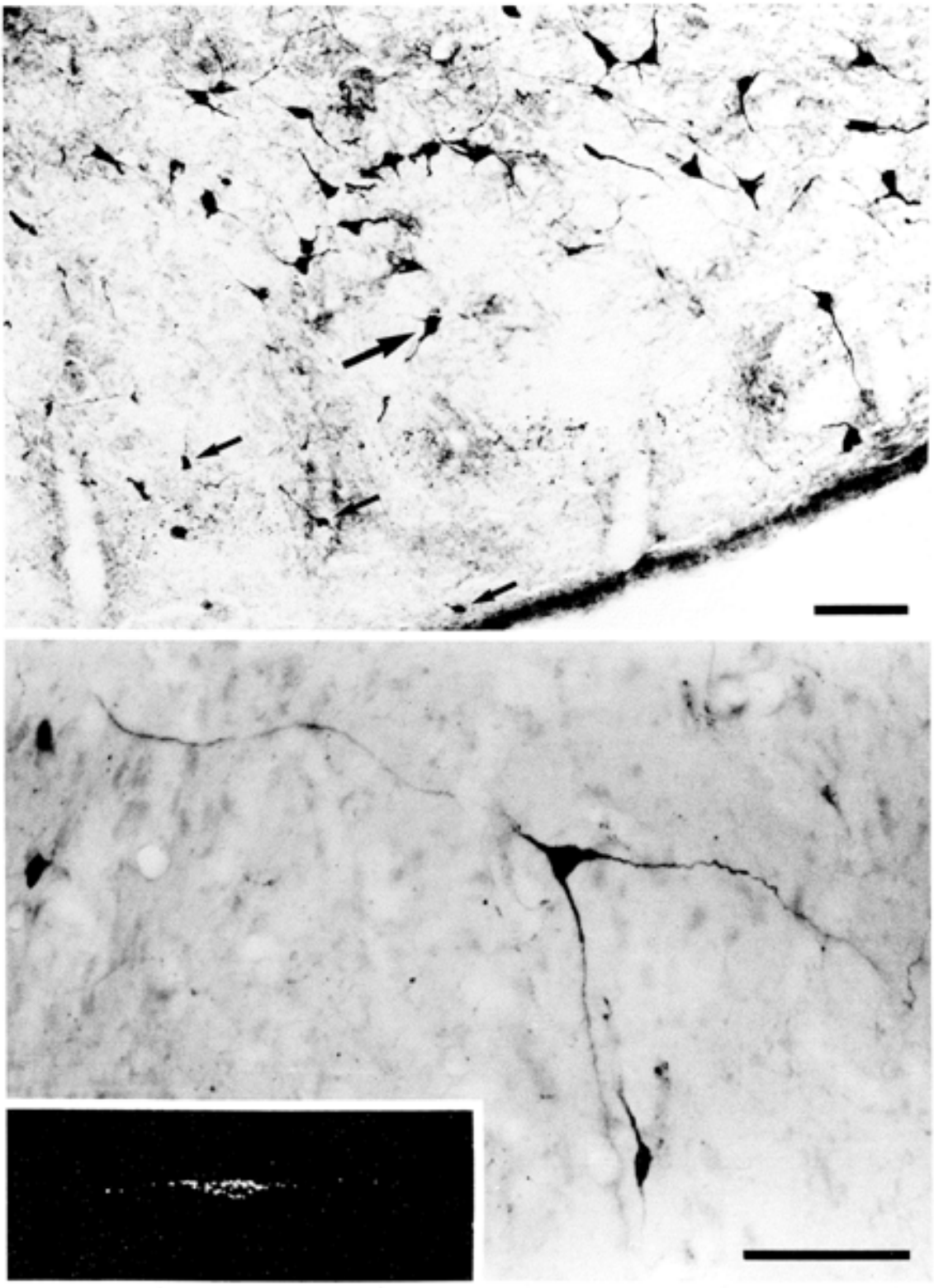
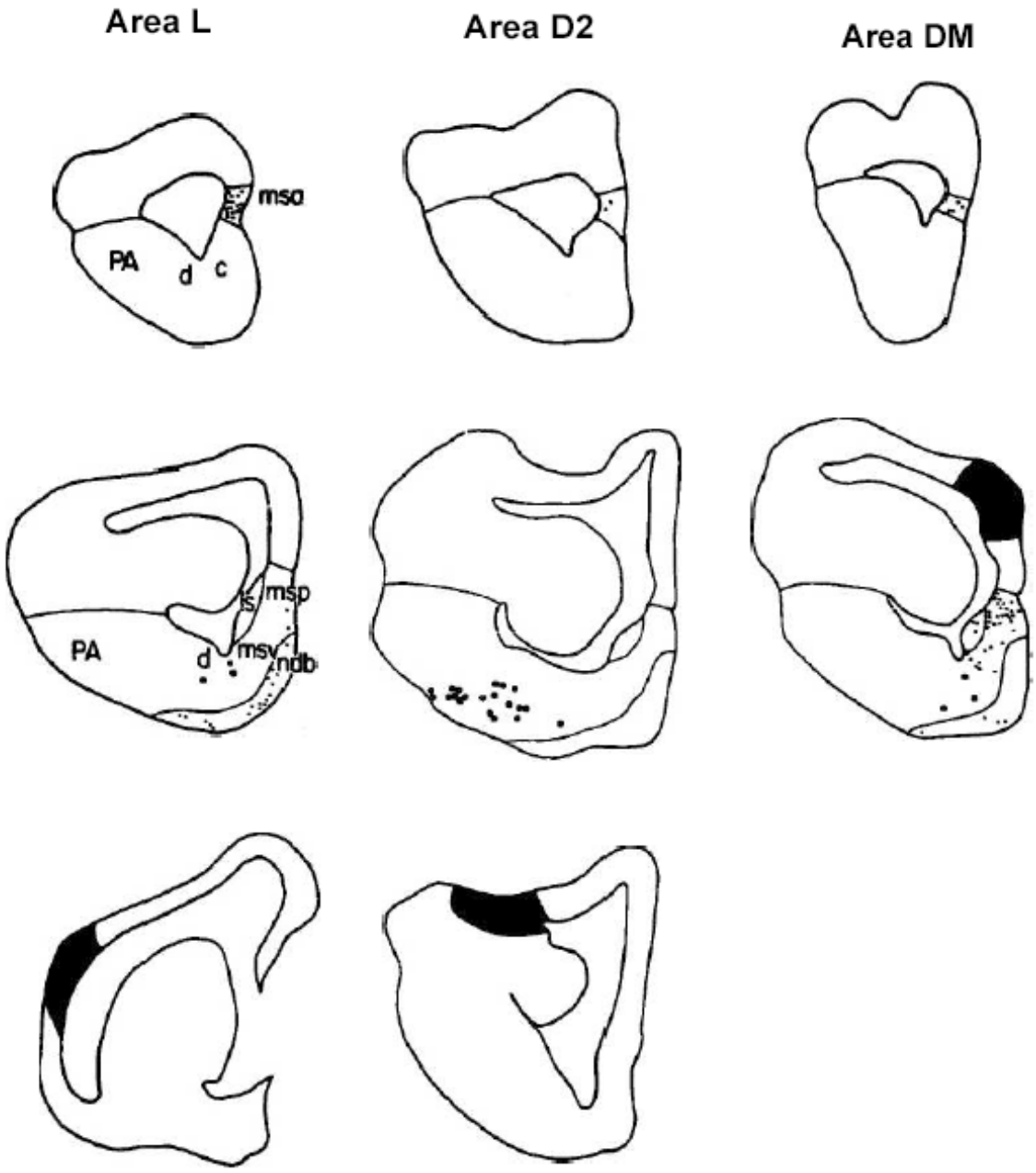


Figure 24. Distribution of cells in the basal forebrain labelled by HRP injections in areas L, D2 and DM.

The cells projecting to areas L, D2 and DM are indicated on sections through the anterior septum and posterior septums. Each section represents 120 microns of tissue. The injection site is marked in black on an additional tracing below, except the for injection in DM which was close to the level shown. Large dots: cells in the nucleus basalis.


Area L
Area D2
Area DM
mso
PA
d
c
msp
PA
d
msv
ndb

These regions also project to the striatum. The medial striatum receives frrm dma as well as a small number of scattered perirotundal cells. The lateral striatum receives from both dma and the perirotundal cells more equally.

The geometric arrangement of this system must have functional consequences. The perirotundal cells projecting to area D2 lie in the neuropil of the nucleus rotundus and every nucleus bordering it, and potentially project tectofugal and polysensory information to area D2. The diffuse system neurons projecting to area D1 lie in dma and presumably receive whatever information dma receives. Conversely, the lateral striatum receives not only input from the dma but also whatever information the perirotundal cells gather.

H. CHOLINERGIC NEURONS IN FOREBRAIN

Sections of turtle brain and spinal cord were reacted with two antibodies against the enzyme choline acetyltransferase. Three brains were reacted with AV8, a monoclonal antibody against mammalian ChAT raised by Wainer and Levy, using a peroxidase anti-peroxidase detection method. That work was performed in collaboration with Dr. Elliott Mufson and Dr. M.-Marsel Mesulam at the Department of Neurology at the Beth Israel Hospital. One brain was reacted with a polyclonal serum against CAT developed by Eckenstein (1983), using a biotin-avidin detection method. The results obtained with either method were similar.

In the forebrain clearly labelled cells were present only in the basal telencephalon. Three populations of cells were found. The largest population of cells lie along the external edge of the striatum. Such cells are present from just behind the level of the olfactory tubercle to just before the level of the amygdala. Most are located between the striatum and the lateral forebrain bundle or in the lateral forebrain bundle itself. These cells are large and stain intensely for ChAT. Their dendrites frequently stain and are long, slender and seldom branch. These cells are most numerous at the level of the anterior commissure, where they form a group obvious in Nissl-stained sections. Some cells are located in the diffuse outer edge of the nucleus accumbens. These cells are smaller and stain lightly for ChAT. Their dendrites did not stain. No cells were stained in the main body of the striatum.

Reactive cells were also observed in the nucleus of the diagonal band. These cells were small and lightly stained, and resemble tie cells of the nucleus accumbens. Such cells were common in the horizontal limb of the diagonal band but rare in the vertical limb. Stained cells of the nucleus basalis and nucleus of the diagonal band are compared in Figure 23B.

Finally, similar small, lightly staining neurons were occasionally observed in the septum. Most of these were in the medial septum. They were found in all parts of that area, including its dorsal and ventral extremes where it bends laterally to contact the ventricle. A few were located in the ls, usually rostrally. A small number of faintly labelled cells were observed in the same regions as the more distinctly labelled cells.

There were no clearly labelled neurons in the diencephalon.

In the midbrain and brainstem labelled neurons were intensely labelled in the motor nuclei of nerves III, V, VI, VII, X (dorsal motor nucleus) and XII, and in an area resembling the nucleus ambiguus of mammals in location. There were a few other areas of labelled cells. Unexpectedly there were labelled cells in the spinal nucleus of the trigeminal.

Spinal cord motor neurons were intensely reactive. Some cells stained in the ventral horns.

Sections reacted with an irrelevant immune serum showed no labelled cells.

I. BASAL FOREBRAIN INPUT TO CORTEX

The projection of the basal forebrain to the cortex was examined in the same series of cortical HRP injections used for the analysis of thalamic projections. In general, only the larger injections labelled basal forebrain neurons, and the labelled neurons usually were lightly labelled and few in number. In the summary figure each outline represents 120 microns of tissue traced from several sections. An anterior level through the anterior medial septum (msa) is illustrated, as is a posterior level through the posterior medial septum (msp), ventral medial septum (msv), lateral septum (ls), nucleus of the diagonal band (ndb) and the nucleus basalis (nb).

Following injections into area D2, labelled cells were observed in the msa and the nucleus basalis. The cells in the msa were concentrated at its anterior end. The cells in the nucleus basalis were more numerous and more heavily labelled in the lateral half of the nucleus; they were present over much of its anteroposterior extent. The cells were very large. Their dendrites, when filled, were thin, staight and unbranching (Figure 23C). In some cases a few cells were seen in the septum or nucleus of the diagonal band.

Following injections in areas DM and M, retrogradely labelled cells were found in all of the sectors of the medial septum, msp, msv and msa. Most of the cells in the septum were located dorsally in the msp, just medial to the fornix. Occasional cells were seen in the ls. Cells were also found in the external portions of the nucleus accumbens and the neighboring part of the nucleus basalis, scattered in the path of the descending fornix as it enters the forebrain bundle (described in the section on Efferent Connections of the Cortex). These cells resemble the nucleus basalis cells labelled from area D2. A small number of cells were seen in the nucleus of the diagonal band.

Injections in area D1 resembled either injections in D2 or DM-M. When the injection site was in the lateral half of D1, the pattern of labelled cells resembled that produced by injections of D2, except for the presence of relatively more cells in the medial half of the nucleus basalis and the septum. When the injection site was in the medial half of D1, the labelling pattern was identical to that following injections of DM-M.

Following injections in area L, labelled cells were present in both the vertical and horizontal limbs of the nucleus of the diagonal band. These cells were usually large and bipolar. An example is shown in Figure 23D. Some cells were labelled in the adjacent parts of the nucleus basalis. Cells were also present inn the msa and rostral msp.

J. COMMENT: CHOLINERGIC INPOT FROM BASAL FOREBRAIN

The same basal forebrain nuclei contain cholinergic cells in both turtle and mammal, but the distribution of the cells among the nuclei varies. In turtle, the nucleus basalis and the horizontal limb of the nucleus of the diagonal band contain large numbers of labelled cells. The septum and the vertical limb contain few labelled cells. In mammals the nucleus basalis is largely cholinergìc, at least 90% of its cells being cholinergic (Mesulam, Mufson, Levet' and Wainer, 1983). However, only 1% of the neurons of the horizontal limb of the nucleus of the diagonal band are cholinergic, while 70% of the neurons of the vertical limb are. The mammalian septum contains about 10% cholinergic cells.

Thus, both the turtle and mammal have a largely cholinergic nucleus basalis. However, the concentrations of cells in the two limbs of the diagonal band are exchanged. It is clear that some reorganization has taken place in evolution: the vertical limb of the diagonal band is the main source of the cholinergic afferents to the hippocampus but the lateral cortex is the only cortical area which receives a significant projection from it in turtles. Finally, the turtle septum contains far fewer cholinergic cells than the mammalian septum. A more sensitive technique might disclose a larger set of cells. In particular, the vaguely labelled cells in the septum may be the edge of a population containing a low level of ChAT or even an altered form of ChAT.

In turtles the septum projects to areas DM and M, the nucleus of the diagonal band to area L, and the lateral nucleus basalis to area D2. In mammals the septum and the vertical limb of the nucleus of the diagonal band project to the hippocampus and entorhinal cortex, the horizontal nucleus of the diagonal band to the olfactory cortex, and the nucleus basalis to the neocortex (Menulam, Mufson, Levet' and Wainer, 1983; Haberly and Price, 1978). Some reorganization of the nucleus of the diagonal band has occurred, which may be related to the shift in the density of cholinergic neurons. The obvious implications about the homology of turtle and mammalian cortical areas are considered in the general discussion.

A striking aspect of these results is the absence of cholinergic cells from the striatum. Apart from some overlap of the nucleus basalis with the outer edge of the nucleus accumbens (area c) or with the medial edge of the globus pallidus, there are no cholinergie neurons in the striatum in turtle. The striatum is the region of greatest AChase activity in the brain and must receive a cholinergic innervation. Perhaps the cholinergic innervation of the striatum in turtle comes from the nucleus basalis, and later in evolution some of these neurons migrate into the striatum. The association of AChase activity with the thalamic termination zones in the cortex suggests that nucleus basalis neurons project to these zones. They may have the same relationship with the striatal thalamic input.

K. ADDITIONAL INPUTS TO CORTEX

In the series of cases of injections of HRP in cortex, cells were also labelled in the DVR. Injections in are D1 label cells in the medial and ventral subdivisions, as defined by Balaban and Ulinski (1981). Injections in area D2 label cells in these subdivisions and in the dorsal subdivision. Injections in lateral area D2 label these same subdivisions, but very large numbers of cells in the dorsal subdivision. In good cases, the cells were well labelled in clear neuropil and appear to be labelled by specific transport from the cortex. However, the possibility that these cells were labelled by spread from the injection site into the ventricle cannot be ruled out. If anyone ever reads this, I'd like to hear. Please look me up in the Society for Neuroscience Directory and drop me a line. Thanks.

Retrogradely labelled cells were also present in the mammillary region of the hypothalamus. Only cases with injections involving the medial areas labelled large numbers of these cells.

CHAPTER THREE

Previous workers using degeneration methods have detected an orderly set of corticocortical connections in the snake and lizard. The lateral, dorsal and dorsomedial cortical areas in these species were found to project to progressively deeper zones in the molecular layer of the medial cortical area. The medial cortical area then projects back to a deep zone in the molecular layer of the more lateral cortical areas. This chapter describes a study of corticocortical connections in the turtle using small injections of anterograde and retrograde tracers. The first aim was to discover if the turtle possesses a pattern of cortical organization like that of other reptiles. The second aim was to study these connections at a higher resolution than possible with degeneration methods: in particular, to label these connections without any artifacts caused by lesions of fibers of passage, to determine the topographic organization and cells of origin of these projections, and perhaps detect connections within cortical areas.

The results indicate that a similar set of corticocortical projections exists in the turtle. The lateral and dorsal cortex project densely to the upper and middle third, respectively, of the molecular layer of the the medial areas DM and M, which in turn project back to the deep levels of the lateral and dorsal cortex. However, the present results expose several additional aspects of this system. The projection to the medial wall originates from the dorsal parts of area L, not the entire area; and from area D1, not from the entire dorsal cortex. Area D2 projects instead to a small region in D1 just dorsal to the medial wall areas. Secondly, different cell populations in the medial areas project back to these cortical areas L and D1. Third, these projections between cortical areas are crudely topographic, connecting portions of these areas at similar anteroposterior levels. Finally, each cortical area is connected with itself by a dense axonal plexus running along its whole length. These longitudinal projections run in the middle third of the molecular layer in areas L, D2 and D and the deep third in areas DM and M.

A. METHODOLOGICAL CONSIDERATIONS

Injections or iontophoretic deposits of HRP, HRP plus lysolecithin (HRP-11), HRP conjugated to wheat germ agglutinin (HRP-WGA) and radioactive proline were made in the areas of the turtle cortex. These tracers seem to diffuse relatively more in the turtle brain than in the mammalian brain. Injections of HRP alone yielded an extensive diffuse injection site surrounded by a large region of granularly labelled cells. Many bulk filled axons radiated form the injection site. Very small injections of HRP with lysolecithin were more useful, because the lysolecithin seemed to enhance the retrograde transport of the HRP more than its diffusion, and even small injections produced vigorous retrograde labelling. Moreover, the lysolecithin dramatically enhanced the axonal labelling obtained. The results below indicate that the labelled axons represent primarily the axons of cells within the injection site, rather than axons passing through the injection site or terminating in it. Iontophoresis of HRP produced a delimited injection site but less strong retrograde transport. In comparison to the injection of HRP-11, it gave very detailed filling of a smaller number of axons and thus was useful for analyzing the morphology of axons. This technique did not appear to favor filling of axons originating at the injection site. Iontophoresis of HRP-WGA produced the most compact deposits of tracer and the most specific labelling of anterograde projections. Anterogradely transported 1abe1 appeared as fine particles over the terminal regions, with little bulk-filling of intervening axons and terminal arbors. The intensity of the anterograde and retrograde transport was weak. Injections of tritiated proline could be used only for tracing long distance projections, as a large region around the injection site was involved in a halo of diffused proline.

Injections of HRP-11 were used in most of the experiments, because this tracer indicates efficiently both anterograde and retrograde connections. A typical injection site consisted of a dense region about 800 microns in diameter in which processes could not be resolved and a surrounding region about 1000-2000 microns in diameter in which axonal and dendritic processes occupied the full thickness of the cortex. Some experiments were done with iontophoresis of HRP for best visualization of axonal morphology. Iontophoresis of WGA-HRP was used for tracing the connections of the medial wall, where precise injection sites were needed.

Regions of terminal and non-terminal axons were distinguished as described in Methods. Figure 25 compares regions with and without terminal specializations.

This description is based on 30 cases with injections of HRP or conjugates of HRP limited to particular cytoarchitectonic zones: in D2, 11 cases; in D1, 7 cases; in DM-M, 8 cases; in L, 4 cases. The results of cases with larger injections not confined to particular zones were consistent. In addition, 7 cases with injections of tritiated proline were available, 5 in areas D2-D1 and 2 in area L.

The connections within the cortex wi11 be described first, and the descending connections of the cortex described in a subsequent section.

Figure 25. Comparison of terminal and non-terminal labelling patterns in anterogradely-filled axons.

Each panel represents the molecular layer of the area M. The top panel is further rostral and illustrates the terminal field of the projection of the area D2 to DM; the bottom panel is further caudal and shows only axons of passage.

Scale bar is 100 microns.

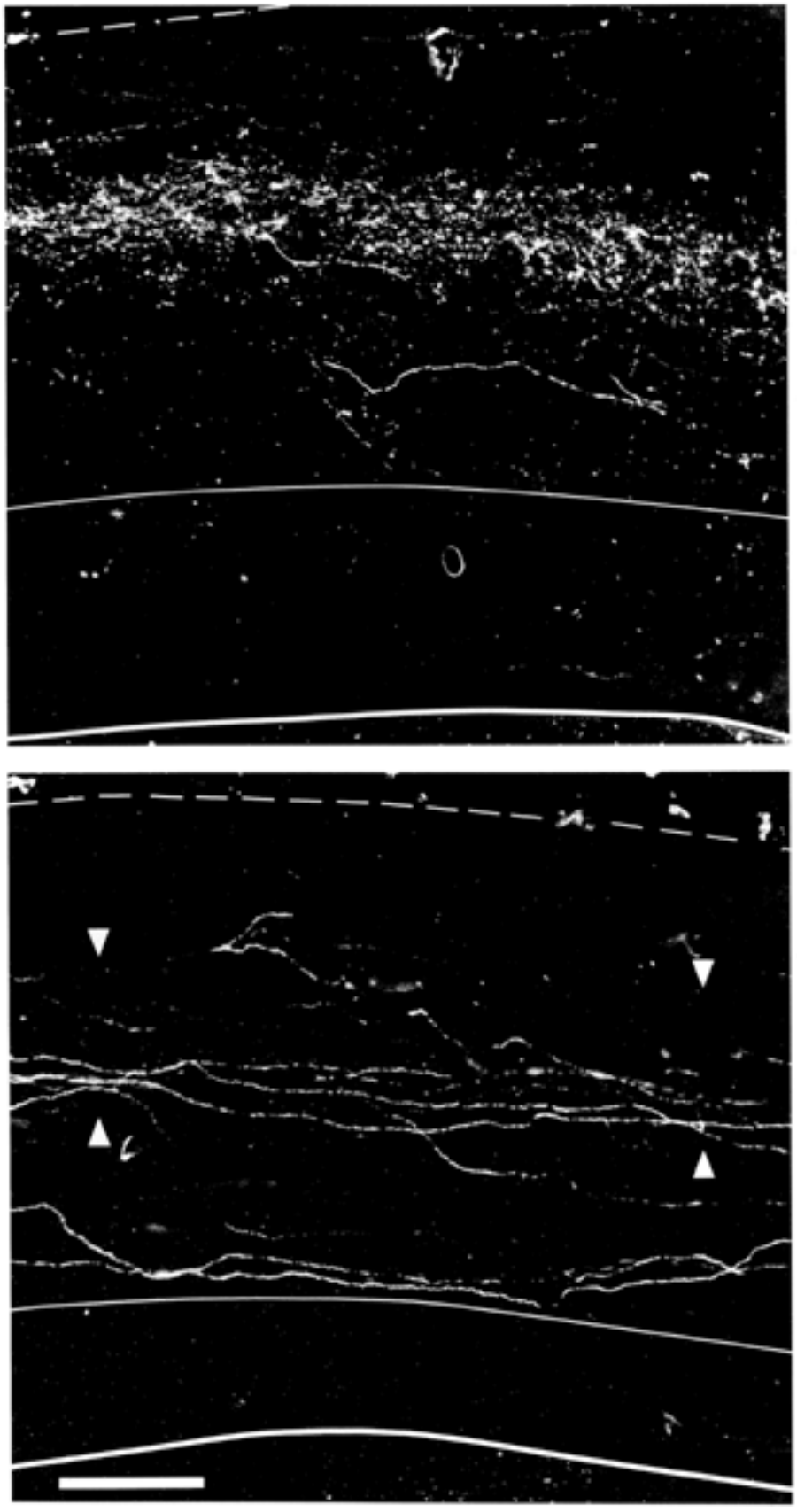


Figure 26. Comparison of labelling following injections in caudal and rostral area 1.

The location of terminal-like labelling (dots) and axons of passage (lines) have been plotted on five levels through the hemisphere. Retrogradely labelled cells are indicated by triangles. Each triangle represents several cells.

The upper row represents an injection of caudal area L.

The lower row represents an injection in a more rostral part of area L.

Scale bar, 500 microns.

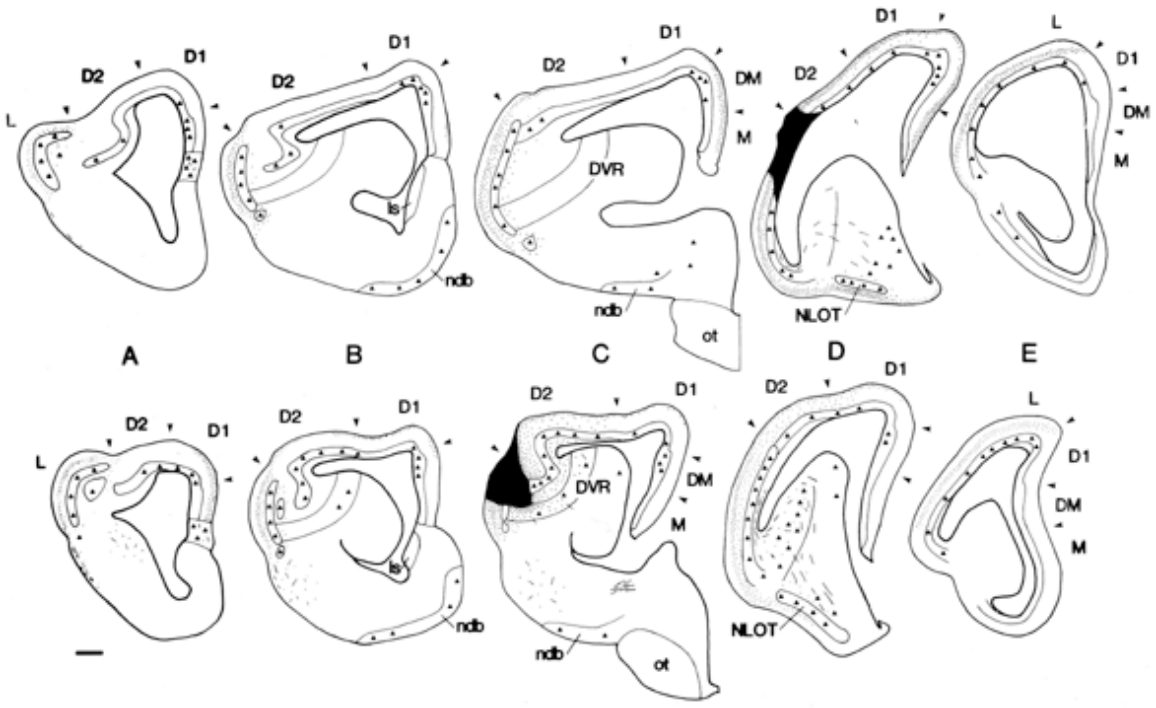

D2
D1
L
ls
ndb
DVR
DM
M
ot
NLOT
A
B
C
D
E

Figure 27. Axonal projections of area L.

The ventricular surface of the cortex has been traced with a thick white line. The cellular layer has been outlined with a fine white line. The pial surface has been emphasized with a dashed line when necessary.

Panels A, B and C are from the same experiment.

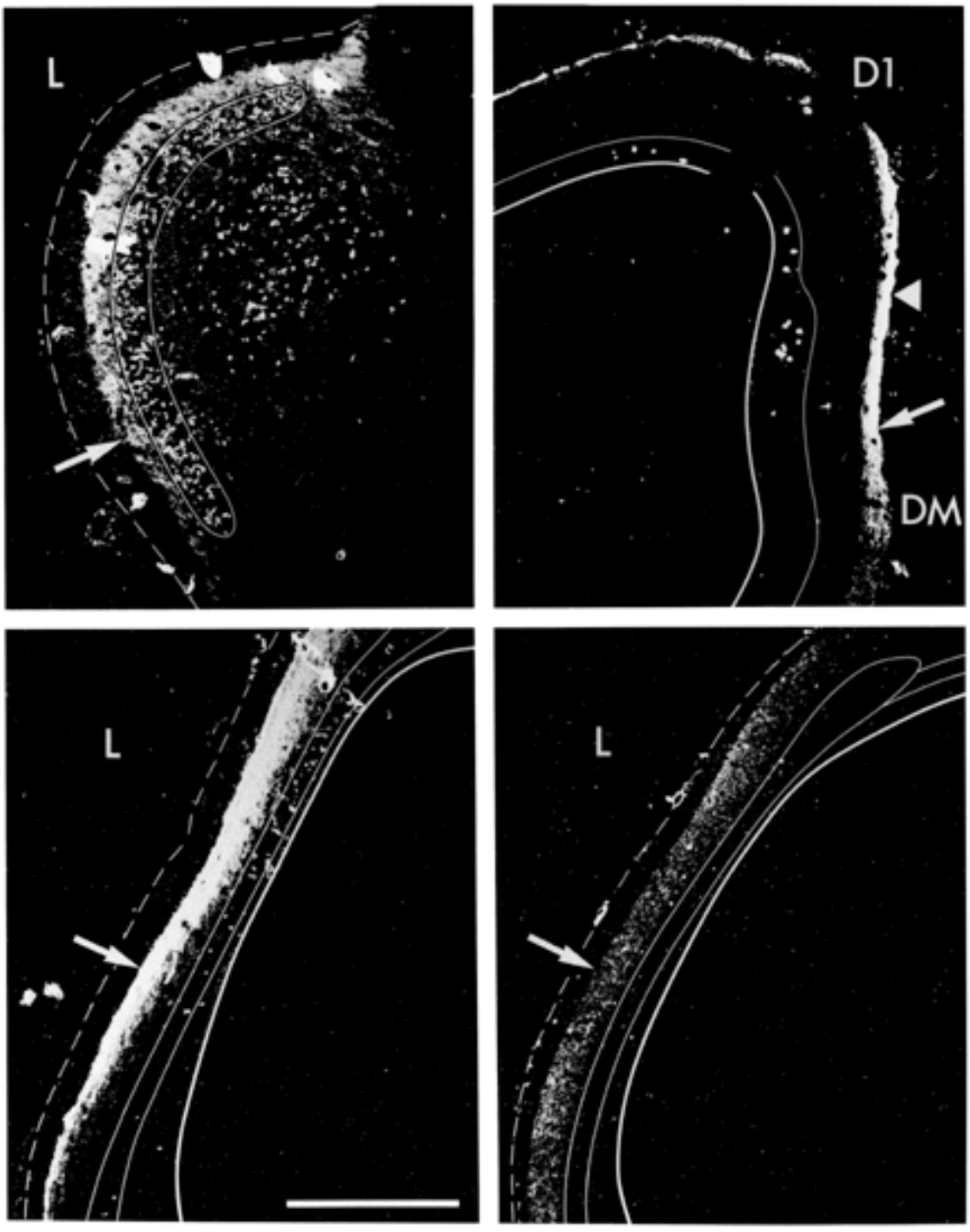
A. Upper left. Labelled terminal plexus (arrow) is present in the inner half of the molecular layer of rostral area L following an injection of HRP in caudal area L. Labelled cells are present in the cellular layer and in the space below the cell plate.

B. Upper right. Labelled terminal plexus occupies an external zone of the molecular layer of DM after an HRP injection in area L. Labelled cells are present around the boundary between DM and D1.

C. Lower left. Labelled axons form a dense plexus in the middle third of the molecular layer of caudal area L after an injection more rostral in L.

D. Lower right. A similar axonal plexus is labelled by a tritiated proline injection in rostral area L.

The scale bar applies to all panels and indicates 500 microns.


L
D1
DM
L
L

B. INTERNAL CONNECTIONS OF THE CORTEX

Injections of HRP-11 into area L label two systems of axons. These are seen in a reconstruction of an experiment in which HRP-11 was injected in the rostral part of area L (Figure 26, lower row). The first set of axons forms a plexus intrinsic to area L. The plexus extends through all of area L rostral, caudal, medial and lateral to the injection site. Its fibers have a terminal morphology. Around the injection site labelled axons are present throughout the thickness of the cortex. Beyond this zone axons and terminal specializations become concentrated in a horizontal lamina in the cortex. The upper border is located at about one third of the thickness of the molecular layer below the pia and is relatively sharp. The lower border is less precise and some processes are located in the cellular and subcellular layer. At greater distance from the injection site the lamina becomes thinner through an upward movement of its lower border. For example, at caudal levels of the area L the plexus occupies the middle third of the thickness of the molecular layer. A detail of this region from this case is shown as Figure 27C. Rostral to the injection site labelled axons become similarly concentrated in the middle third of the molecular layer, but axonal processes remain present in the cell layer and below, over a cluster of cells located internal to the lateral cortex proper (Figure 27A).

A second set of axons forms a plexus extrinsic to area L. At about the level of the injection site these axons continue medially from the intrinsic plexus into a thin superficial layer of the molecular layer of the dorsal cortex. They do not form any apparent terminal specializations but may bear periodic varicosities. Whether these axons form synapses within the dorsal cortex cannot be determined. They continue diagonally rostrally and medially to the medial wall areas DM-M, where they expand into a dense plexus occupying the superficial third of the molecular layer (Figure 26, photograph Figure 27B). The terminal field is located rostral to the injection site in L, and is narrow in the anteroposterior dimension.

Injections of tritiated proline in area L label both of these axonal systems (Figure 27D), confirming that the HRP-11 method labels axons mainly in the anterograde direction in this system. The axons of the olfactory bulb projection pass through the injection site but are relatively unlabelled in either the anterograde or retrograde direction. (Neither are the afferent axons from the medial wall areas, described below, significantly filled).

Each of these axonal projections is accompanied by labelled cell bodies. First, labelled cells are present throughout the cellular layer in area L, indicating that all regions of area L project to the region of the injection site. The cell group below rostral area L is also labelled, confirming its functional association with the olfactory cortex. Second, labelled cells are present in the molecular layer on the medial wall, in the dorsal portions of area DM and the adjoining regions of Dl. The cells extend over a substantially greater anteroposterior distance than the region of the terminal plexus.

An injection of HRP-11 placed more caudally in area L also labels two sets of axons. A reconstruction is shown in Figure 26, upper row. First, a similar intrinsic axonal plexus is labelled. As with the former case, this plexus is widest near its origin and decreases in width further away. The external border of this plexus is precise and coincides with the external border of the axonal plexus labelled previously. Second, axons proceeds rostromedially across the top of the molecular layer of dorsal cortex and gives rise to a terminal arborization over areas DM and M. This arborization is also narrow anteroposteriorly, and is located caudal to that labelled in the previous case. Retrogradely labelled cells are scattered through the molecular layer of area L, and present in dorsal area DM and adjacent area D1 over a wide anteroposterior extent.

INJECTIONS OF AREA D2

Injections of HRP-11 into the either dorsal cortical area D2 or D1 label four sets of axons: a projection within the area injected, a projection medially, and two sets of axons leaving the cortex.

First, such injections label an intrinsic axonal plexus much like that in area L. A plexus labelled after an injection in rostral D2 illustrates typical features (Figure 28). In the vicinity of the injection site labelled axons are spread throughout the cortex. At greater distances the axons become progressively restricted. Injections at other levels of D2 produce a similar pattern of labelling. The upper border of the plexus is the same in all experiments. It varies systematically at different anteropoterior levels, and appears to correspond to the lower border of the thalamocortical termination zone. The intrinsic plexus extends to the rostral and caudal limits of D2. Injections in medial or lateral D2 produce axonal labelling which is stronger in medial or lateral D2: there is some topographic organization of these axons within D2. Retrogradely labelled cells are present in the cellular layer under the plexus along the length of D2.

The plexus of axons and the area of retrograde cell labelling sometimes continue a short distance into area D1 near the level of the injection, so these regions are freely interconnected.

Secondly, some axons run medially from the plexus in area D2. A typical case is reconstructed in Figure 29, upper row. Around the level of the injection site some fibers course medially in the molecular layer of D1 with minimal terminal

specializations. These fibers ascend and form a terminal plexus in the superficial molecular layer of the medialmost portion of D1 adjacent to DM, termed the longitudinal plexus (Figure 30A). This plexus contains many axons running in an anteroposterior, direction, and extends along the edge of D1 anteriorly and posteriorly. Anteriorly it increases in size and extends onto the very rostral medial wall: this extension follows the curve of the cortical areas so that this plexus is always on the border of DM (the plexus is visible on the medial wall in Figure 29, upper row, A). Posteriorly the plexus decreases in size but may reach to the caudal end of D1.

In addition, scattered axons which may have periodic varicosities but not explicit terminal features run from the longitudinal plexus across the molecular layer of DM and M (Figure 29, upper row, B). These axons run in the lower two thirds of the thickness of the molecular layer. Such axons are present mainly at levels near the injection site. (Figure 25 compares these axons with those in the extension of the longitudinal plexus onto the medial wall).

Third, a group of axons leaves the injection site or the intrinsic plexus and run rostrolaterally out of the cortex. These axons run straight courses without terminal features. Most gather under the pia, a lesser number at the ventricular surface and some travel at intermediate depths in the cortex. These axons fan out in the pallial thickening and proceed out of the cortex. They are further described in the section on the projections of cortex. Some of these fibers may be retrogradely filled axons from the thalamus, but many originate from deeper axons in cortex which are unlikely to be thalamocortical. A group of these axons is marked by double arrows in Figure 28.

Fourth, some axons run in a direct path along the ventricle to the exit of the fornix at the level of the septum. These axons are labelled by injections of tritiated proline in the dorsal cortex, so that such bundles must largely contain corticofugal fibers. Their eventual course is described in the next section.

INJECTIONS OF AREA D1

Injections of area D1 also label four sets of axons, of which three are similar to those labelled by injections of area D2. A typical case is shown in Figure 29, lower row.

First, injections of D1 label an intrinsic plexus like that of D2 (Figure 30B). Injections near the border with D2 label a rostrocaudal plexus which extends across the border; the intrinsic axon systems of the two areas seem to be continuous. As in areas L or D2, retrogradely labelled cells are present in association with the anterograde plexus.

Figure 28. Intrinsic axonal plexus of area D2.

Axonal labelling is illustrated at levels 1200 microns apart after an injection in area D2. The top photograph is the furthest caudal. The injection site is visible in the third panel.

The axon]. plexus becomes progressively concentrated in the middle third of the molecular layer with increasing distance from the injection site.

Scale bar indicates 500 microns.

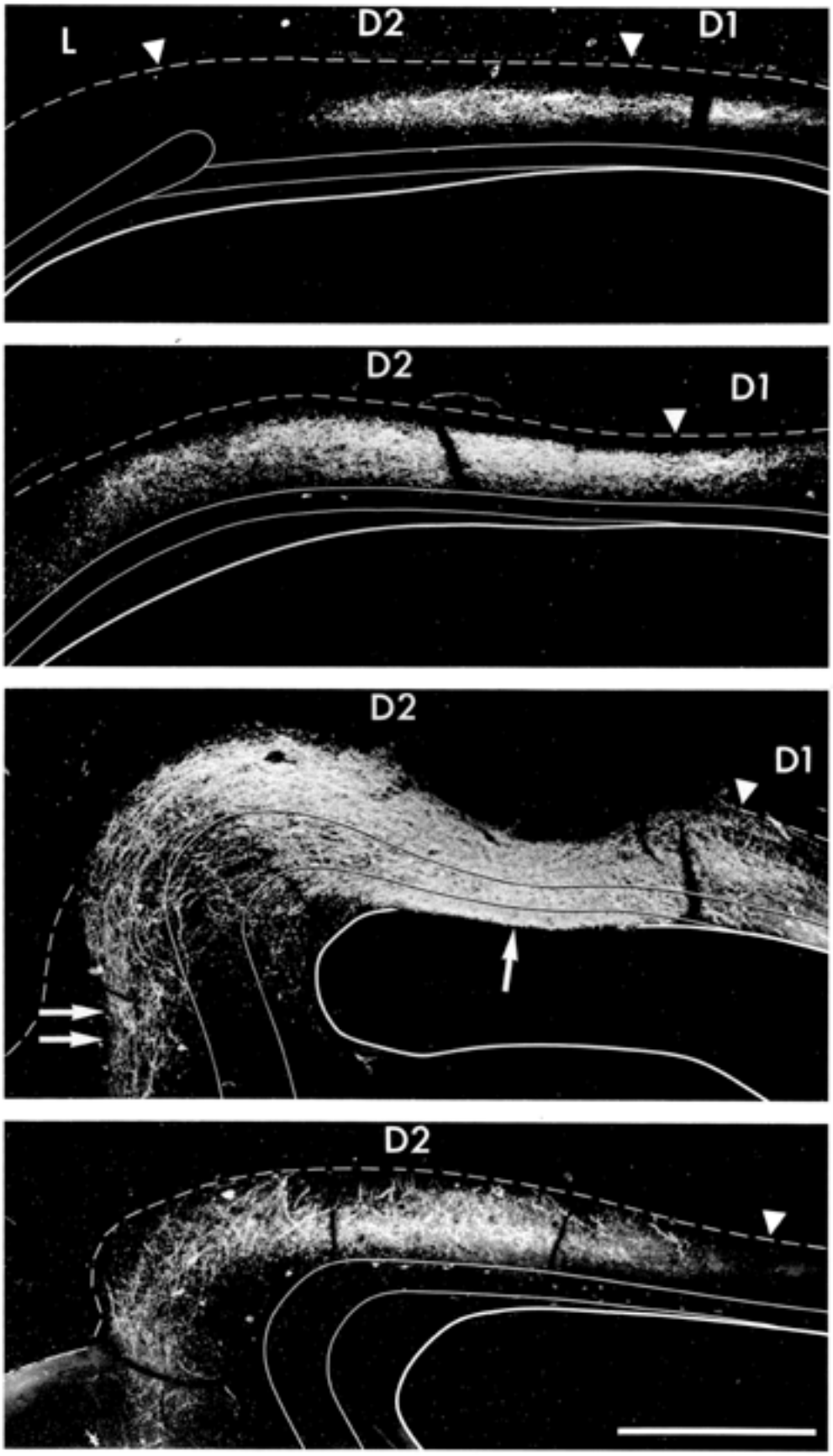

L
D2
D1
D2
D1
D2
D1
D2

Figure 29. Comparison of labelling following injections in areas D2 and D1.

The location of terminal-like labelling (dots) and axons of passage (fine lines) bas been plotted on five evenly spaced levels of the hemisphere. Retrogradely labelled cells are indicated by triangles.

The upper row represents an injection of area D2.

The lower row represents an injection of area D1.

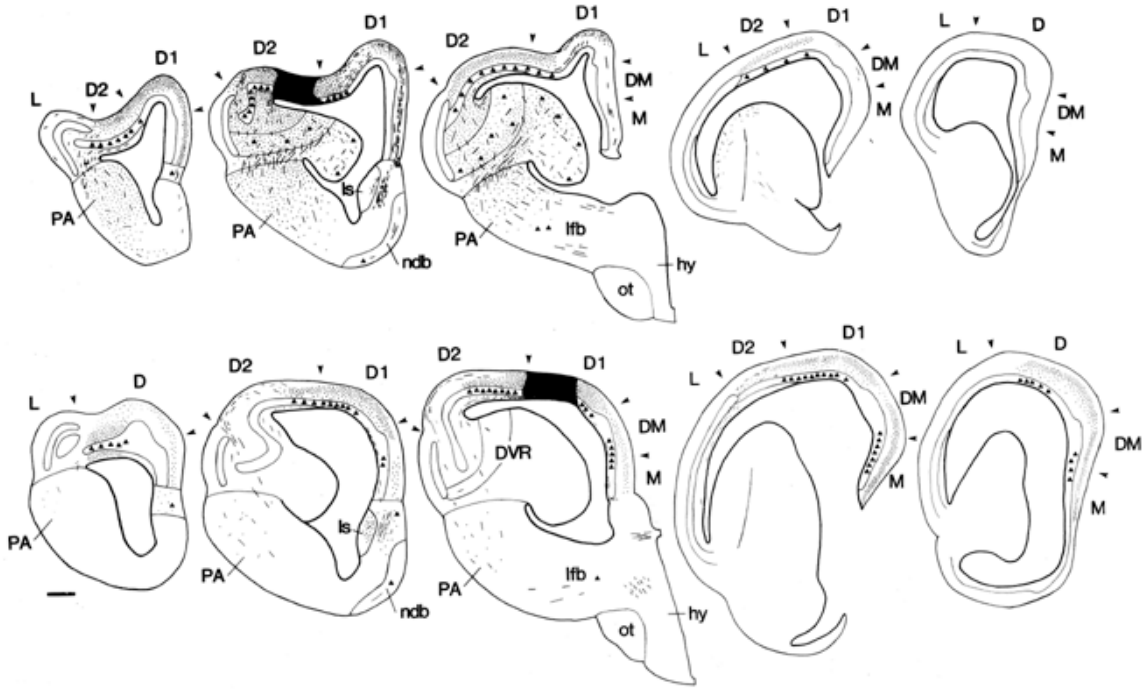

L
D2
D1
PA
ls
ndb
DM
M
lfb
hy
ot
D
DVR

Figure 30. Axonal projections of dorsal cortical areas.

Landmarks indicated as in Figure 27.

A. Upper left. Axons from area D2 cross Dl and form a plexus (arrow) near its medial border.

B. Upper right. Caudal to an injection in area D1, axons are limited to the middle depths of the molecular layer. None are present in the zone of thalamic terminations, whose border is indicated by an arrow.

C. Lower left. Axons from area D1 form a dense plexus in the middle third of the molecular layer in areas DM and M.

D. Lower right. The same projection as in C. is labelled by an injection of radioactive proline in the dorsal cortex.

Scale bar measures 500 microns.

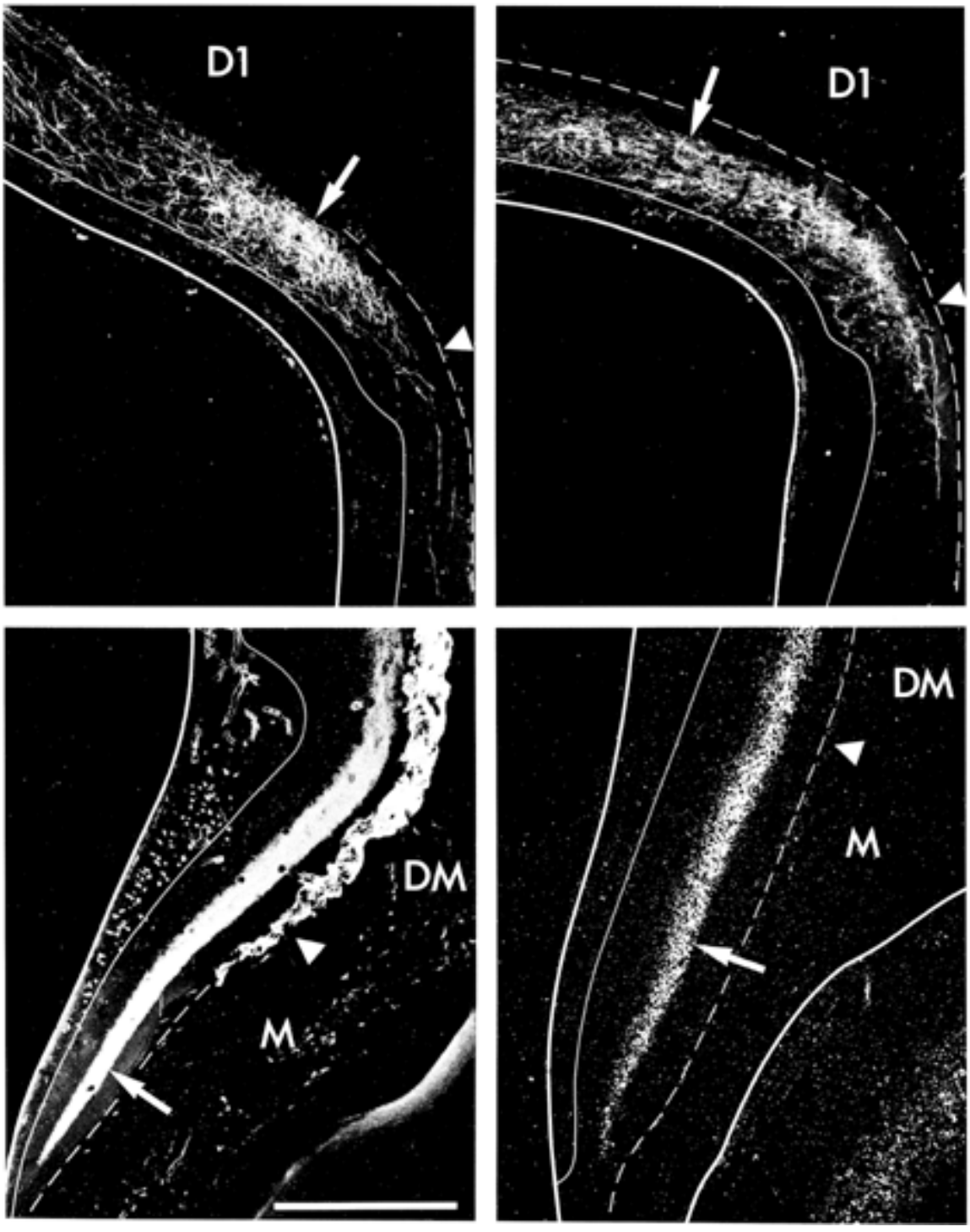

D1
D1
DM
M
DM
M

Figure 31. Distribution of cells on the medial wall projecting to area L and to area D2.

The medial wall has been flattened in this representation. The thick line represents the ventral edge of area M, the thin lines the boundaries between areas M, DM and D1. Each dot represents one cell. In areas of dense labelling the cells are represented by double and triple rows of dots.

The upper panel shows an experiment in which area L was injected. The labelled cells straddle the border between D1 and DM. The lower panel shows an experiment in which area D1 was injected. The labelled cells straddle the border between DM and M, and do not overlap significantly with those projecting to area L.

Scale bar, 500 microns.

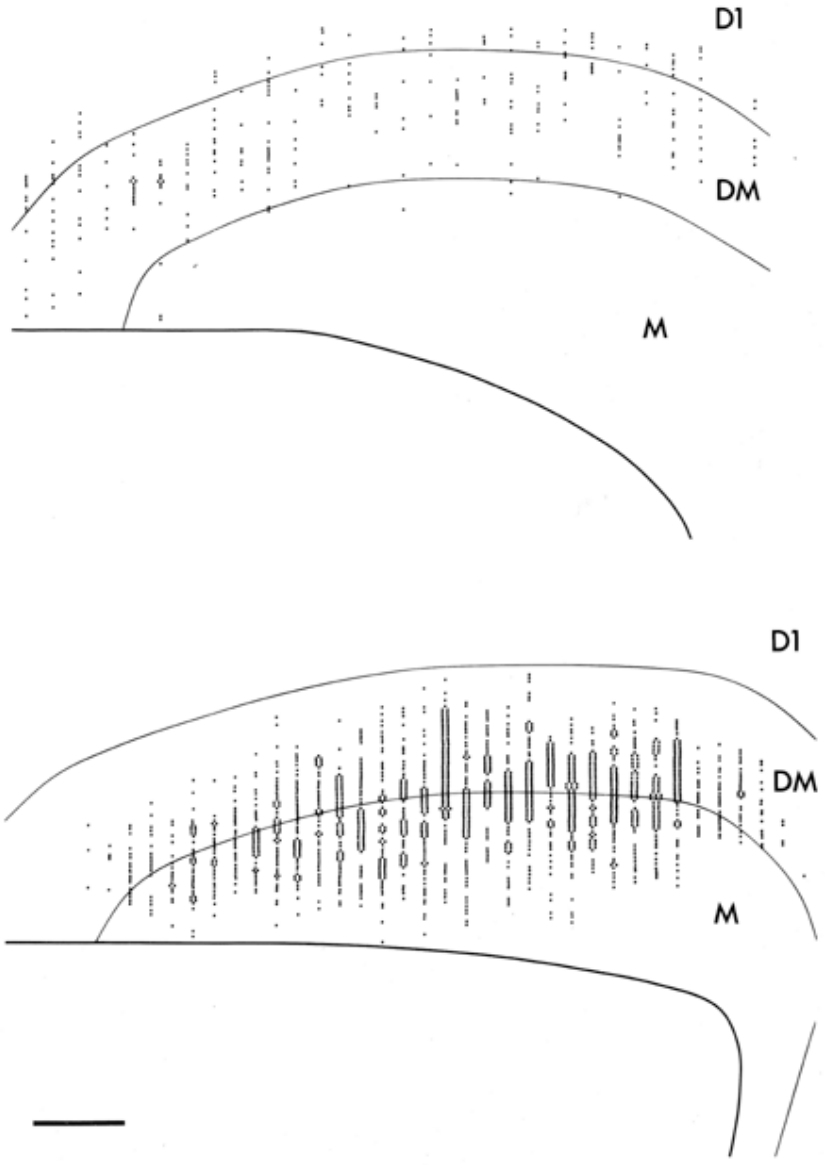

D1
DM
M
D1
DM
M

Figure 32. Reconstruction of injection in area M.

Scale and conventions as in Figure 29.

The injection site is in area DM at the caudal end of the hemisphere.

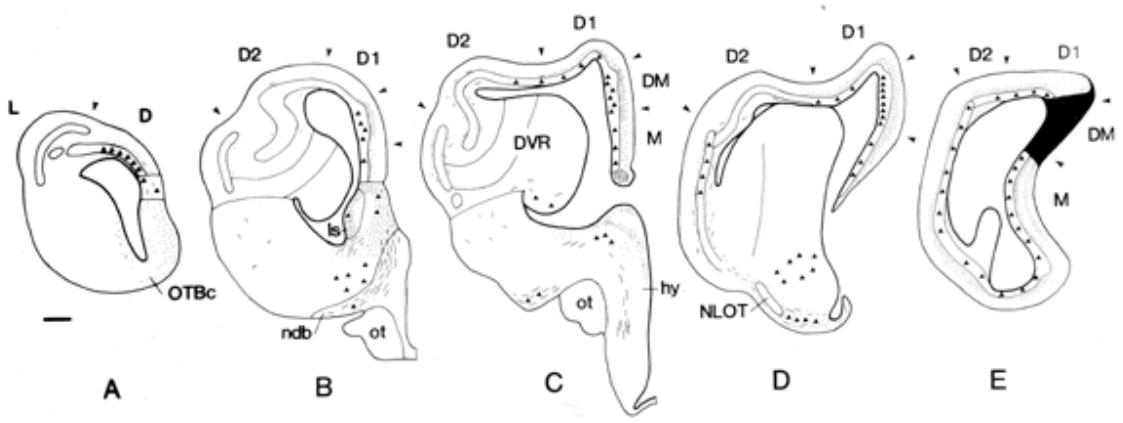

L
D
OTBc
A
D2
D1
ls
ndb
ot
B
D2
D1
DVR
DM
M
ot
hy
C
D2
D1
NLOT
D
D2
D1
DM
M
E

Figure 33. Axonal projections of area M.

The edges of the cortex and its landmarks are indicated as in Figure 27.

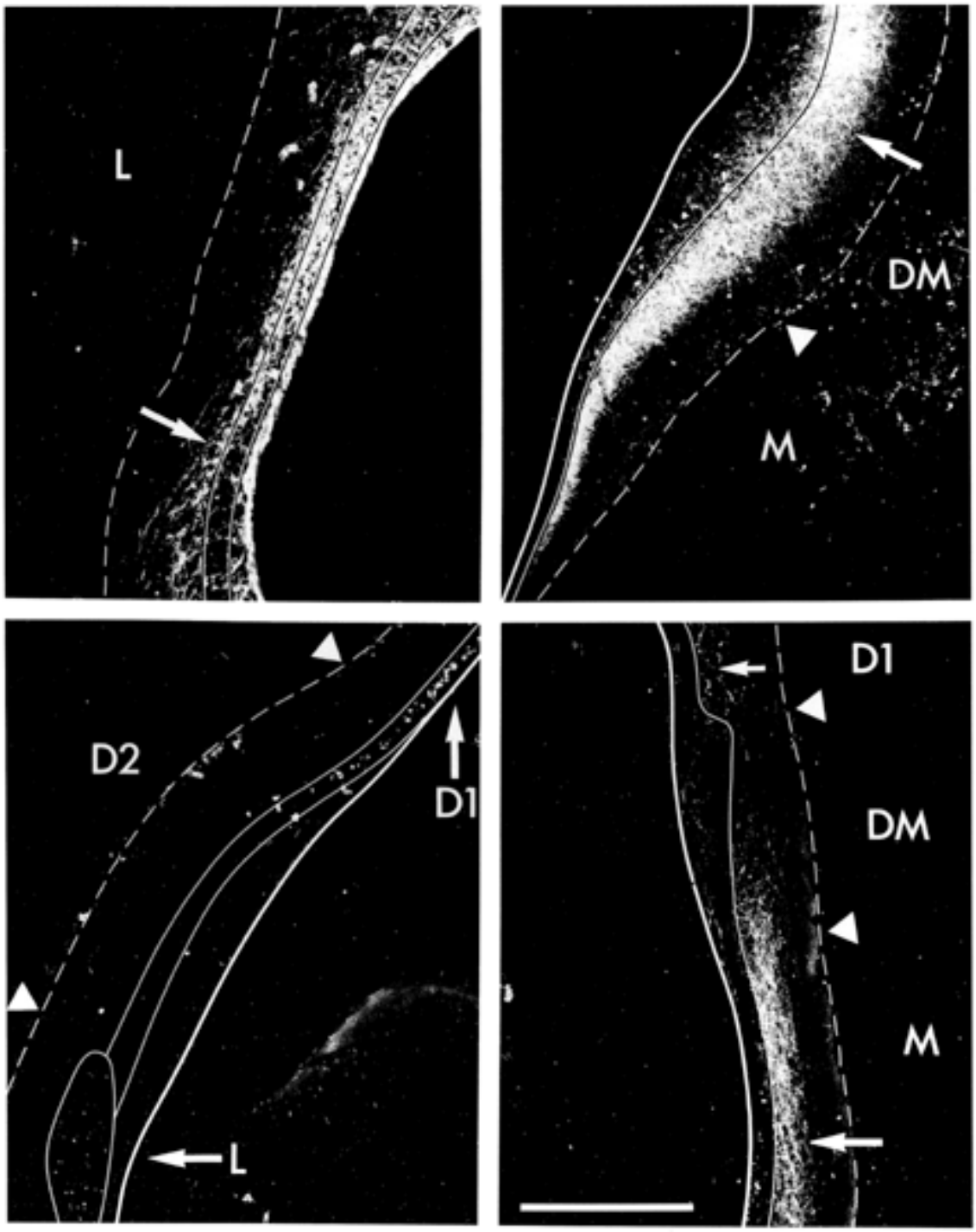
A. Upper left. Labelled axons are limited to the deepest part of area L after an injection in area DM.

B. Upper right. Labelled axons are limited to the deepest third of the molecular layer after a more rostral injection in the medial areas.

C. Lower left. Retrogradely labelled cells are seen in areas L and D1 but not in area D2 after an injection in the medial areas.

D. Lower right. The axonal plexus is neatly limited to area L and to the bottom third of the molecular layer after an injection in caudal area M.

Scale bar, 500 microns.


L
DM
M
D2
D1
L
D1
DM
M

Second, unlike area D2, area D1 gives rise to a dense projection to the medial areas, DM and M. This projection arises from the axons of the intrinsic plexus and terminates in the middle third of the molecular layer of DM and M (Figure 30C). All of the injections of area D1 were at middle levels of the hemisphere. Following injections of moderate size, the terminal field on the medial wall was most dense slightly caudal to the injection, but sparse terminal labelling could be found over cost of the length of the upper limb of the curve formed by the medial wall areas.

These projections were confirmed in cases injected with tritiated proline (Figure 30D).

As in area L, the projection onto the medial wall is reciprocal: labelled cells are present at the boundary between DM and M over a large anteropogterior extent. Figure 31 is a reconstruction of the location of cells labelled after injections in D2 and L on an unfolded view of the medial wall (in fact, these are the cases drawn in Figure 26, upper row, and Figure 29, lower row). The cell populations projecting to area D1 and L overlap only at their edges.

Finally, injections of D1 label axons leaving the cortex by a lateral path and axons running medially along the ventricle like those labelled by injections of D2.

INJECTIONS OF AREAS DM AND M

In most of the present cases DM and M were injected together, as it is difficult to confine an injection to area DM and even more difficult to inject a deeply buried area M. Injections of area DM-M label two sets of axons in the cortex, one intrinsic and one extrinsic.

First, an axonal plexus is located in the inner third of the molecular layer of the medial wall areas. Near the injection site the plexus covers both DM and M. Rostral and caudal to the injection site the plexus gradually decreases its extent, so that it occupies a zone analogous to the locus of the injection. Thus Figure 32 is a reconstruction of an injection in caudal DM: at caudal levels both DM and M contain labelled axons (section D) but at rostral levels only DM contains heavy label (section B). Conversely, Figure 33D is a photograph of the rostral medial wall after an injection in caudal area M. The label is neatly limited to a the inner third of the molecular layer of area M. Intermediate injection sites produce intermediate bands of label: the deep axonl plexus of the medial wall is continuous in the same manner as that in D1 and D2. The external boundary of the labelled zone corresponds to the inner boundary of the afferent plexus from area D1 (e.g., compare Figure 33B and 30C). Retrogradely labelled cells accompany the labelled plexus.

Second, sparse labelled axons with terminal specializations are found in two patches. The first is located in the sub-cellular layer, the cellular layer and the innermost edge of the molecular layer in area L at or caudal to the locus of the injection site (Figure 33A). The second is located in similar lamina in area D1 at or just rostral to the level of injection (Figure 33D). Only a few scattered fibers are present in D2. Retrogradely labelled cells are situated among the fibers of these patches in area L and D1, but few or no cells are labelled in D2 (Figure 33C).

Some contralateral projections were labelled in these experiments.

After some injections in D2 a few axons were traced to the anterior commissure (by the lateral pathway described below) but lost in the contralateral basal forebrain. Scattered cells were labelled in the contralateral pallial thickening.

Injections in the medial parts of area D1 label cells is the contralateral medial wall. These cells are identical in location to the cells projecting to area D1 from ipsilàteral medial wall.

Injections in the medial wall label a similar population of cells at the DM-M border. The cell labelling is most dense at the level of the injection. Sparse fibers and terminal 3peeialization is present in the inner third of the molecular layer in areas DM and M. In some cases this labelling extended into adjoining D1.

B. COMMENT: INTERCONNECTIONS OF THE CORTEX

In summary, the cortex of the turtle is interconnected by two sets axons. The first set of axons runs mainly lengthwise, and interconnects each area with itself. The second set runs roughly perpendicular the first and interconnects the different areas. Areas L and D1 project to areas DM and M; area D2 projects to a longitudinal band in D1. Areas DM and M project back to L and D1.

The first set of axons, the intrinsic plexus, extends deeper in the cortex near its origin than far rostral or caudal in the lateral or dorsal cortical areas. This suggests that it is composed of axonal arborizations which slope up from their point of origin. The axon collaterals which ascend obliquely into the middle of the molecular layer in the Golgi method might represent the morphological basis of such intrinsic connections. The axon collaterals which form the association plexus of the medial areas must run horizontally in the bottom third of the molecular layer.

In the dorsal and medial cortex, the plexus extends longitudinally in front and behind the injection site regardless of its location in the mediolateral plane. Consequently, the arborizations of individual cells must be asymmetric in the longitudinal direction. In the lateral cortex, the intrinsic plexus is always filled to all the borders of the area, and presumably many cells have axonal arborizations over the whole lateral cortex.

The projection of L onto the medial wall is topographic and directed to a zone on the medial wall anterior to the injection site. Such a topography may be necessary so that area L which increases in width caudally can map evenly onto the medial areas. A ventral region of L does not contain labelled cells after any injection in the dorsal part of the curve of DM and M on the medial wall. Perhaps this region projects to the ventral limb of DM and M.

The heaviest projection of D1 onto the medial areas is directed to a zone caudal to the injection site. This trajectory is approximately perpendicular to the long axis of the cortical areas, which run rostromedially to caudolaterally. However, an injection of reasonable size in central D1 labels scattered axons over the entire length of the dorsal part of the curve of DM and M on the medial wall: the divergence of this connection is considerable.

Some aspects of the topography of the projections between D1 and the medial wall areas have not been established conclusively. First, the topography of the projection of D1 to the medial areas has only been studied with retrograde labelling after injections in caudal or central DM and M. All of the injections in D1 were at central rostrocaudal levels, and labelled the same region of DM and M. No information on the projection of the rostral D1 to the rostral DM and M is available. Contersely, the projection of rostral area M, which is very difficult to inject, has not been labelled. The lamellar topography observed in the caudal hemisphere probably prevails in the anterior hemisphere.

Area D2 has a complex medial projection. First, its axons arborize in a long plexus at the medial edge of D1. Presumably this plexus serves as an afferent to area D1, and the information it carries is relayed to the medial areas. Secondly, the longitudinal plexus increases in size anteriorly, implying that it collects rostral going axons. At the rostral end of the cortex it occupies the medial wall, apparently a rostral extension of DM. Thirdly, scattered axons are found in the medial areas at levels around the injection site. Many of these travel in the middle third of the molecular layer where D1 afferents terminate, but others run in the inner third. Such axons may have varicosities but whether these axons make synapses or a significant number of synapses is unclear. Certainly the magnitude of this input to the medial areas is trivial compared to the input from D1. Fourth, area D2 must be coupled to area D1 by the axonal arborizations of cells near the border between them.

These results agree with previous studies of the intracortical connections of other reptiles in finding that the lateral, dorsal and dorsomedial cortical areas project to successively deeper tiers in the neuropil of the medial cortical area (Lokman and Mentink, 1972; Lokman and Van Woerden-Verkley, 1976; Ulinski, 1976). The present results differ in that the projections from the lateral and dorsal cortex in the turtle terminate in similar layers in the dorsomedial (DM) area as well. In turtle, the lateral part of dorsal cortex or D2 does not project to the medial wall: as noted in the previous chapter, D2 is probably homologous to an area of the DVR in other reptiles.

Ulinski (1976) observed a projection from the medial cortex to all three more laterally lying cortical areas: to the thickness of dorsomedial cortex except perhaps the most superficial molecular layer, to the inner two thirds of the molecular layer of dorsal cortex, and all of the molecular layer of lateral cortex In turtle the projection of medial cortex includes areas DM, D1 and L but is limited to the cellular layer and inner third of the molecular layer in these areas. Lotman and Mentink (1972) found a projection to the dorsal cortex terminating throughout its thickness except the superficial and deep edges. The lamination of the projection of the medial cortex in snakes and lizards is thus not as precise as in turtles. Lokman and Mentink (1972) did not observe any projection to the lateral cortex, which may reflect a true species difference or a failure of the silver-stain method. In turtles the projection to the dorsal cortex derives from cells straddling the DM/M boundary, and the projection to the lateral cortex derives from cells near the DM/D1 boundary, while in snakes and lizards the lateralward-projecting cells are restricted to the medial cortex, area M, alone. The organization of area DM is apparently radically different in turtles.

Each of the connections described by Ulinski (1976) in the snake is characterized by a distinctive topography: following a lesion in the lateral, dorsal or dorsomedial areas degeneration is present in the medial cortex at the same level as the lesion and at all levels further caudal. Similarly, different levels of the medial area project to similar or more caudal levels of the dorsomedial or dorsal areas kowever, particular levels of the medial cortex project to similar levels of the lateral cortex only. Lohman and Mentink (1972) found different levels of lateral and dorsal cortex to project to the same and more caudal levels of medial cortex; by contrast, the dorsomedial cortex projects to more rostral levels of medial cortex. In turtle each level of lateral, dorsal and dorsomedial cortex projects most densely to a particular level of medial cortex, like the projection of the medial cortex upon the lateral cortex in the snake. Moreover, individual levels of dorsal cortex (D1) project diffusely to a large extent of the medial wall areas.

Finally, the plexus of axons which interconnects each of the cortical areas in turtle has not been described in snake or lizard. Perhaps the organization of these areas is very different, or degeneration methods do not adequately stain the fine axons of the cortical neuropil.

These axon systems of the cortex connect each point of the cortex with every other in one or two synapses. While the sensory inputs to the cortex are quite precise, these connections suggest that the cortex acts as a polysensory unit. The laminated termination zones of these projections and the laminated inputs from the thalamus and olfactory bulb are complementary to each other. Together they occupy almost the whole molecular layer of the cerebral cortex. The major unoccupied dendritic territory is the basal arborization of area D2 cells: perhaps these dendrites receive the afferent input from the dorsal division of the DVR, the major remaining afferent to area D2.

C. DESCENDING PROJECTIONS OF THE CORTEX

These experiments also labelled some projections of the cortical areas within the telencephalon.

Following injections of the dorsal cortical area D2 two sets of projections are labelled.

The first set derives from the axons, mentioned above, which leave the cortex laterally. These axons fan out through the dorsal division of the DVR, forming profuse terminal structures (Figure 29, section B, C). In the caudal hemisphere where the dorsal division of the DVR is not wrapped around the lower edge of D2, the axons detour long distances in order to innervate the whole extent of the dorsal DVR (section D). A lesser density of axons and terminal-like material is present in the remainder of the DVR.

These axons continue to the lateral portion of the striatum, PA, and form a dense terminal field there (Figure 29A, B, C). In the caudal levels of the striatum the terminal field occupies only a lateral portion of PA. In rostral levels the terminal field occupies almost all of PA. Scattered axons arborize among the cells of the nucleus basalis.

Some of these axons continue to descend in the lateral forebrain bundle, and join the descending fibers of the fornix described below. A small group of fibers runs to the anterior commissure, as noted above.

The second set of axons derives from axons which traverse the molecular layer of the medial wall areas. Upon reaching the ventral edge of the cortex these axons run forward to the exit of the anterior fornix (defined below). The axons leave the cortex as a distinct fascicle and proceed directly through the septum and into the lateral forebrain bundle. In some cases there seemed to be sparse terminal-like structures near the fornix in septal areas msa and msp. Some scattered axons are present coursing along the curve of the nucleus of the diagonal band.

In contrast, injections of the medial wall areas labelled several axon systems innervating much of the medial telencephalon.

First, axons exit the cortex all along its rostral border with the septum. These axons form a terminal plexus over all the septal areas, and over the striatal areas c and OTBc. The label over the striatal region extends past the ventral tip of the lateral ventricle just slightly. It is approximately complementary to the terminal field labelled from area D2 at this level.

Second, axons run along the curve of the nucleus of the diagonal

Third, two bundles of axons leave the cortex. They are termed here the anterior fornix and posterior fornix. The anterior fornix exits in the rostral msp, the posterior fornix immediately behind it. The anterior formi proceeds through the septum and into the lateral forebrain bundle. The posterior fornix derives largely from axons located in the periventricular level of the cortex. It runs to the anterior commissure, but some axons leave the main bundle and enter the lateral forebrain bundle. The cell-free zone in the septum extending from the cortex to the anterior commissure corresponds to the posterior fornix.

Injections in area D1 label an intermediate pattern. Lateral injection sites label relatively more of the area D2 pattern, medial injection sites more of the medial wall area pattern.

Injections in area L label fibers to cell groups located ventrally adjacent to area L: areas al, c1 and NLOT. The projection to NLOT is confined to the deep half of the molecular layer, and is approximately complementary to the olfactory bulb projection.

D. COMMENT: DESCENDING PROJECTIONS OF CORTEX

This informal study of the projections of the cortical areas makes two claims First, the major cortical input to the septum is from the medial wall areas and medial D1. Second, a projection to the AChase-rich basal telencephalon is a major output path for both area D2 and areas DM-M. The two parts of the cortex project to distinct fields. If one allows that the nucleus accumbens of the turtle is a striatal area like the mammalian nucleus accumbens, (Heimer and Wilson, 1975; Nauta, 1979), then a primitive version of a topographic projection onto the striatum exists.

DISCUSSION

The inputs, interconnections and outputs of the cortical areas of the turtle are highly specific. The olfactory bulb projects to area L, the thalamus to areas D2, D1, DM and M. Each area receives an input from cell groups in the basal telencephalon: area L mainly from the nucleus of the diagonal band, area D2 from the lateral nucleus basalis, areas DM and M from medial nucleus basalis and the septum, area D1 from an intermediate combination of the preceding two patterns. Area L projects to a superficial lamina in areas DM and M, area D1 to a deeper lamina. Area D1 receives afferents from area D2, from the dorsal division of the DVR by way of area D2, and probably direct input from the remaining divisions of the DVR. Area DM and M project back to the areas L and D1. Each cortical area is interconnected with itself by a rostrocaudal plexus of axons. Area D2 projects to the lateral division of the striatum, while areas DM and M project to the medial division of the striatum and to the olfactory tubercle. Area D2 has only a limited projection to the septum, areas DM and M an extensive one. The descending projections of area D1 are intermediate between those of its neighbors.

This discussion considers whether these results justify any homologies between the cortical areas of the turtle and the cortical areas of mammals. To say that areas in two species are homologous means that they both derive from one area in a common ancestor. In this case we are comparing the cortex of the turtle, which we assume to be relatively close to the common ancestor, with the cortex of the mammal, which is highly evolved. In evolution an area may become more complex, acquire new afferents or efferents, form subdivisions. The central assumption behind assigning homologies is that an area serves a particular purpose in the brain, and so certain aspects of its structure and connections will remain constant during that evolution. An area may become more complex or differentiated into subdivisions in evolution: the simpler system is then homologous to a more complex one or to multiple subdivisions taken together as a group.

The olfactory bulb in mammals projects to a collection of contiguous regions with corticoid structure: the anterior olfactory nucleus, the olfactory tubercle, the piriform cortex, the nucleus of the lateral olfactory tract, the cortical nucleus of the amygdala, the lateral entorhinal cortex and the tenia tecta (Price, 1973). In all of these regions the olfactory input ends in the superficial molecular layer, Ia. These regions are connected together by an association system whch ends in the deep molecular layer, Ib, and in the deeper cellular layers. In some regions the association projections seem to run in broad strips; these strips cross boundaries between areas (Haberly and Price, 1978). This organization resembles the lamination of superficial afferent connections and deep association connections in all of the turtle cortical areas. The elongate association connections resemble the rostrocaudal connections of dorsal or medial cortex.

The projection of the thalamus in mammals is complex. Herkenham (1980) summarizes the patterns of these projections. Afferents from the intralaminar nuclei terminate primarily in layers V and VI. The ventromedial nucleus projects to layer I of almost all neocortex. A number of nuclei project to layer I in multiple cortical areas, with projections to deeper layers differing in the different areas: the visual lateral posterior nucleus, the somatosensory posterior nucleus, the auditory magnocellular geniculate nucleus, the complex ventral anterolateral nucleus, the lateral dorsal nucleus which projects to retrosplenial cortex. Similarly, the nucleus reuniens projects to layer I in the hippocampus, layers I and III in the entorhinal cortex (Segal and Landis, 1974; Herkenham, 1978). Lastly; the ventroposterior, medial and lateral geniculate and mediodorsal nuclei project to layers III or IV, and possibly layers I or VI. Projections to layer I are perhaps a more fundamental pattern than projections to the middle depths of cortex.

The projection of nucleus dma and the perirotundal cells to the striatum and cortex in turtles may be homologous to the similar projection of the intralaminar nuclei in mammals (Jones and Leavitt, 1914). As in mammals, the projection to the cortex is diffuse, and the projection to the striatum dense. The projection of the perirotundal cells to area D2 and to the lateral striatum are brought in register by the projection of area D2 to the lateral striatum, as occurs in the mammalian imtralaminar projection (Jones and Leavitt, 1974). Ulinski and Balaban (1981) found that the nucleus dma projects to the full thickness of the medial wall cortex, unlike the projection of other thalamic nuclei to a superficial zone of the molecular layer. The nature of the projection from the perirotundal cells to area D2 may be similar.

The specific projections of the thalamus to the cortex and DVR in the turtle may be homologous to particular thalamic projections to neocortex in mammals. The projection of nucleus reuniens to the hippocampus and entorhinal cortex may be similar to the projection of the nucleus dla to the medial wall and adjacent area D1. Whether the sensory thalamocortical projections of the turtle are homologous to the expansive projections to layer I or the lemniscal projections to layer IV of the thalamus of mammals cannot be decided. In fact, the many thalamic projections of

mammals presumably originate from a simpler primitive set, so that multiple projections in the mammal can be homologous to one in the primitive set. Moreover, the common ancestor of turtles and mammals may have had only an undifferentiated thalamocortical projection, so that turtles and mammals may be evolving an unrelated set of projections.

The thalamostriatal projection is larger than the thalamocortical projection in the turtle brain. Perhaps the thalamostriatal projection came first, and the thalmocortical projection derived from it. It is hard not to see movement in the partially separated cell groups of the turtle brain: for example, to see the lateral geniculate nucleus and the nucleus dorolateralis anterior separating from the surface of the dma-perirotundal system.

The main input to the mammalian hippocampus is not the thalamus but the cortex. Two general studies of the pattern of corticocortical connections in the monkey demonstrated the step-wise progression of association areas from each of the primary sensory areas (Pandya and Kuypers, 1969; Jones and Powell, 1970). These progressions converge in the parahippocampal gyros. Other studies have shown that many association areas project directly and indirectly to regions of the parahippocampal gyros, and that these regions project to the entorhinal cortex (review: Van Hoesen, 1982).

The entorhinal cortex is the main input to the hippocampus (Cajal, 1911; Lorente de No, 1934; Hjorth-Simonsen, 1972; Hjorth-Simonsen and Jeune, 1972; Steward, 1976). The entorhinal cortex consists of two subdivisions, medial and lateral. The lateral entorhinal cortex, which receives direct and indirect olfactory input, gives rise to the lateral perforant path. The medial entorhinal cortex receives neocortical input and gives rise to the medial perforant path. The lateral perforant path projects to the superficial part of the molecular layer of the dentate gyrus and the field CA3, while the medial perforant path projects to the middle of the molecular layer of these sectors. Both the lateral and medial entorhinal cortex project to the subiculum and the field CA1, via the temporoammonic path. The medial and lateral entorhinal areas and the regions of the hippocampus form narrow strips aligned parallel to one another. The projection of the entorhinal cortex, as well as the intrinsic connections between the hippocampal areas, are roughly topographic and run perpendicular to these strips; the intrinsic and commissural projections terminate in the deepest part of the molecular layer of the hippocampal areas (Cajal, 1911; Lorente de No, 1934; Raisman, Cowan and Powell, 1965; Blackstad, Brink, Hem and Jeune, 1970; Hjorth-Stmonsen, 1973; Swanson and Cowan, 1978).

There are striking similarities in the organization of the medial wall areas in the turtle. Area L projects to the superficial third of the molecular layer, relaying olfactory information. Area D1 projects to the middle third of the molecular layer. Area D1 receives input from area D2, from the dorsal division of the DVR by way of D2, and probably direct information from the other sectors of the DVR. The projections to the medial wall areas are crudely topographic, running across the strips of the cortical areas. Commissural and associational axons end in the deep third of the molecular layer. The turtle cortex accomplishes in one or two synapses the convergence of information which requires an entire cortex in mammals.

This information suggests a model of the homologies between the cortical areas of the turtle and mammal. Area L is homologous to all or part of the olfactory cortex of mammals, including the lateral entorhinal area which gives rise to the lateral perforant path. Area D1 is homologous at least to the medial entorhinal area, which gives rise to the medial perforant path. Areas DM and M are homologous to the hippocampal sectors as a group. Area D2 and the sensory areas of the DVR are homologous to the remaining cortex, both neocortical and perhaps less differentiated areas.

This model is consistent with the pattern of afferent input from the nuclei of the basal telencephalon, as described in Chapter 2: the septum in the turtle projects mainly to cortical areas DM, M and the medial parts of D1, while the large distinctive cholinergic cells scattered in the lateral forebrain bundle project to the remainder of the dorsal cortex. The nucleus of the diagonal band projects to olfactory cortex in turtle, although only the horizontal limb of that nucleus does so in mammalian brain.

The model is also consistent with several additional observations of the thesis. Among the cortical areas the medial areas receive the largest input from the mammillary region of the hypothalamus; in mammals the hippocampus receives projections from the supramammillary region, as first described by Segal and Landis (1974). Only the medial areas have a substantial projection to the septum, like the mammalian hippocampus. The medial areas also project to the medial portion of the AChase-rich basal forebrain, the nucleus accumbens; in mammals this region receives an input from the hippocampus (Heimer and Wilson, 1975).

In specifying these homologies, we are concluding that the organization of the cerebral cortex is much the same in mammals and reptiles. We are thus confirming the original report of Spitzka who in 1880 "having, through a piece of good fortune, come into possession of a living iguana, and thence obtained the brain and cord in a perfectly fresh condition" saw that the medial cortical areas of that reptile were separated from the rest, gave rise to a fornix, and thus must be hippocampus.

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