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Salman Afsharpour

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MORPHOLOGICAL ANALYSIS OF SUBTHALAMIC NEURONS AND THE ORGANIZATION OF CORTICOSUBTHALAMIC PROJECTIONS IN THE RAT

BY

Salman Afsharpour

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To my dearest mother and mother- and father-in- law, who made everything possible by their continued support.

To my wife Shukoh, who has devoted her life to furthering my educational career.

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ABSTRACT

MORPHOLOGICAL ANALYSIS OF SUBTHALAMIC NEURONS AND THE ORGANIZATION OF CORTICOSUBTHALAMIC PROJECTIONS IN THE RAT

BY

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Neuronal organization of the rat subthalamic nucleus (STH) was studied using Golgi, Nissl and autoradiographic techniques. Golgi impregnated STH neurons were analysed in sagittal and frontal sections under a light microscope. The somatic shape was either fusiform, oval, or polygonal. The cross-sectional areas of the soma (SA) varied between 140-440μm^2. Some of the cells had a few somatic spines. Two to six proximal dendrites usually gave rise to tapering secondary dendrites extending up to 500μm in length. These dendrites were sparsely covered with spines. Statistical analysis of the somatic size and the number of proximal dendrites showed unimodal distributions.

Neurons located in the central portion of STH had oval dendritic fields. Some of these neurons had one or two dendrites extending beyond the nuclear boundary into the zona incerta, the lateral hypothalamus or the cerebral peduncle (CP). Neurons located at the borders of STH had dendritic fields extending parallel to the borders and were generally confined within the nucleus except for those

neurons adjacent to CP which had some dendrites extending into CP.

Two types of afferent fibers were observed entering STH. One type arose from CP while the other entered STH from the rostral border after crossing the internal capsule.

The cytoarchitectonic characteristics of STH neurons were studied on 25um thick sections stained with cresyl violet. The densely packed somata appeared fusiform in frontal sections (6 to 32um in diameter), and round or polygonal in horizontal and sagittal sections (10 to 28um in diameter).

Corticosubthalamic projections were investigated using autoradiographic tracing techniques. Tritiated amino acids were injected unilaterally into frontal, parietal and occipital cerebral cortices. The rostral part of the medial agranular frontal cortex projected throughout the ventral two-thirds of the medial half of STH. The lateral agranular frontal cortex projected to the lateral portion of the rostral two-thirds of STH. The area between the medial and the lateral agranular cortex projected mainly to the medial half of STH. The caudal part of the medial agranular frontal cortex projected to the dorsolateral part of the caudal two-thirds of STH. The caudal part of the lateral agranular cortex projected to the ventral aspect of the middle third of STH. No projection from somatosensory, visual and association areas to STH were detected.

The results of the present study suggested that 1) The

rat STH is an open nucleus in contrast to other species (i.e. man, monkey, cat). 2) The rat STH may consist of only one type of neuron. 3) The somatic size and the shape of STH neurons are dependent on the plane of section. 4) The cortico-STH projection is unilateral, arising mainly from the frontal agranular cortex, and is topographicaly organized.

CHAPTER 1. INTRODUCTION

Although previous anatomical studies have described the existence of a number of neuronal types within the subthalamic nucleus of various species, the morphological descriptions and classification of these neurons are inconsistent. Furthermore, although the connections of STH with other subcortical structures has been intensively studied, information regarding the topographical organization of the cerebral cortical projections to this nucleus is surprisingly lacking. The aims of the present study were to investigate the morphological characteristics of subthalamic neurons, and the topographical organization of the corticosubthalamic projections in the rat at the light microscopic level. Golgi preparations were used to study the neuronal types in the subthalamic nucleus, and Nissl stained preparations were used to analyze the cytoarchitectonic features of the nucleus. An autoradiographic tracer method was used to analyze the topographical organization of the cortico-subthalamic projections.

BACKGROUND:

A. Gross Anatomy Of Subthalamic Nucleus

The subthalamic nucleus (STH) was first discerned by Luys (1865) and was subsequently named "Corpus Luysii" by Forel (1877) (cited by Yelnik & Percheron, 1979). As described by Rioch (1930) STH is a lens shaped mass situated

on the dorsomedial aspect of the cerebral peduncule (CP). Rostrally, it is bounded by the internal capsule (IC); Caudally, it is separated from the substantia nigra (SN) by an area consisting mainly of fibers and scattered cells termed "the accessory nucleus of Luys" (Watanabe & Kawana, 1982). The ventromedial part of the nucleus extends toward the lateral hypothalamic area (LHA).

Embryologically, STH is considered to arise from a cell column that extends from the ventral thalamus to the mammillary nucleus (Shanner, 1930). Kuhlenbeck (1948, 1949) described the STH as a derivative from the dorsocaudal part of the lateral hypothalamic cell column. Richer (1965) described STH as arising from the "subthalamic longitudinal zone" along with both segments of the pallidum. The anlage of STH lies caudal to that of both pallidal segments, and is said to mature before either the globus pallidus or the substantia nigra (cited by Carpenter et al., 1981).

In humans STH has a volume of approximately 158 mm3 which is about 1500 times the size of rat STH, but its size relative to the total brain is the same in both species (Bonin & Shariff, 1951).

Clinico-pathologically, STH is known to play an important role in motor regulation. Lesions of STH result in dyskinesia, predominantly a choreoid activity (dancing movements) or ballism (flinging movements) in the monkey (Whittier & Mettler, 1949; Carpenter et al., 1950; Carpenter & Carpenter, 1951; Mettler, 1962; Hammond et al., 1979) and in man (Martin, 1927; Whittier, 1947; Carpenter, 1955) (see

also literature review by Carpenter & Sutin, 1983). This dyskinesia is characterized by its violence, persistence and association with marked hypotonus. The dyskinesia can be ameliorated and abolished by subsequent stereotaxic lesions in the medial globus pallidus, the ventral lateral nucleus of the thalamus, or the motor cortex (Whittier & Mettler, 1949). STH dyskinesia is considered to be a physiological expression of inhibitory influences which normally act on the medial segment of the globus pallidus (Carpenter et al., 1950). Results from experimental investigations have suggested that STH is somatotopically organized since lesions in different parts of the nucleus produced dyskinesia in different parts of the body (Carpenter & Whittier, 1950; Carpenter & Carpenter, 1951; Mettler & Stern, 1962).

B. Subthalamic Intrinsic Organization

a) Light Microscopic Study Of Nissl Preparations

Light microscopic analysis of Nissl stained sections have revealed that cell bodies of the STH neurons in the monkey and man are either round, polygonal or fusiform in shape. The smallest somata were found to be 10 X 10μm in Macaca and 11 X 11μm in man. The largest were 33 X 6μm in Macaca and 40 X 9μm in man (Yelnik & Percheron, 1979). Both Luys (1865) and Forel (1877) commented on the masses of brownish pigment in the cytoplasm of human STH neurons while such pigment in STH neurons of the rhesus monkey is absent (cited by Carpenter et al., 1981). Rafols & Fox (1976)

however, have noted the presence of such cytoplasmic lipofuscin granules in all cell profiles of the principal STH neurons in the squirrel monkey (Saimiri sciureus) and pig tail monkey (Macaca menestrina) while in the monkey (Macaca mulatta) the lipofuscin granules were present in some nerve cell profiles and absent in others. In Nissl studies by Whittier & Mettler (1949) in the monkey, STH neurons appeared to be large with large nuclei that contained chromatin and a massive nucleolus. Differences in cell size and concentrations between medial and lateral parts of the nucleus have also been recognized. Foix & Nicolesco (1925) were the first to indicate, on Nissl material in human, that STH neurons look smaller in the ventral and external parts than dorsal and internal parts of the nucleus (cited by Yelnik & Percheron, 1979). However, in subsequent Nissl studies, the opposite observation was reported by Kodama (1928) in man, and Whittier & Mettler (1949) in monkey. The latter authors described large cells within the lateral portions and small cells in medial portions of STH. Rafols & Fox (1976) localized the smaller cells of the Macaca in a narrow strip along the ventral border at the medial end of the nucleus. However, Yelnik & Percheron (1979) have described STH neurons as having different sizes and shapes, and being more numerous in the oral and medial portions than in caudal and lateral portions of STH. Neurons with different sizes and shapes are intermingled so that no part of STH contains only one specific cell size or shape. The latter authors in addition

noted more fusiform appearing somata in sagittal sections and more round and polygonal appearing ones in frontal sections.

b) Light Microscopic Studies Of Golgi Preparations

Studies on subthalamic neurons using Golgi techniques have described a number of neuronal types in several species. However, the classification and description of these neurons have remained controversial. In early Golgi impregnation studies, STH appeared to contain only Golgi type I neurons in rat and mouse (Kolliker, 1896), in man (Mirto, 1896)and in the kitten (Ramon y Cajal, 1910-1911). Raman y Cajal was the first to illustrate drawings of four radiating STH neurons of an 8-day-old kitten. Subsequently, Golgi impregnated STH neurons of cat and dog similar to drawings by Cajal were illustrated by Ramon Moliner (1962) and Leontovich & Zhukova (1963).

Recently, more Golgi studies have been done on STH and different cell types have been described in the monkey based on the somatic shape, size and dendritic features. Rafols & Fox (1976) described two types of principal neurons and one type of local circuit neuron. Principal neurons were referred to as either radiating or elongated fusiform types. Radiating neurons had a few delicate somatic spines and 5 to 8 dendritic trunks that gave rise to branched, tapering dendrites extending over 400μm. Dendrites of these neurons were thinner than those seen on pallidal or nigral neurons and occasionally spines were seen on dendritic trunks.

Elongated fusiform neurons were less numerous than those of the radiating type and were found mainly near the capsule of the nucleus. They had polar dendrites. Small local circuit neurons had relatively few long undulating dendrites, but axons arising from these neurons have not been identified. In the kitten, Iwahori (1978) has identified three varieties of Golgi type I STH neurons based on somatic size. All had polygonal shaped cell bodies. Axons of these neurons were found to arise from the soma, but occasionally emerged from a proximal dendrite. Many axon-collaterals were observed to arise from the parent axons of STH neurons either intranuclear or extranuclearly coursing in both rostral and caudal directions. Iwahori classified afferent fibers entering STH as 1) ascending afferents, the axon-collaterals arising from ascending fibers in CP; 2) descending afferents, the axon-collaterals of descending fibers in CP; and 3) afferents from Meynert's commissure which crosses CP. Yelnik & Percheron (1979) analyzed their Golgi data of STH neurons by means of both algebraic and geometric statistical parameters, and suggested that STH is a homogeneous closed nucleus which consists of only Golgi type I (projection) neurons that are morphologically identical in the cat, monkey and man. The cell bodies of these neurons were shown to be ovoid in shape giving them a fusiform appearance when observed parallel to their long axis, and round or polygonal when viewed in other directions. On the average, seven proximal dendrites were observed originating from various points of STH perikarya; they branched out in successive

bifurcations giving rise to a mean of 27 dendritic tips. Axon-collaterals of STH neurons were also observed to course in medial and lateral directions.

c) Labeling With Intracellularly Injected Markers

The recently developed technique of intracellular labeling with either the enzyme HRP or fluorescent markers have enabled direct analysis of the morphology of neurons which have been physiologically characterized through intracellular recording techniques (Kitai et al., 1976). Intracellular recordings in the rat (Kitai & Deniau, 1981) has demonstrated that Golgi type I STH neurons were monosynaptically excited by the cerebral cortex. On the other hand, STH neurons received inhibitory inputs from the globus pallidus (Kita et al., 1983).

Light microscopic analysis of HRP labeled neurons has confirmed and extended previous Golgi studies (Iwahori, 1978, in the kitten; Yelnik & Percheron, 1979, in the monkey) concerning the extent of the elaborate axonal collateral arborizations of STH Golgi type I neurons (Kita et al., 1983). Branches of the main axons were traced into the entopeduncular nucleus, the globus pallidus and the substantia nigra. Kita et al. (1983) distinguished two types of STH neurons in the rat based on axonal branching patterns. 1) STH neurons with intranuclear branching axons. These neurons were characterized by numerous dendritic arborization and axons which gave rise to collaterals before leaving the confines of the nucleus. 2) STH neurons without

intranuclear branching axons. This group of STH neurons had less dendritic branching than the other group.

d) Electron Microscopic Studies

Rafols & Fox (1976) recognized two different STH neurons in their electron microscopic investigation of normal material in the monkey. Both of their principal neurons, radiating and elongated fusiform Golgi type I, were observed to have the same ultrastructural morphology of cytoplasmic and nuclear contents. The Golgi type I STH neurons in the monkey, were frequently characterized by cytoplasmic lipofuscin granules. The few Golgi type II (local circuit neurons) neurons were characterized by funnel-shaped protrusions of the cell bodies at the base of the primary dendrites, and also a large quantity of free polyribosomes, profiles of the Golgi apparatus were located at the base of some dendrites and in the rim of the cytoplasm. The mitochodria appeared to have a darker matrix, with slightly thinner cristae than the mitochondria in the Golgi type I neurons. However, only one type of STH neuron was observed in the rat (Chang et al., 1983). The somata were found to be often closely apposed to each other without any intervening glial processes. Membrane appositions were also found between some somata and dendrites as well as dendrites and dendrites. The somata size were observed to range between 10 and 25um in diameter and all somata were characterized by an abundance of organelles. Some of the somata had cilia. The nuclei had

deeply invaginated membranes and pale nucleoplasm with little heterochromatin. Most of the STH neurons had filamantous nuclear rods.

Electron microscopic studies have revealed that several types of terminals synapse on STH neurons. Following pallidal lesions in the cat (Nakamura & Sutin, 1972; Romansky et al., 1979), in the rat (Kooy et al., 1981), and in the baboon (Usunoff et al., 1982), degenerated terminals were found to be associated with symmetrical synapses formed with large dendrites, dendritic spines, somata and somatic spines. These terminals were large in diameter and contained large-sized elliptical vesicles. Another type of terminal was demonstrated after cerebral cortical lesions (Romansky et al., 1980). The degenerated terminals were observed to be associated with asymmetrical synapses mainly on thin dendrites and their spines. These terminals were small in diameter and contained medium-sized round vesicles. In an recent electron microscopic study on rat STH normal material Chang et al. (1983) described at least two types of terminals similar to those reported by Nakamura & Sutin (1972) and Romansky et al. (1979, 1980) from lesioned material. However, Hassler et al. (1982) in an electron microscopic study of the synaptic organization of the baboon STH, described nine different types of synapses. Type I (F) was the most frequently encountered and was involved either in axo-dendritic or axo-somatic symmetrical synapses. This type was characterized by scattered flat vesicles and many large mitochondria. The

terminals were either elongated, tuble shaped (type IF enpassent) or spindle shaped (type IFa). The type IF terminals have been shown to degenerate after pallidal lesions (Usunoff et al., 1982). Type IIF was a smaller terminal with flattened vesicles, which showed a tendency of clustering together. Type III (elongated), IV (star shaped) and V (oval shaped) all contained small, round vesicles, and were associated with asymmetrical synapses formed with dendritic terminals or spines. Type VI (SO) terminals which contained larger, pleomorphic vesicles made synapses on dendritic terminals. Type VII (LO) showed a looser arrangement of vesicles intermingled with more dense core vesicles. Type VIII was a dendritic terminal with loosely arranged, flat vesicles and was in contact either with a type VI or a type VII terminal. Type IX was an axo-spinous microsynaptic one, which partly degenerated after contralateral pallidal lesion. The origin of these terminals except type I (F) have not yet been examined.

e) Biochemical, Histochemical and Immunocytochemical Studies

Biochemical and histofluorescent studies have revealed that STH contains dopamine (DA) receptors, DA-containing axons as well as catecholaminergic cell bodies. In a study by Brown & Wolfson (1978) using [14C]deoxyglucose as a metabolic tracer in rats, it was shown that a DA agonist, apomorphine, markedly increased glucose utilization in STH. Based on these findings they suggested a direct action of

apomorphine on DA receptors in STH. In a subsequent study by the same authors (Brown et al., 1979), catecholamine-containing axons and varicosities were observed in most parts of STH. However, DA-containing cell bodies were not observed in rat STH, although they have been described in the caudal part of STH in the cat, using the Falck-Hillarp histofluorescent technique (Meiback & Katzman, 1979). The latter authors have also found DA-containing dendrites and terminals in the cat STH.

In an immunofluorescence study, the rat STH was found among many other nuclei and areas of the central nervous system to be densely innervated by serotonin nerve terminals in its caudal portion (Steinbusch, 1981). In the cat, Nauta & Cuenod (1982) have autoradiographically demonstrated GABA-containing STH cell bodies following pallidal injection with tritiated GABA. However, they did not observe any STH neuron labeled after tritiated D-asparate and tritiated serotonin injections into the pallidal complex.

C. Subthalamic Afferent Connections

Although it is difficult to determine the connections of this nucleus because of its location in the brain and relatively small size, different techniques have been used to determine the afferent connections of STH in various species. The main afferents to STH arise from the globus pallidus and the cerebral cortex. Some afferents also arise from the pedunculopontine nucleus, the center median-parafascicular nucleus, and the substantia nigra.

(for more detailed review on the subthalamic connections, see Grybiel & Ragsdale, 1979; Carpenter & Sutin, 1983).

a) The Globus Pallidus

There is general agreement in the literature that the major afferents to STH arise from the ipsilateral lateral pallidal segment (LPS), (the globus pallidus in subprimates). This has been demonstrated in the pig (Shanner, 1936) using a sensitive silver impregnation technique; in the monkey (Ranson, 1939; Ranson et al., 1941; Whittier & Mettler, 1949), in the cat (Fonnum et al., 1978), and in the rat (Kooy et al., 1981) using degeneration techniques; in the monkey (Kim et al., 1976; Nauta, 1979) using autoradiographic tracer techniques; in the monkey and the cat (Rinvik et al., 1979) using retrograde axonal transport of horseradish peroxidase (HRP); and in the rat Carpenter & Fibiger (1978), and in the monkey (Carpenter et al., 1981) using combined anterograde and retrograde axonal transport techniques. Carpenter et al. (1968) and Rinvik et al. (1979) using degeneration and HRP techniques in the monkey and the cat, have shown some fibers arising from the internal pallidal segment (the equivalent of the entopeduncular nucleus in subprimates) and terminating on the most caudal and medial parts of STH. However, Carpenter et al. (1981) and Kim et al. (1976) have noted no projection from the internal pallidal segment to STH. The largest number of lateral pallidal neurons projecting to STH lie in the rostral and central divisions of this segment, however

fibers are also derived from the cells in the caudal division of the LPS. Both the degenerating and the autoradiographic studies in the monkey indicated that pallido-STH projections have a rostrocaudal organization (Carpenter et al.,1968; Carpenter et al., 1981, 1981). Neurons in the rostral two-thirds of the LPS project to the rostral two-thirds of STH, and neurons in the caudal division of the LPS project to the caudal third of STH. Only the medial and caudal pole of STH appears to be void of LPS projections (Carpenter et al., 1981). The topographical arrangement of pallido-STH projections was also shown in the monkey by Nauta & Mettler (1966) and in the cat by Grofova (1969). McBride & Larsen (1980) described a mediolateral laminar origin of pallido-STH projections. Their observations suggested that neurons in lateral lamina of the globus pallidus projected to the lateral portion of STH and that neurons in medial lamina projected to medial parts of STH. A study on the pattern of distribution of the pallido-STH projections in normal and experimental animals (i.e. lesioning globus pallidus) revealed that the pallido-STH fibers terminated mainly on dendrites. No particular part of STH was found to receive a specific type of synapse. Overall 80.3% of the synapses were axodendritic, 12.3% were axoaxonic, and 7.4% made axosomatic contacts. No axoaxonic degenerated terminals were seen after pallidal lesions (Nakamura & Sutin, 1972). Synaptic vesicles in terminal boutons varied in shape within the same terminal. Dense core vesicles were evident in about 20% of the terminals.

Investigations by Romansky et al. (1980) on pallido-STH synaptic relationships, using the degeneration methods, revealed that the pallidal inputs terminated on perikarya of large STH neurons, on proximal dendrites, and rarely on dendritic spines. On the other hand, they did not observe any degenerated terminals synapsing on the perikarya of small STH neurons or the other vesicle-containing profiles.

b) The Cerebral Cortex

The projections from the cerebral cortex to STH were generally denied in early studies using fiber degeneration methods in monkey (Levin, 1936, 1949; Verhaart & Kennard, 1940). However, Meyer (1949) in man and Mettler (1945); Devito & Smith (1964); Petras (1965, 1969) in monkey, dog and cat and Auer (1956) in cat, using degeneration methods demonstrated projections from various motor cortical areas to STH. Siegel et al. (1971) also found some projections from the temporal cortex to STH in the cat. Studies by Kunzle & Akert (1977) and Kunzle (1978) using the autoradiographic tracing techniques in the monkey, have found some projections from the area 8 (frontal eye field) to ipsilateral STH which had been previously denied by Astruc (1971) using fiber degeneration methods in the same species. An autoradiographic study by Hartmann Von Monakow et al. (1978) demonstrated that the corticosubthalamic fibers were somatotopically organized in the monkey. The frontal motor cortex, area 4, was shown to project ipsilaterally to the lateral and dorsal parts of STH, and

this projection seemed to display an orderly arrangement with the face area lying laterally and the leg area medially. The projection of area 6 of the frontal motor cortex was relatively circumscribed within the intermediate portion of STH. Area 8 of the prefrontal cortex projected to the ventral part of STH, and area 9 of the prefrontal cortex projected to the middle part of the ventral portion of the nucleus. Confirming previous findings from a degeneration study by Petras (1965, 1969) and an autoradiographic study by Kunzle & Akert (1977), Hartman Von Monakow et al. (1978) also found no projection to STH following tritiated amino acid injections into various parts of the post central gyrus. Rinvik et al. (1979) found some retrogradely labeled cerebral cortical cell bodies following HRP injections into the ipsilateral STH in the cat and monkey. A recent electrophysiological study by Kitai & Deniau (1981), using a combined technique of intracellular recording and intracellular labeling of neurons with horseradish peroxidase, demonstrated that cerebral cortical neurons exert monosynaptic excitatory actions on STH projection neurons. Electron microscopic studies, in the cat, indicate that cortical influences upon STH neurons are mediated by thin myelinated and unmyelinated axons that terminate in small round boutons on small dendrites and spines (Romansky et al. ,1979).

From previous anatomical studies of the cortico-STH projection, it is clear that there has been no report concerning the organization of this projection in the rat,

while the majority of physiological and anatomical data on the function of the basal ganglia and its related nuclei (i.e STH) have been obtained in the rat. Thus, the data from the present investigation could be correlated with physiological data obtained in previous studies and should contribute to a better understanding of the role of STH in basal ganglia function.

c) The Pedunculopontine Nucleus

Studies utilizing both retrograde HRP trasport and autoradiographic tracing techniques suggested that only a small number of scattered pedunculopontinal afferents end in STH. This has been demonstrated in cat (Nomura et al., 1980; Rinvik et al., 1979) and in monkey (Carpenter et al., 1981; Rinvik et al., 1979). Tritiated amino acid injections in pedunculopontine nucleus produced labeling in only the ventromedial part of rat STH (Veazey et al., 1980). However, the synaptic relationships and the physiology of this input to STH has not been investigated.

d) The Substantia Nigra

Although SN has been demonstrated to receive projections from STH in various species (see Subthalamic efferent to SN in following pages), there is little evidence that this connection is reciprocal. No STH afferents were observed to arise from either part of SN in degeneration studies in monkey, cat and rat (Nauta & Mettler, 1964; Carpenter et al., 1964; Carpenter & Peter, 1972; Faull &

Carman, 1968) as well as autoradiographic studies in monkey (Carpenter et al., 1975; Carpenter et al., 1976). Physiological data in the rat also did not support the existence of a nigro-STH projection (Hammond et al., 1978). Afifi & Kaelber (1965) in a degeneration study in the cat, and Rinvik et al. (1979) in a retrograde HRP axonal transport study in the cat and the monkey, are the only authors to report of nigral projections to STH, although nigral efferents may traverse or bypass STH (Deniau et al., 1980; Carpenter et al., 1976; Carpenter et al., 1981). The projections they found were not limited to any specific part of STH (Afifi & Kaelber, 1965).

e) The Centre Median-parafascicular Nucleus

The projection from the centre median-parafascicular nucleus was first described by Sugimoto et al. (1982) using both anterograde and retrograde axoplasmic transport techniques in the cat and the rat. Tritiated amino acid and HRP injections in the centre median-parafascicular complex revealed dense labeling in the ipsilateral STH, over its rostral one-third portion. A few scattered centre median-parafascicular neurons were retrogradely labeled following HRP injection into the cat STH.

f) Other Less-studied Subthalamic Afferents

Studies based on retrograde HRP axonal transport techniques have described various other STH afferent areas, including the locus coeruleus (Rinvik et al., 1979;

Carpenter et al., 1981), dorsal raphe, the hypothalamus and the central nucleus of amygdala (Rinvik et al., 1979). However, since these pathways have not been confirmed by further studies using other anatomical techniques (i.e autoradiography), their true nature remains unclear.

g) Physiology Of Subthalamic Afferents

Previous electrophysiological studies (Frigyesi & Rabin, 1971, in the cat; Ohye & Feger, 1976, in the monkey), have indicated that striatal stimulation induces excitatory responses in STH neurons. However, in a recent intracellular recording study by Kita et al. (1983) of rat STH neurons after decortication globus pallidus stimulations induced only short latency monosynaptic IPSPs. This result suggested that the excitations reported in earlier studies were most likely due to the activation of cortico-STH fibers coursing through the caudate-putamen and the pallidum. In an electrophysiological study in the cat by Tsubokawa & Sutin (1972), it was found that most STH neurons which showed spontaneous unit activities were inhibited by the stimulation of the globus pallidus or the ventral tegmental area of Tsai.

The neurotransmitter of the pallidosubthalamic pathway was biochemically demonstrated to be gamma-amino butyric acid (GABA) (Fonnum et al., 1978). Evidence from physiological data have supported the above finding that GABA might be the neurotransmitter of the pallido-STH pathway (Rouzaire-Dubois et al., 1980). However, Kooy et al. (1981) have denied the

possibility of either GABA or acetylcholine as neurotransmitters in pallido-STH fibers using histochemical techniques. Thus, the nature of the neurotransmitter of pallido-STH fibers is still controversial.

The cerebral cortical activity on STH neurons was studied by Kitai & Deniau (1981), using a combined technique of intracellular recording and labeling with HRP in the rat. They demonstrated that cerebral cortical neurons exert monosynaptic excitatory actions on STH projection neurons.

No physiological studies concerning the effects of the substantia nigra, the pedunculopontine, the centre median-parafascicular nucleus and other sparse STH afferents were carried out.

D. Subthalamic Efferent Connections

The major STH efferent targets are the pallidum, the substantia nigra, and to a lesser extent the pedunculopontine nucleus, the cerebral cortex and other nuclei.

a) The Pallidum

Subthalamic projections to the medial pallidal segment (MPS) were first described by Kolliker (1896). A subsequent study by Mirto (1896) confirmed this projection and also postulated that STH projects to both pallidal segments using a Golgi method in human material. Other studies also

indicated that STH projects to both segments of the pallidum. This has been demonstrated in the monkey (Glees & Wall, 1946; Whittier & Mettler, 1949; Carpenter et al., 1967) using degeneration techniques, in the monkey (Devito et al., 1980) using retrograde HRP tracing technique, in the monkey (Nauta & Cole, 1974, 1978); in the rat (Ricardo, 1980); and in the monkey and the rat (Carpenter et al., 1981) using autoradiographic tracing techniques, and in the rat (Deniau et al., 1978; Kita et al., 1983) using intracellular recording and labeling with HRP. Axons of STH neurons were found to send collaterals to the entopeduncular nucleus, the globus pallidus and the substantia nigra (Kita et al., 1982). Autoradiographic studies in the monkey demonstrated the massive nature of the STH-pallidal projection, and indicated that terminals end in arrays parallel to the medullary lamina suggesting a topographical organization (Nauta & Cole, 1978; Carpenter et al., 1981). Although profuse terminations were found in both pallidal segments, the number of arrays and the silver grain density were much greater in the medial pallidal segment (Carpenter et al., 1981). In the monkey, rostromedial parts of STH were found to project to the rostral and the medial portions of LPS, while lateral parts of STH projected to the central division of LPS. In the cat, STH-pallidal projections cover both the entopeduncular nucleus and the globus pallidus, but did not exhibit a laminar pattern (Nauta & Cole, 1978). The STH projection to the cat entopeduncular nucleus was also greater than that to the globus pallidus. Although the MPS

receives a profuse projection from STH, the connections between STH and pallidum in the primate are not totally reciprocal (Carpenter et al., 1981). On the other hand, the interconnections between STH and LPS appear to be reciprocal and organized topographically in a rostrocaudal sequence (see also Perkins & Stone, 1980).

b) The Substantia Nigra

Subthalamonigral projections have long been suggested, (Kolliker, 1896). In subsequent experimental studies using degeneration techniques several authors have described STH-SN projections in various species (Morgan, 1928; Glees & Wall,1946; Whittier & Mettler, 1949). Injection of HRP in various parts of SN revealed labeling of cells in STH (Kanazawa et al., 1976). The latter authors found that the largest number of HRP positive cells in STH were associated with HRP injected predominantly into the pars compacta. However, most of the evidence suggests that STH-SN projections are confined mainly to the pars reticulata (Nauta & Cole, 1978; Deniau et al., 1978; Carpenter et al., 1981). Antidromic and orthodromic stimulations of STH-SN fibers support the view that these projections end mainly on cells of the pars reticulata (Hammond et al., 1978). Nauta & Cole (1978), in their autoradiographic study of STH efferent fibers, noted that the labeled fibers converged on the medial part of STH and entered SN directly from the dorsal side, or via fibers interposed between CP and SN. Anatomical and physiological evidence in the rat indicated

that a single STH neuron supplied axonal collaterals to both the pallidum and the SN (Deniau et al., 1978; Hammond et al., 1978; Kooy et al., 1980; Kita et al., 1983). Recent data from the anterograde and retrograde axoplasmic transport studies (Ricardo, 1980; McBride & Larsen, 1980; Carpenter et al., 1981; Carpenter et al., 1981) have supported the existence of STH-SN projections.

c) The Pedunculopontine Nucleus

A subthalamo-pedunculopontine fiber pathway was suggested by Woodburn (1946). Both retrograde and anterograde axoplasmic transport studies in the monkey and the cat suggested that PPN may receive relatively small projections from STH (Nauta & Cole, 1974, 1978; McBride & Larsen, 1980; Numora et al., 1980; Jackson & Crossman, 1980). Based on their autoradiographical data in the cat and the monkey, Nauta & Cole (1978) concluded that the lateral part of STH projects to the pars compacta of PPN. This projection was confined to both dorsal and ventral aspects of the brachium conjuctivum at the rostrocaudal level of the trochlear nucleus. Using a combined retrograde HRP transport and autoradiograpgic tracing techniques, in the rat, Jackson & Crossman (1980) noted an appreciable projection from STH to PPN. The number of cells labeled in STH by retrograde HRP transport was heavier than the number of labeled cells in entopeduncular nucleus.

d) Other Less-studied Subthalamic Efferents

Studies based on retrograde HRP axonal transport techniques have described a direct projection from STH to the cerebral cortex in the rat (Jackson & Crossman, 1981). The retrogradely labeled STH neurons were located only on the ipsilateral side and were restricted to the lateral half of the nucleus. A few STH neurons were labeled from HRP injection in the rat insular cortex (Afsharpour et al., unpublished). These STH neurons were confined to the lateral, but more dorsal aspect of STH. Nauta & Cole (1978), in an autoradiographic study of STH efferents, noted sparse projections to the ipsilateral putamen, the ventralis lateralis and the ventralis anterior complex of the thalamus, and the midbrain. Although certain studies suggested that STH influences the excitability of the midbrain reticular formation (Adey et al., 1959; Shibazaki et al., 1980), there is little information regarding the pathways from STH to this region.

From previous studies on the connections of the STH with the motor cortex, basal ganglia related nuclei (e.g. pallidal complex, substantia nigra), the pedunculopontine nucleus, the locus coeruleus, and the centre median parafascicular nucleus, one can speculate that the STH most likely integrates these inputs and subsequently conveys this information to the pallidum and the substantia nigra. Since the output of the basal ganglia appears to be through either the pallidal complex or the substantia nigra, the control exerted by STH over basal ganglia function may be of a powerful nature. In view of this, it is not surprising that

lesions of STH results in the most extreme of movement disorders (hemiballism)-a consequence of basal ganglia dysfunction.

e) Physiology Of Subthalamic Efferents

Data from the physiological study of Yoshida (1971), suggests that STH may be the origin of the entopeduncular inhibition in the cat. Following STH stimulation, suppression of activity was detected in most entopeduncular neurons projecting to both the lateral habenula (73%) and the ventralis anterior of the thalamus (15%) (Larsen & Sutin, 1978) (see also Perkins & Stone, 1980). In the rat, STH stimulation produced three types of responses in the entopeduncular nucleus: 1) a short latency inhibitory response, 2) a short latency activation and 3) long latency inhibitory responses (Shibazaki et al., 1980). These authors suggested that STH neurons inhibit entopeduncular neurons projecting to both the lateral habenula and the pedunculopontine nucleus. In an attempt to determine the transmitter or transmitters involved in the subthalamopallidal pathway, Nauta & Cuenod (1982), using an autoradiographic technique, have suggested that the neurotransmitter of this pathway is mainly GABA. The possibility that the neurotransmitter in the subthalamopallidal pathway of glycine origin has been suggested by Yoshida (1974).

The response of SN neurons to STH projecting fibers was studied by Hammond et al. (1978). They demonstrated

excitatory responses of SN neurons in both pars compacta and pars reticulata following STH stimulation in the rat.

E. Research Objectives

Although the rat has been a widely used animal in recent experimental studies of the basal ganglia and its related nuclei, there have been very few studies concerned with the intrinsic anatomical organization of the rat STH. For example, there have been no studies concerned with the classification of cell types or the organization of afferent inputs such as the cortico-STH fibers. Thus, the elucidation of the morphology of rat STH neurons, the cytoarchitectural features of the nucleus and the relationship of STH with various cerebral cortical areas at the light microscopic level should serve to clarify some of the controversies with respect to the anatomical organization of STH.

The Specific Aims Of This Study Are:

1) To perform a detailed light microscopic analysis of morphological and cytoarchitectural features of rat STH neurons using classical Golgi impregnation techniques and Nissl staining procedures.

2) To determine the topographical organization of the cerebral cortical projections to the subthalamic nucleus using the tritiated amino acid autoradiographic tracing technique.

CHAPTER 2. METHODS

A. Golgi Study

Aproximately 200 Sprague-Dawley rats (5-75 days old) weighing 5-400 grams were used in this study. The rats were anesthetized with intraperitoneal administrations of Avertine (0.015ml/gm body weight) for newborn and Nembutal (50mg/kgm body weight) for adults. The animals were perfused intraventricularly with saline (0.9% Nacl) followed by a fixative (e.g. 3% glutaraldehyde in 0.15M phosphate buffer; 2% glutaraldehyde with 2% paraformaldehyde in 0.15M phosphate buffer(pH 7.3); 10% formaldehyde in saline; or chloral hydrate). Dissected brains were post-fixed for 12-72 hours. Blocks 3-5mm thick which contained STH were processed for the Golgi impregnating methods(e.g. rapid Golgi "Valverde, 1970"; Golgi Kopsch "Gobel, 1978a"; Chloral hydrate "Moliner's modification, 1957" and mixing rapid Golgi with Golgi Kopsch method). Frontal or sagittal 80-120μm thick sections were cut using an Oxford Vibratome. The serial sections were dehydrated in two changes of 100% ethanol for 10 minutes, cleared in two changes of fresh Xylene for 5 minutes each, mounted on glass slides with a synthetic neutral medium (Protex or Histoclad), and then analysed by light microscopy.

Well impregnated STH neurons were selected and detailed tracings of these elements were made using an Olympus microscope and a drawing tube. They were also photomicrographed using a Leitz Orthoplan microscope.

The somatic cross-sectional areas were measured from previously drawn neurons with the aid of a graphic analyzer attached to a PDP-11 computer. The existence of somatic spines and the density of dendritic spines and dendritic appendages were noted. The number of primary dendrites and the branching pattern forming the dendritic field were analysed. Any correlation between the location of the neurons in STH and their dendritic shape was also noted.

B. Nissl Study

The cytoarchitectural characteristics of rat STH were investigated on 25 to 30μm thick sections cut in frontal, sagittal and horizontal plans. Sections were first dehydrated in graded alcohols (70%, 95%, 100%), defatted in fresh Xylene, rehydrated in graded alcohols (100%, 95%, 70%), rinsed in distilled water and then stained with cresyl violet. The sections were dehydrated, cleared in fresh Xylene and cover slipped. Selected samples from frontal, sagittal and horizontal planes were photographed using a Leitz Orthoplan microscope. The cytoarchitectonic features of STH in different planes of sections were then analyzed.

C. Autoradiographic Study

Twenty male Sprague-Dawley rats weighing 230-488g were anesthetized with sodium pentobarbital (Nembutal, 50mg/kg i.p.). A mixture (1:1) of two amino acids of the following

were stereotaxically injected into the various agranular and granular regions of the frontal, parietal and occipital cerebral cortex: 3H-Proline(L-[5-3H]-Proline, specific activity 54 ci/mmole), 3H-Leucine (L-[3,4-3H(n)]-Leucine, specific activity 58 ci/mmole and 3H-Lysine (L-[4,5-3H(n)-Lysine, specific activity 10 to 51 ci/mmole) (from New England Nuclear). The original solutions of tritiated amino acids were first evaporated then diluted to concentrations of 15 to 20uci/ul with 0.9% saline. Injections of 0.25ul to 0.4ul of this concentration were carried out by a Hamilton 1 microliter syringe driven by a micromanipulator over a period of 15 to 30 minutes. The needle was left for 10 to 15 minutes at the injection sites after the end of the injections. Table 1 lists the injection areas, injected radioactive amino acid quantities, concentrations and survival times following the injections. After a survival times of either 4 or 7 days, the animals were deeply anesthetized and perfused with 0.9% saline, followed by either 10% formaldehyde in 0.9% saline or 4% formaldehyde in phosphate-buffered saline (pH 7.2). Brains were dissected and post fixed in 10% formaldehyde with 30% sucrose for 12 to 24 hours. Frozen sections 25 to 30um in thickness were cut in the frontal (15 cases), sagittal (2 cases) or horizontal planes (3 cases). Two sets of alternating serial sections were mounted on subbed glass slides, air dried, dehydrated through graded alcohol, defatted by fresh xylene, rehydrated, and then air dried. The slides were dipped in Kodak NTB2 nuclear emulsion

diluted 1:1 with distilled water. The individual slides were allowed to dry 3 hours or overnight in a humid atmosphere to prevent emulsion cracking. They were stored in light-tight plastic slide boxes containing a small amount of Drierite, and exposed for 6 to 15 weeks at 4 degrees centigrade . The properly exposed slides were developed with full strength D-19 (Kodak) at 18 to 20 degrees centigrade for 2 to 5 minutes, washed in tap water for 30 seconds, fixed in rapid fix for 5 minutes and washed in tap water for 1 to 3 hours. The sections were subsequently counterstained with cresyl violet and examined with both light and dark-field microscopy. Micrographs were taken by a light and dark-field microscope, Wild-M400. Camera lucida drawings from serial sections were also made using an Olympus microscope. Cerebral cortical areas were identified according to the cytoarchitectonic observations made from Nissl stained sections in frontal and sagittal planes (Donoghue & Wise, 1982; Hall & Lindholm, 1974). Subcortical nuclear regions were identified according to the atlas of Pellegrino et al. (1979).

CHAPTER 3. RESULTS

A. Golgi Study

In the present Golgi study the quantity and quality of impregnation of neurons in the rat STH varied considerably from block to block of brain tissue as well as from method to method. The Goli Kopsch method gave the majority of the present results with the rest from a mixed Golgi Kopsch-rapid Golgi technique. The fixative also influenced the amount of impregnation. Three percent glutaraldehyde appeared to be the best for light microscopic Golgi impregnation studies. Although the Golgi methods are undoubtedly capricious, and the small size of the rat STH which is enwrapped by bundles of passing fibers make it difficult for the chemicals to penetrate into the nucleus, we have succeeded by staining enough neurons which stand out sharply on a clean background. Some double impregnated sections that were stained with the mixed Golgi method gave a better STH outline, thus sharply delineating it from the structures bordering it.

The rat STH is a disk shaped nucleus that lays obliquely on the dorsomedial surface of CP. Two roughly equidistance axes (1-1.5mm long) of STH are directed rostrocaudally and dorsolateral-ventromedially. The intermediate portion is the thickest part (250-270μm width) of the nucleus (Fig.5 and 7). The STH is separated rostrally from the entopeduncular nucleus by the internal capsule; caudally, it is separated from the substantia nigra

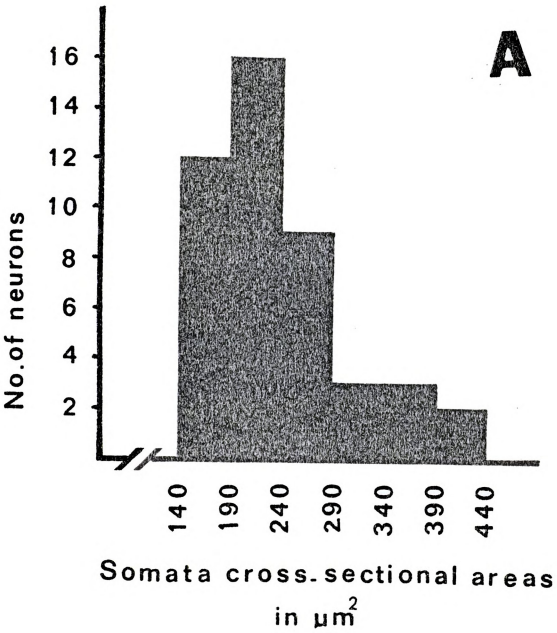
by an area consisting mainly of fibers with scattered cells. The lateral part of the posterior hypothalamus borders STH ventromedially (Fig.5 and 7).

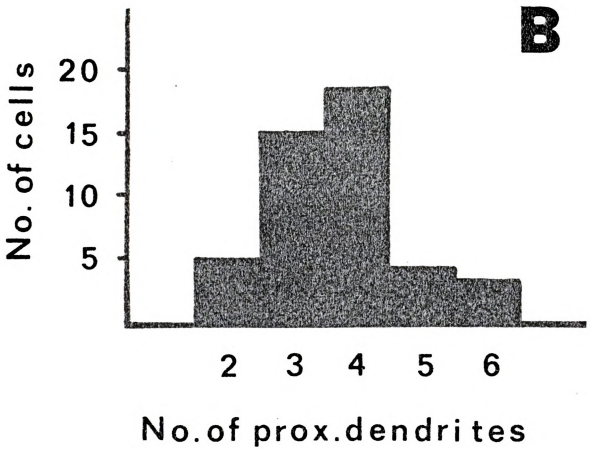
In the present study the distributions of the somatic cross-sectional areas (SA) and the number of proximal dendrites were statistically analysed. The results showed a unimodal distribution of each parameter (Fig.1) and indicated that rat STH consists of only one type of neuron. These neurons are distributed rather randomly within the nucleus. Some dendrites of almost all neurons located at the lateral border of STH cross over this border into CP (H in Fig.4 and B, J, K, M in Fig.6). Some dendrites of centrally located STH neurons spread out beyond the nucleus either dorsomedially into ZI and/or ventrolaterally into CP (C, G in Fig.4 and E in Fig.6) or some of the dendrites cross over the dorsal or the dorsomedial boundaries of STH to extend into ZI only (B, E in Fig 4). Other dendrites of these neurons spread out beyond the ventromedial border of STH into the lateral hypothalamic area (C in Fig.6). Neurons similar to STH neurons are also observed embedded within the fibers of CP (D in Fig.4). None of the neurons which are located at the dorsomedial, medial or the ventromedial borders of STH had dendrites which cross these borders (F in Fig.6).

In the frontal or sagittal planes, three different varieties of neurons were distinguishable on the basis of the origin and the number of proximal dendrites. One variety possessed three to five proximal dendrites and were

Figure 1.

Histograms: A, Somata size distribution of 45 Golgi impregnated rat STH neurons. B, number of proximal dendrites per neuron.


A
No.of neurons
16
14
12
10
8
6
4
2
140
190
240
290
340
390
440
Somata cross-sectional areas
in µm²


B
No. of cells
20
15
10
5
2
3
4
5
6
No. of prox. dendrites

the most frequently impregnated neurons (see histogram B in Fig.1). They were medium in size (average: 225μm). The main characteristic feature of this variety was that one of the dendrites originated from the mid-section of the cell body (A in Fig.2 and 3; E, F in Fig.4; C, E, F, J, K, N in Fig.6) while the remaining dendrites arose mainly from opposite poles of the cell body. The other two varieties were less frequently impregnated and were characterized by having either two proximal dendrites originating from opposite poles of a fusiform shaped cell body (C in Fig.2, 3 and 4; M in Fig.6), or four to six proximal dendrites originating from the cell body without any specific orientation (B in Fig.2 and 3; A, G, H in Fig.4; A, G, L in Fig.6). All of these neurons were randomly distributed within the nucleus without showing any specific pattern of arrangement. Somatic cross-sectional areas (SA) ranged between 140μm^2 and 440μm^2 (see histogram A in Fig.1). The somata were fusiform, oval, polygonal round or multipolar shaped. Some of the neurons had delicate somatic spines (C in Fig.2 and 3; A, B, D in Fig.4; I, K in Fig.6). The diameters of proximal dendrites were between 1.4um and 3.6um. Bifurcation of the proximal dendrites occurred at variable distances (3 to 90μm) from the cell body (see neuron C in Fig.6). Frequently, the secondary dendrites further bifurcated at distances between 18 to 100μm from the cell body, and extended to well over 500μm from the point of branching (C in Fig.6). Somtimes there were long tapering secondary dendrites that extended approximately 400μm from

Figure 2.

Reconstruction of the representative rat STH neurons: A. The most frequently impregnated neuron with oval shaped cell body and variable dendritic spines. Note the single dendrite originates from the mid-region of the cell body. Arrowhead points to a filiform process. B. A radiating neuron with an ovoid shaped cell body and dendrites studded with variable amounts of spines. a, axon; arrowheads point to filiform processes; s, points to a dendritic spine with two heads. C. A bipolar neuron with fusiform-shaped cell body and a few dendritic spines. Arrowheads point to filiform processes; s, somatic spines.

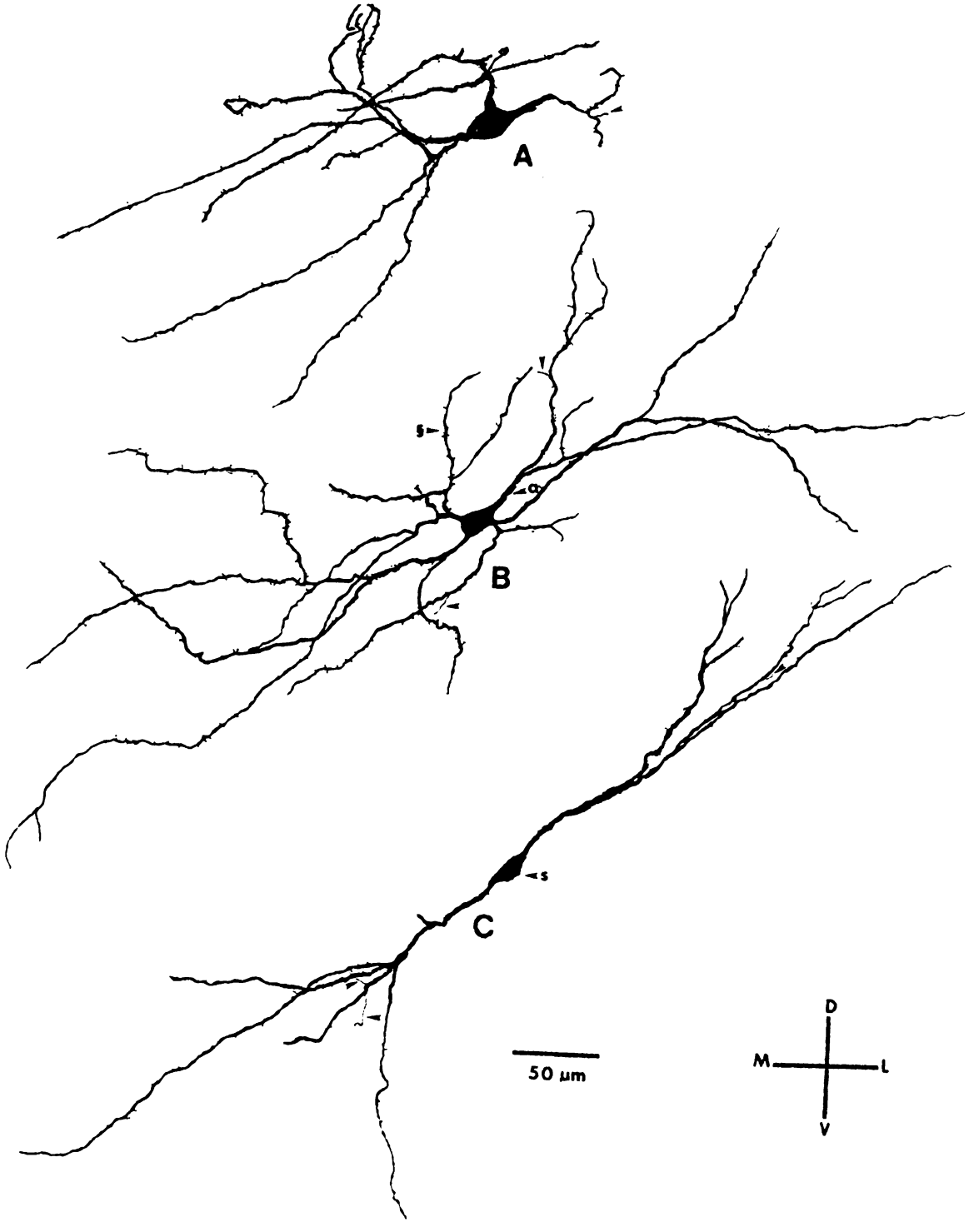
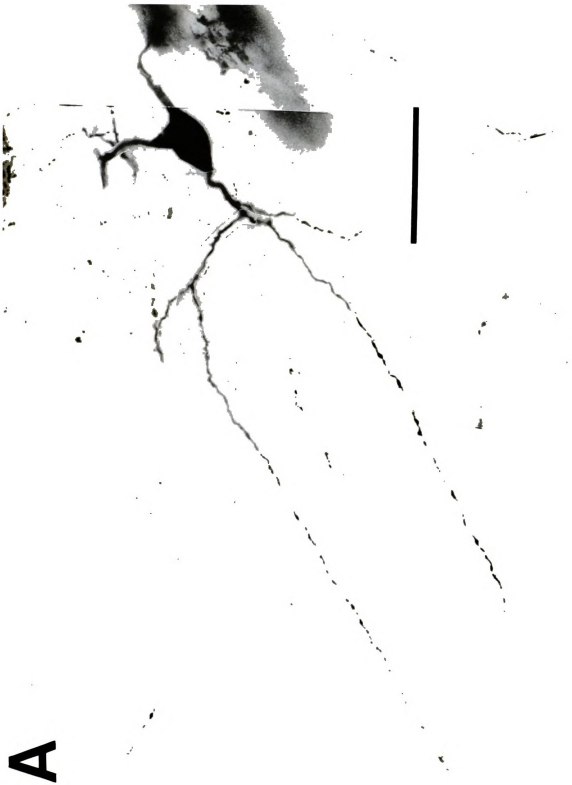
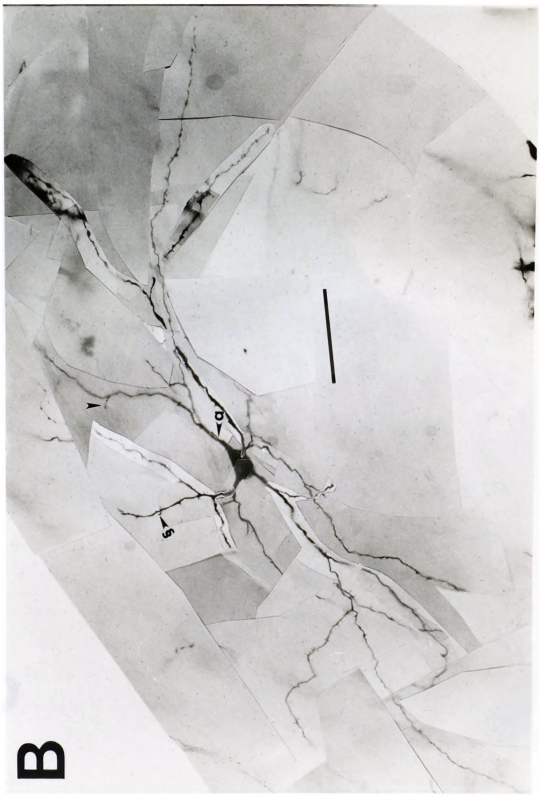

A
B
C
50 µm
D
M
L
V

Figure 3.

Photomicrographs of Golgi impregnated STH neurons illustrated in Fig. 2. Scale bar = 50μm.


A


B
a
s


S
C

the branching point without futher bifurcation (F in Fig.6). Occasionally, a single unbranched dendrite, 1.3 to 3μm in diameter, originated from the cell body and extended over 410μm (B in Fig.4). Beadings in some of the distal dendrites were observed in all Golgi impregnated rat STH neurons. Dendrites which crossed beyond STH borders were usually beaded (see Fig.4 and 6). The dendrites of all STH neurons were covered by spines. The quantity of the spines varied from neuron to neuron. Usually, proximal dendrites had few spines, while secondary and tertiary dendrites had sparse to moderate amounts of spines (Fig.2, 4, 6). Even within the same neuron, some dendrites could have more spines than others (Fig.2). Dendritic spines were pedunculated. Spine shafts were 1 to 3um long, ending with a small bulbous head. Dendritic appendages were also frequently seen in rat STH neurons. These filiform-shaped processes measuring 3 to 60um long and less than lum in diameter, frequently arose from the distal dendrites, and occasionally from proximal dendrites (see arrow heads in Fig.2, 4 and 6).

In the present study, the axons of rat STH neurons were not impregnated beyond their initial segments. They arose from either the cell body (A, E, G in Fig.4; B, N in Fig.6) or from a proximal dendrite (B in Fig.2 and 3; D in Fig.4; K in Fig.6).

Afferent fibers:

Quite a few sagittal sections that were prepared by

Figure 4.

Reconstructions of Golgi impregnated rat STH neurons from sagittal sections. Rostral is to the left, dorsal is at the top of the figures. Note the position of different varieties of STH neurons with respect to the shape of the nucleus (A-H). Also note that centrally located neurons A, F and H extend some of their dendrites into CP; neurons B and E extend some dendrites into ZI; C and G, extend dendrites into both ZI and CP. Neuron D has its cell body and about half of its dendrites completely embedded within CP. a, points to axons; arrow heads point to filiform processes; s, somatic spines. CP, cerebral peduncle; EP, entopeduncular nucleus; OT, optic tract; SN, substantia nigra; STH, subthalamic nucleus; TH, thalamus; ZI, zona incerta.

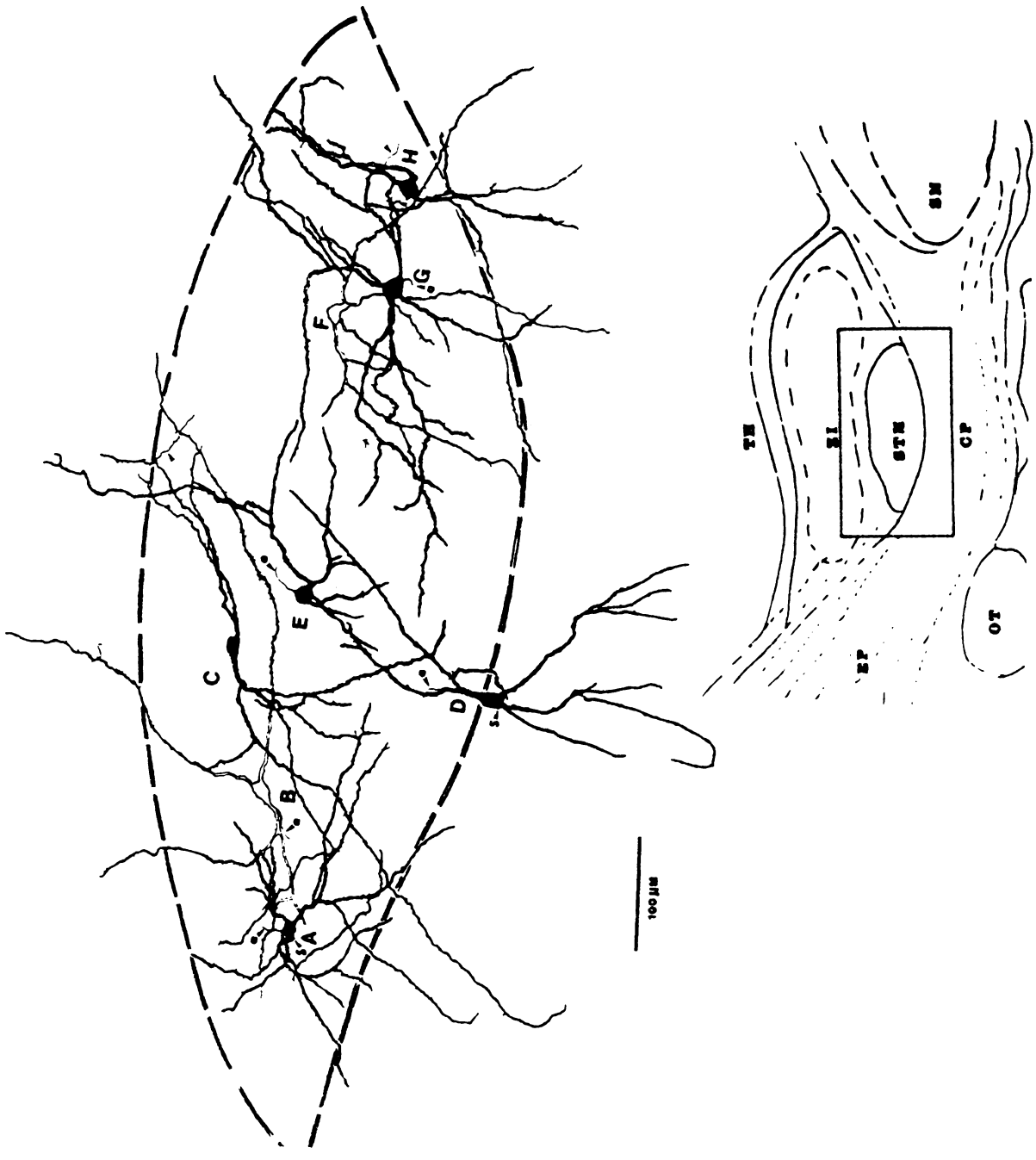

A
B
C
D
E
F
G
H
100 µm
TH
ZI
STH
CP
SN
EP
OT

Figure 5.

Photomicrograph of Golgi impregnated STH neurons some of which are illustrated in Fig. 4 (i.e. neuron B corresponds to neuron B, and neuron H to neuron H in Fig. 4). In this figure dendrites which extended beyond the confine of STH to ZI, IC and CP and also some of ZI neurons' dendrites extending into STH (arrow heads) are visible. CP, cerebral peduncle; EP, entopeduncular nucleus; IC, internal capsule; SN, substantia nigra; STH, subthalamic nucleus; TH, thalamus; ZI, zona incerta. Scale bar = 200um.

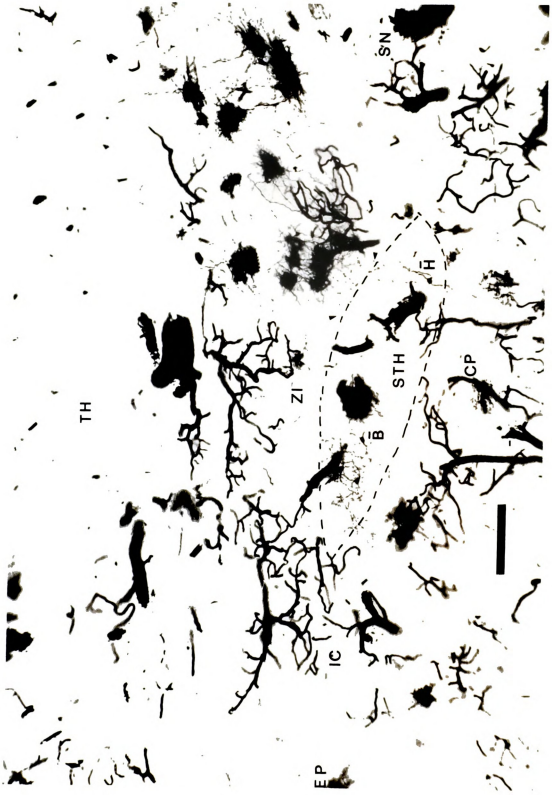
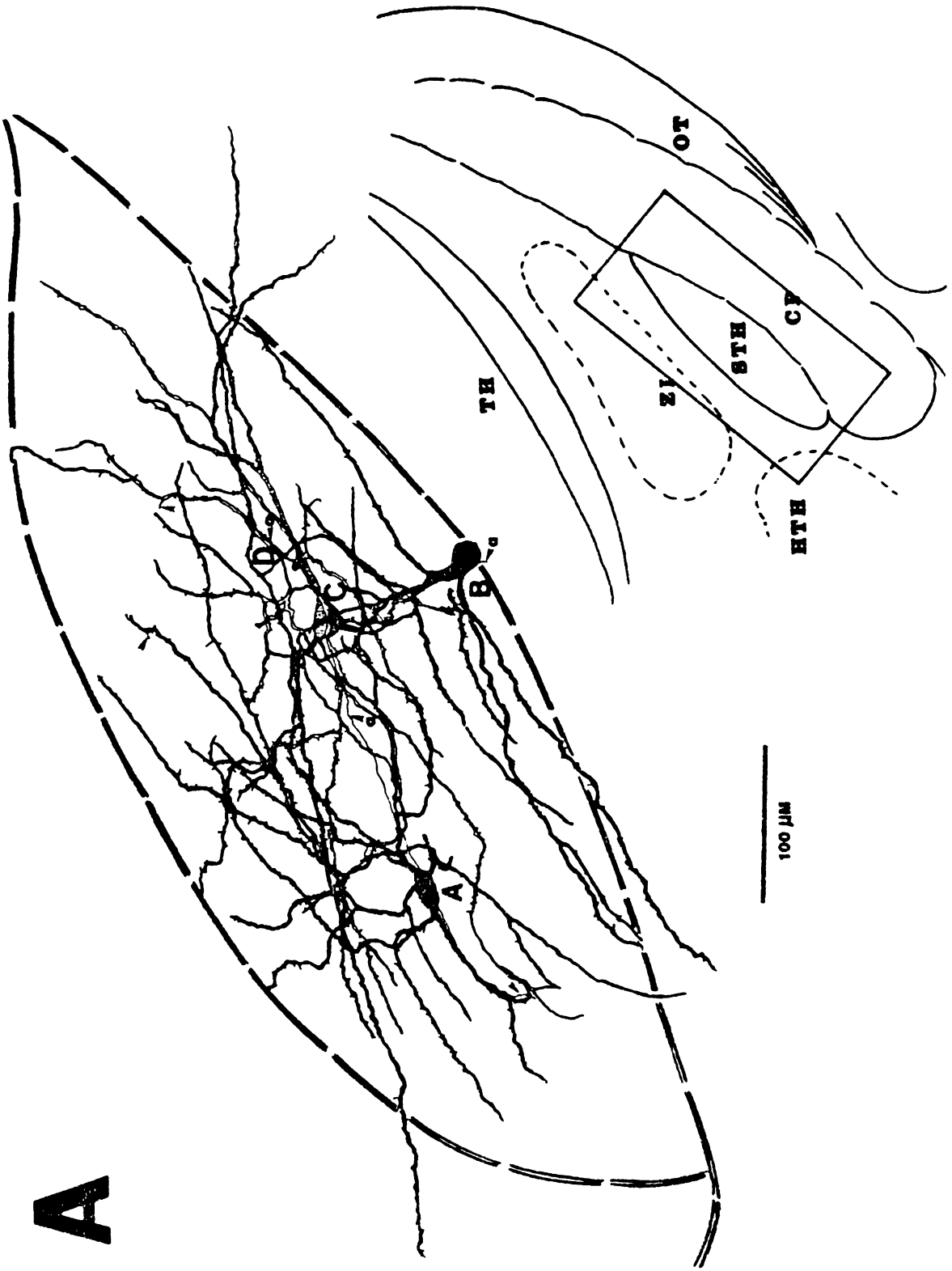

TH
ZI
EP
IC
B
STH
H
CP
SN

Figure 6.

Reconstructions of Golgi impregnated rat STH neurons from frontal sections: A, Rostral. B, Intermediate. C, Caudal. Lateral is to the right, dorsal is at the top of the figures. Note the positions of different varieties of rat STH neurons with respect to the shape of the nucleus (A-N). Some dendrites of neurons A,B,D,E,J,K,M,N, extended into CP. A dendrite of neuron C extended into lateral part of the posterior hypothalamus, and dendrites of a centrally located neuron E, extended into both ZI and CP. a, point to axons; arrow heads, point to filiform processes. CP, cerebral peduncle; HTH, hypothalamus; OT, optic tract; STH, subthalamic nucleus; TH, thalamus; ZI, zona incerta.


A
A
B
C
D
100 µm
TH
ZI
STH
CP
HTH
OT

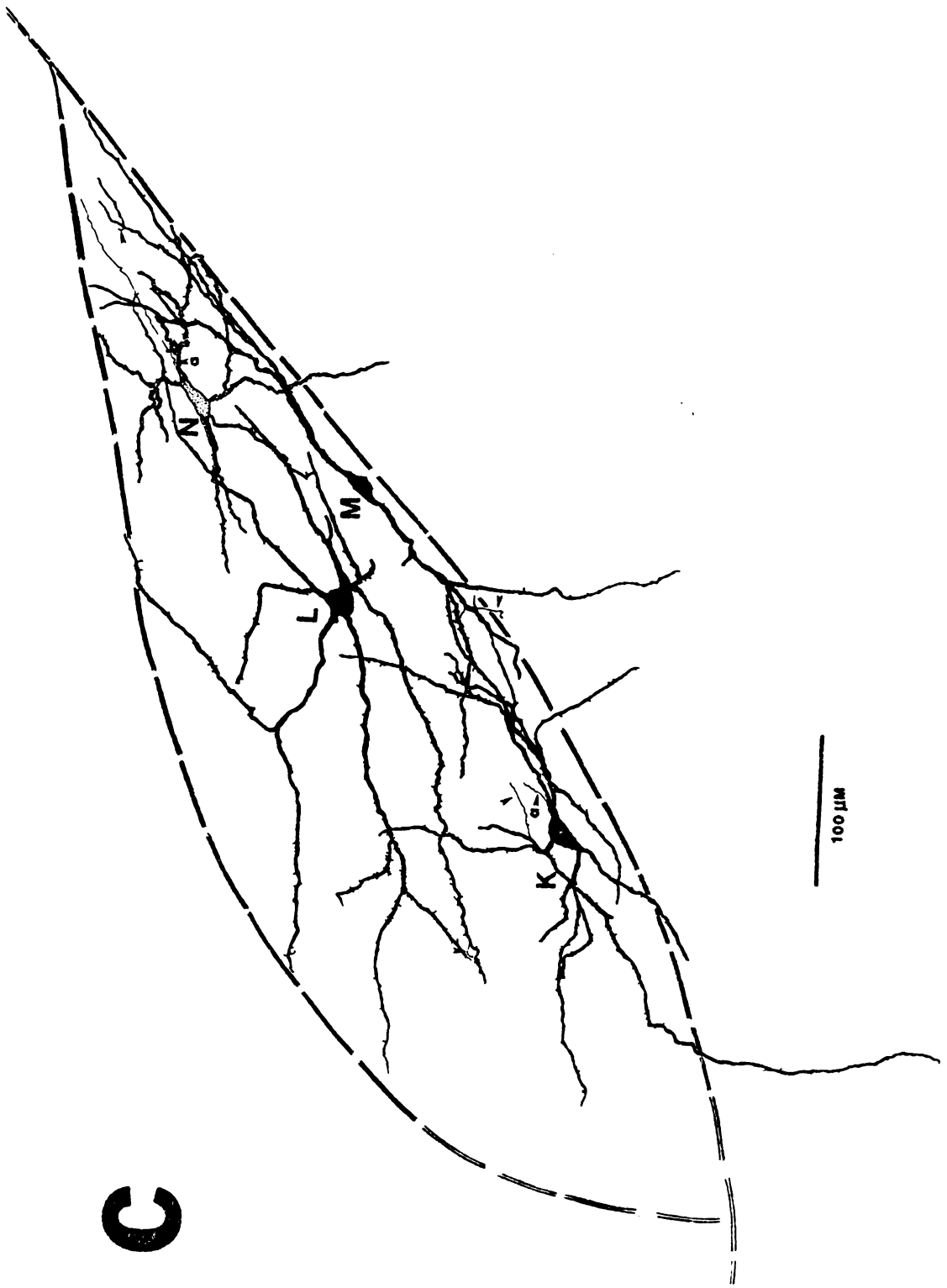
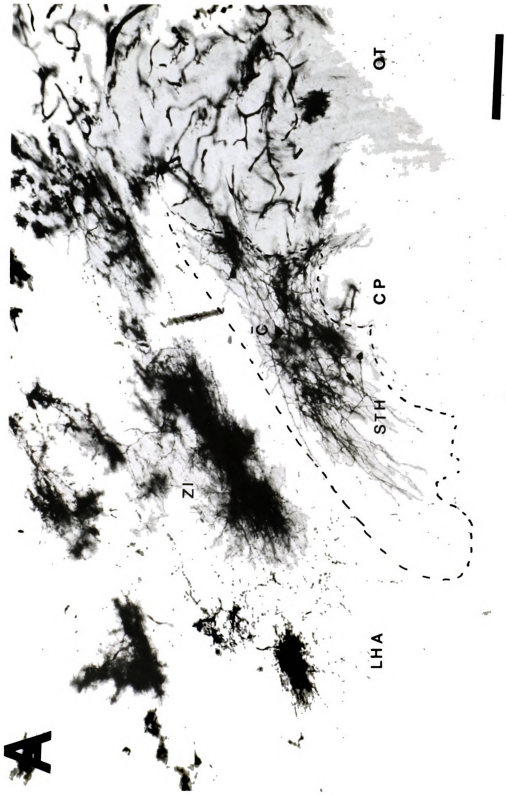
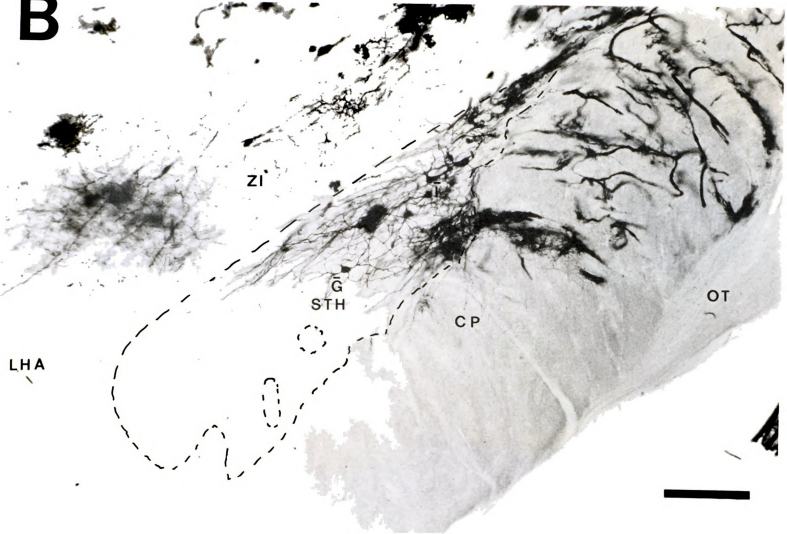

C
N
M
L
K
100 µM

Figure 7.

Photomicrographs of Golgi impregnated STH neurons some of which are illustrated in Fig. 6. A, Rostral. B, Intermediate. C, Caudal. Neurons $\bar{C}$ in A, $\bar{G}$ & $\bar{I}$ in B, and $\bar{L}$ in C of these figures are illustrated as C in A, G & I in B, and L in C of Fig.6 respectively. Note the intermingling pattern between the dendrites of both STH and ZI neurons (arrow head in C). CP, cerebral peduncle; LHA, lateral hypothalamic area; OT, optic tract; STH, subthalamic nucleus; ZI, zona incerta. Scale bar = 200μm.

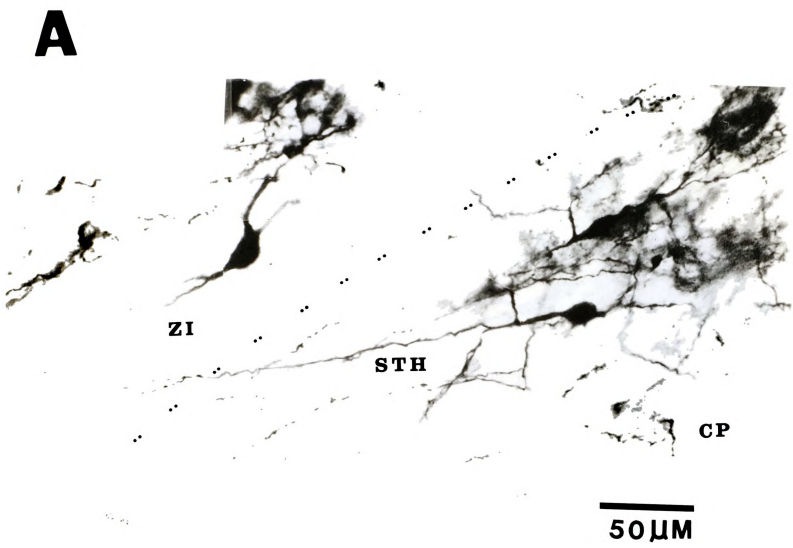

A
LHA
ZI
C
STH
CP
OT


B
ZI
G
STH
CP
OT
LHA

LHA
ZI
L
STH
CP

Figure 8.

Higher magnification photomicrographs of Golgi impregnated rat STH neurons from frontal sections. Lateral is to the right, dorsal is at the top of the figures. A, Illustrates the frontal part of STH. Note Golgi impregnated STH neurons extending some of their dendrites beyond the cytoarchitectural border of the nucleus and entering ZI dorsomedially (upper neuron), and ventromedially (lower neuron). B, Illustrates the midportion of STH. Note the dendrite of the upper STH neuron extends beyond the dorsomedial border of the nucleus and enters ZI. CP, cerebral peduncle; STH, subthalamic nucleus; ZI, zona incerta.


A
ZI
STH
CP
50µM

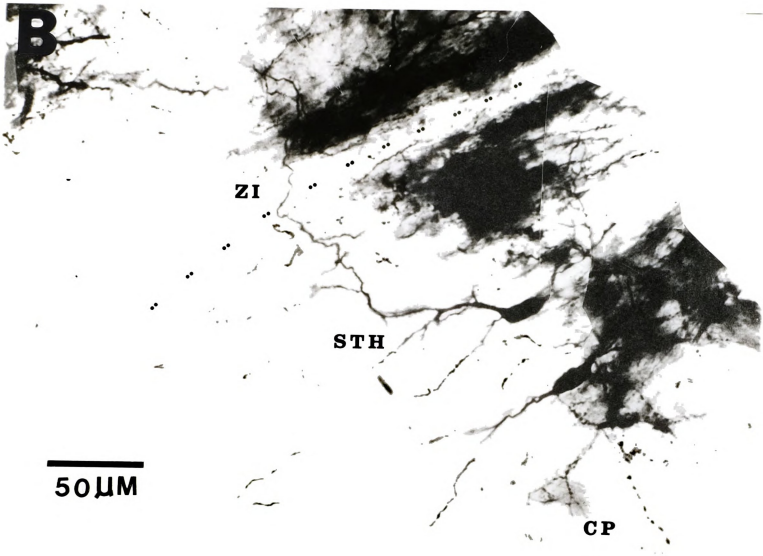
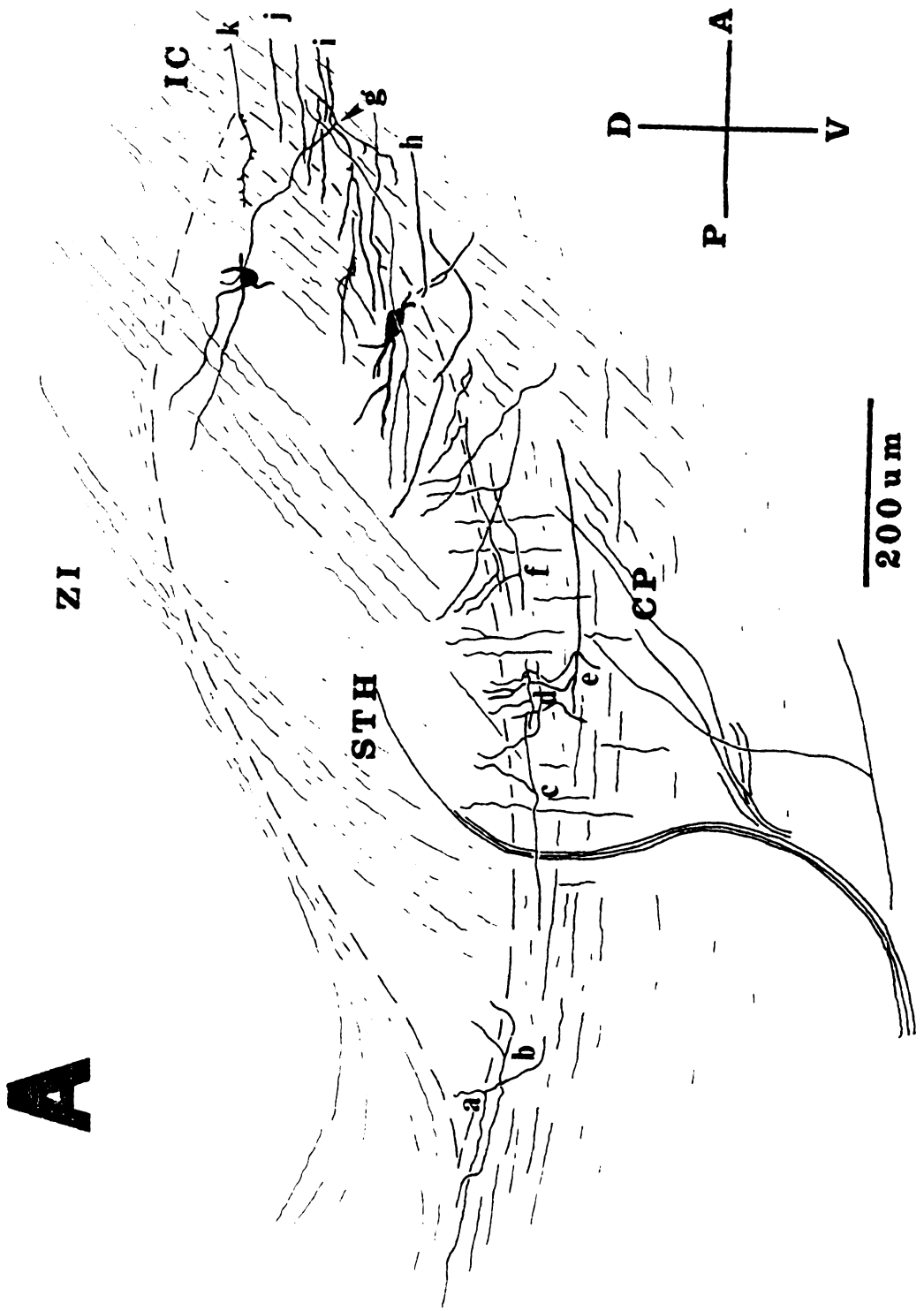

B
ZI
STH
50µM
CP

Figure 9.

Afferents to rat STH as seen from sagittal sections. Rostral is to the right, dorsal is at the top of the figures. A, Reconstructions of some Golgi impregnated rat STH afferents. The majority of the afferents are axon-collaterals of CP fibers. Some of them are derivatives of CP fibers which are directed caudorostrally (a, b, c), and others of rostrocaudally directed fibers (d, e, f). Some descending fibers running through the internal capsule also emit axon-collaterals toward STH (g). Another type of STH afferents are fibers which enter the nucleus from rostral aspect after crossing IC fibers (h, i, j, k). B, Photomicrograph of some Golgi impregnated STH afferents that are illustrated in A. CP, cerebral peduncle; IC, internal capsule; STH, subthalamic nucleus; ZI, zona incerta. Scale bar = 200μm.


A
ZI
IC
STH
CP
k
j
i
g
h
f
e
d
c
b
a
D
V
A
P
200um

B

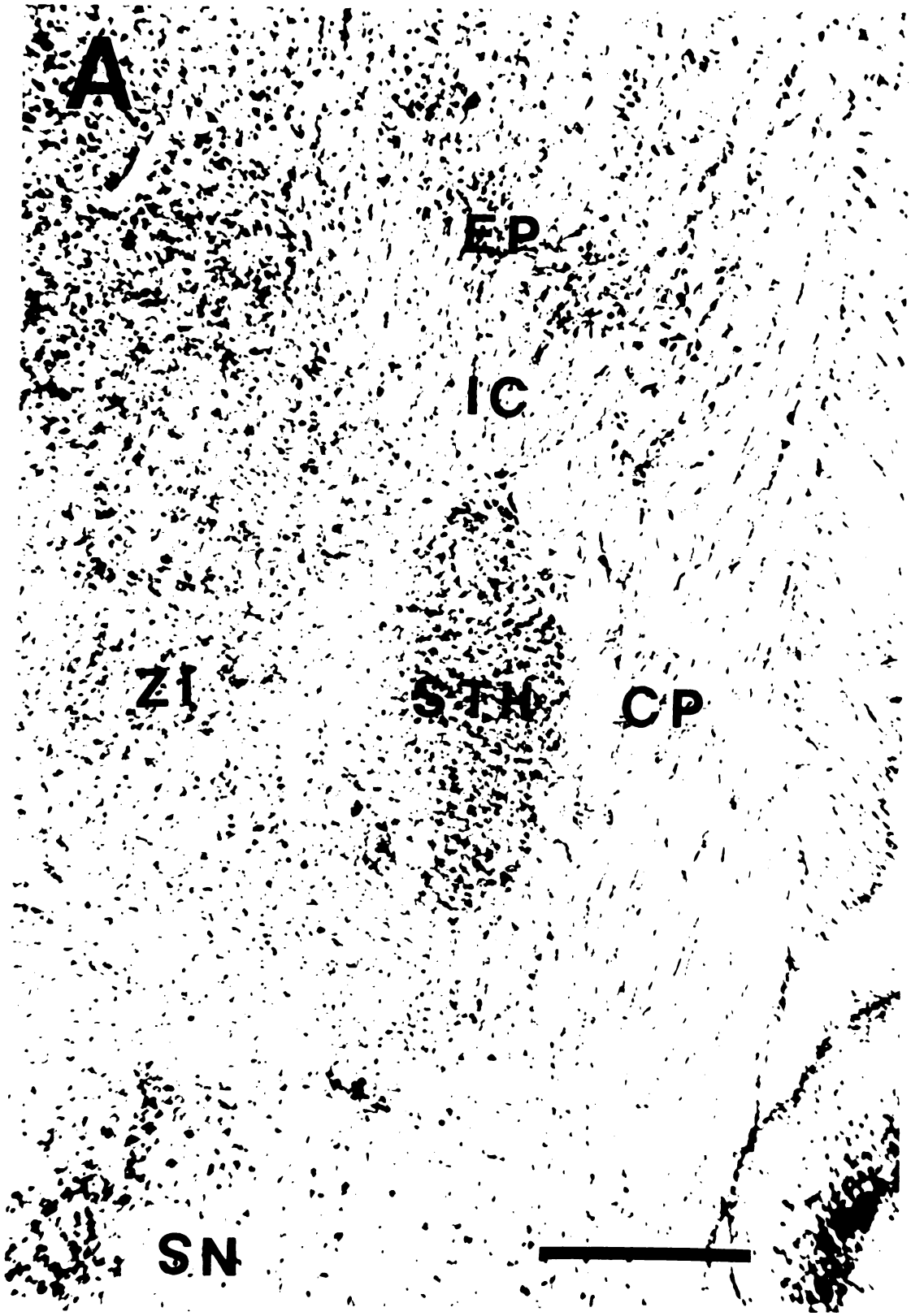
the mixed Golgi Kopsch-rapid Golgi procedure, had well impregnated afferent fibers. A large number of axon collaterals were derived from fibers within CP. They were usually directed dorsocaudally to enter STH (d, e, f in Fig.9A; c, d in 9B). Some of the axon collaterals which originated from fibers within CP at caudal levels of STH were directed dorsorostrally to enter STH (a, b, c in Fig.9A; a, b in 9B). Rostrally, there were two kinds of fibers that entered STH: 1) Axon collaterals and fibers which were relatively smaller in diameter from others, originated from IC fibers and directed toward STH (g in Fig.9A; e in 9B), and 2) fibers that were relatively thicker in diameter crossed IC toward STH (h, i, j, k in Fig.9A; g, h, i, j in 9B). In addition, there were fibers passing through STH without giving rise to any axon collaterals.

B. Nissl Study

The appearance and distribution of the somata of rat STH neurons in various parts of the nucleus are shown in Fig.10, 11, 12. Rat STH cell bodies were densely packed within the confines of the nucleus except at the dorsolateral and more rostral parts of STH. Some scattered cells in CP which could belong to STH were also visible (see arrow in Fig.10A). In frontal sections, STH somata appear to be more elongated at the dorsolateral and the dorsomedial parts of the nucleus. The longest, thinnest and the widest STH cell bodies observed from the frontal sections were

Figure 10.

Photomicrographs of STH from Nissl stained 25um thick horizontal sections. Lateral is to the right, dorsal is at the top of the figures. A, Low magnification photomicrograph showing the STH and the surrounding stractures. Note that STH is packed with somata compared to the other adjacent areas. CP, cerebral peduncle; EP, entopeduncular nucleus; IC, internal capsule; SN, substantia nigra; STH, subthalamic nucleus; ZI, zona incerta. Scale bar = 0.5mm. B, Higher magnification photomicrographs of A. The top photomicrograph is rostral part, lower left is middle part, and the lower right is the caudal part of STH. Note the polygonal or round shaped somata of STH neurons in this plane of section. Many adjacent STH neurons which have somatic membrane relationships are also visible . Scale bar = 50um.


A
EP
IC
ZI
STN
CP
SN

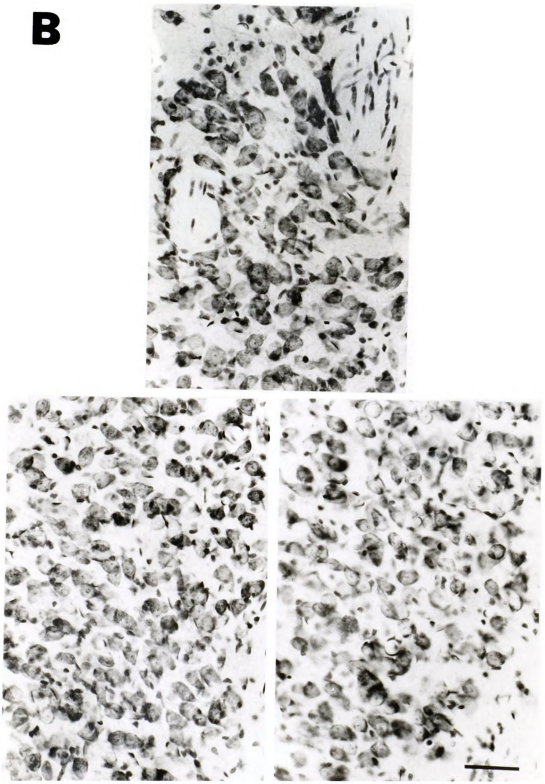
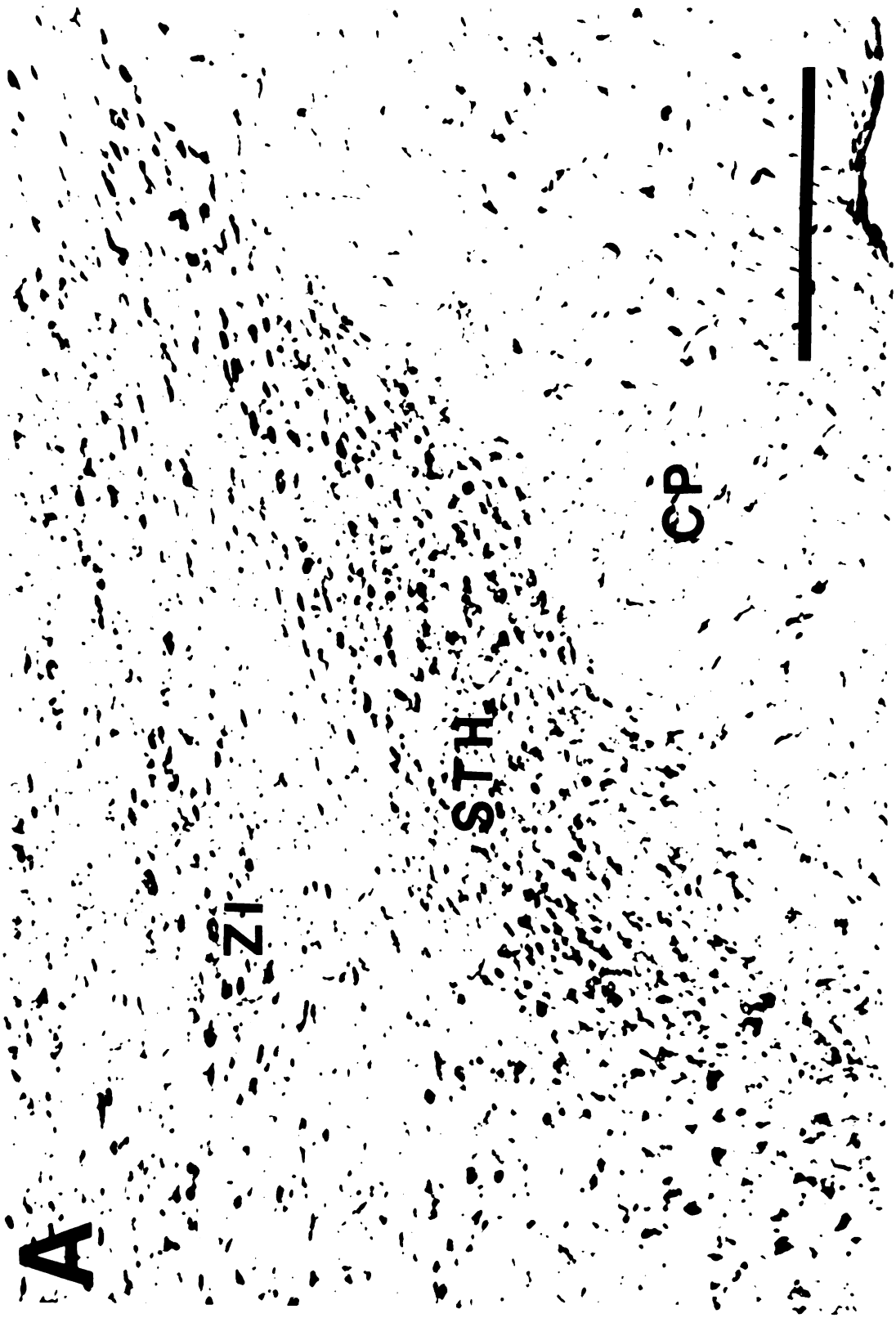

B

Figure 11.

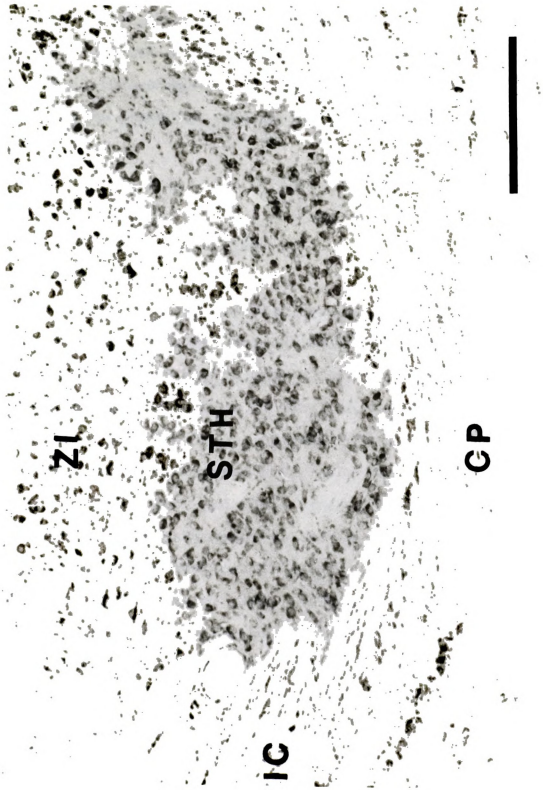
Photomicrographs of STH from Nissl stained 25um thick frontal sections. Lateral is to the right, dorsal is at the top of the figures. A, Low magnification photomicrograph showing the STH and the zona incerta. Note that STH is packed with somata. CP, cerebral peduncle; STH, subthalamic nucleus; ZI, zona incerta. Scale bar = 320um. B, Higher magnification photomicrographs of A. top photomicrograph is the lateral part, lower left is the middle part, and the lower right is the medial part of STH. Note the fusiform shaped somata of STH neurons in this plane of section, especially at the lateral part of STH. Scale bar = 50um.


A
IZ
STH
CP


B

Figure 12.

A low magnification photomicrograph of STH from Nissl stained 25um thick sagittal section. Rostral is to the left, dorsal is at the top of the figure. Note the round or polygonal shaped somata of STH neurons in this plane of section. Some fiber bundle sections are also visible. CP, cerebral peduncle; STH, subthalamic nucleus; ZI, zona incerta. Scale bar = 1/3mm.


ZI
STH
IC
CP

approximately 31μm, 6μm and 16.6μm, respectively. However, in the horizontal or sagittal sections, STH somata were more polygonal or round and measured between 10 to 28μm in diameters. All STH somata within the confines of the nucleus were intermingled in a manner such that no specific part of STH consisted of neurons with a particular size or shape.

C. Autoradiographic Study

Injection sites

The injection sites produced by representative amino acids injections into the various regions of the cerebral cortex are shown in Fig.13. Around the injection sites were uniformly distributed fields of label in the cortical neuropil that resulted in an apparent enlargement of the injection sites when observed under dark field illumination (Fig.13, 14A, 15A, 16A, and 17A). These fields of labels are probably due in large part to the local collateral arborizations of cortical cells (Cajal, 1911). From the cytoarchitectural point of view, the injection sites of all cases appeared to be restricted to the frontal agranular cortex except for CXSTH 23, 25 and 26. In the latter cases the injection sites appear to be limited to the caudal part of the frontal granular, occipital and parietal cerebral cortices respectively (Fig.13, 14A, 15A, 16A, 17A and cases 23, 25 and 26). Labeled projection axons arising from the injection sites were seen coursing caudally within fascicles

through the striatum, the internal capsule and the cerebral peduncle (e.g. Fig.13, CXSTH 12). Labeled fibers arising from IC and CP bundles were observed to reach the ipsilateral STH and resulted in either diffusely distributed silver grains or dense accumulations of labeling in a relatively restricted part of STH. Contralateral labeling was not observed.

Based on the cortical projection pattern into STH, the injection sites of the frontal agranular cortex (Fig.13) have been divided into four regions:

1. Rostral part of the medial agranular cortex

Figure 14C shows the light and diffuse labeling pattern of CXSTH 3, a representative case in which the radioactive amino acids were injected into the rostral part of the medial agranular cortex (Donophue & Wise, 1982), the eye & eyelid representations areas of Hall and Lindholm (1974). As seen from Fig.14C, E and 19A labeling was confined to the ventral two-thirds of the medial half of STH and extended rostrocaudally. The projection displayed a mediolateral ordered arrangement with some overlap. The rostral part of the eye & eyelid field projected ventromedially (cases CXSTH 1 and 2) and the caudal part of the field projected to the ventrolateral aspect of the medial half of STH(case CXSTH 5).

2a. Rostral part of the lateral agranular cortex

The projections from the lateral agranular cerebral

Table 1.

Injection sites and parameters of the radioactive labeling used for tracing corticosubthalamic projections (see also fig.13).

Case numbers	Injection site Bragma point (AP)(ML)	Radioactive lable Leucine Proline	Total Quant. µl	Concent. uCi/µl	Survival time/day
CXSTH,1	+6 & +1	Leu. & Pro.	0.4	20	4
CXSTH,2	+5 & +1	Leu. & Pro.	0.4	20	4
CXSTH,3	+3.5 & +1.5	Leu. & Pro.	0.4	20	7
CXSTH,4	+0.5 & +1	Leu. & Pro.	0.4	20	7
CXSTH,5	+2 & +1	Leu. & Pro.	0.4	20	7
CXSTH,6	-0.5 & +1.5	Leu. & Pro.	0.4	20	7
CXSTH,7	-1 & +2.5	Leu. & Pro.	0.4	20	7
CXSTH,8	-0.5 & +3	Leu. & Pro.	0.25	20	7
CXSTH,9	+0.5 & +3.2	Leu. & Pro.	0.3	20	7
CXSTH,10	+5.8 & +1.9	Leu. & Lys.	0.3	20	7
CXSTH,11	+5.5 & +2.8	Leu. & Lys.	0.4	20	7
CXSTH,12	+5 & +3.5	Leu. & Lys.	0.3	20	7
CXSTH,19	+4.2 & +2.8	Leu. & Pro.	0.3	15	7
CXSTH,20	+2 & +2.6	Leu. & Pro.	0.3	15	7
CXSTH,21	+4.3 & +4	Leu. & Pro.	0.3	15	7
CXSTH,22	+2.3 & +4.5	Leu. & Pro.	0.3	15	7
CXSTH,23	-2.5 & +6	Leu. & Pro.	0.3	15	7
CXSTH,24	-3 & +1.5	Leu. & Pro.	0.3	15	7

CXSTH,25	-6 & +1.5	Leu. & Pro.	0.3	15	7
CXSTH,26	-5 & +5	Leu. & Pro.	0.3	15	7

Figure 13.

Illustration of the site and the extent of the amino acid injection into the various regions of the rat cerebral cortex. Black areas represent the injection site. The stippled areas represent the corona surrounding the center of the injection site which probably corresponds to diffusely spread amino acids. Case 4 and 12 were cut into sagittal sections; 6,7 and 11 horizontal sections; and the rest frontal sections. The schematic drawing of rat cerebral hemisphere at the center of the figure shows the topographical placement of the injection sites. B corresponds to the bregma. One mm spaced lines are drawn in order to show the relative coordinate of various injection sites.

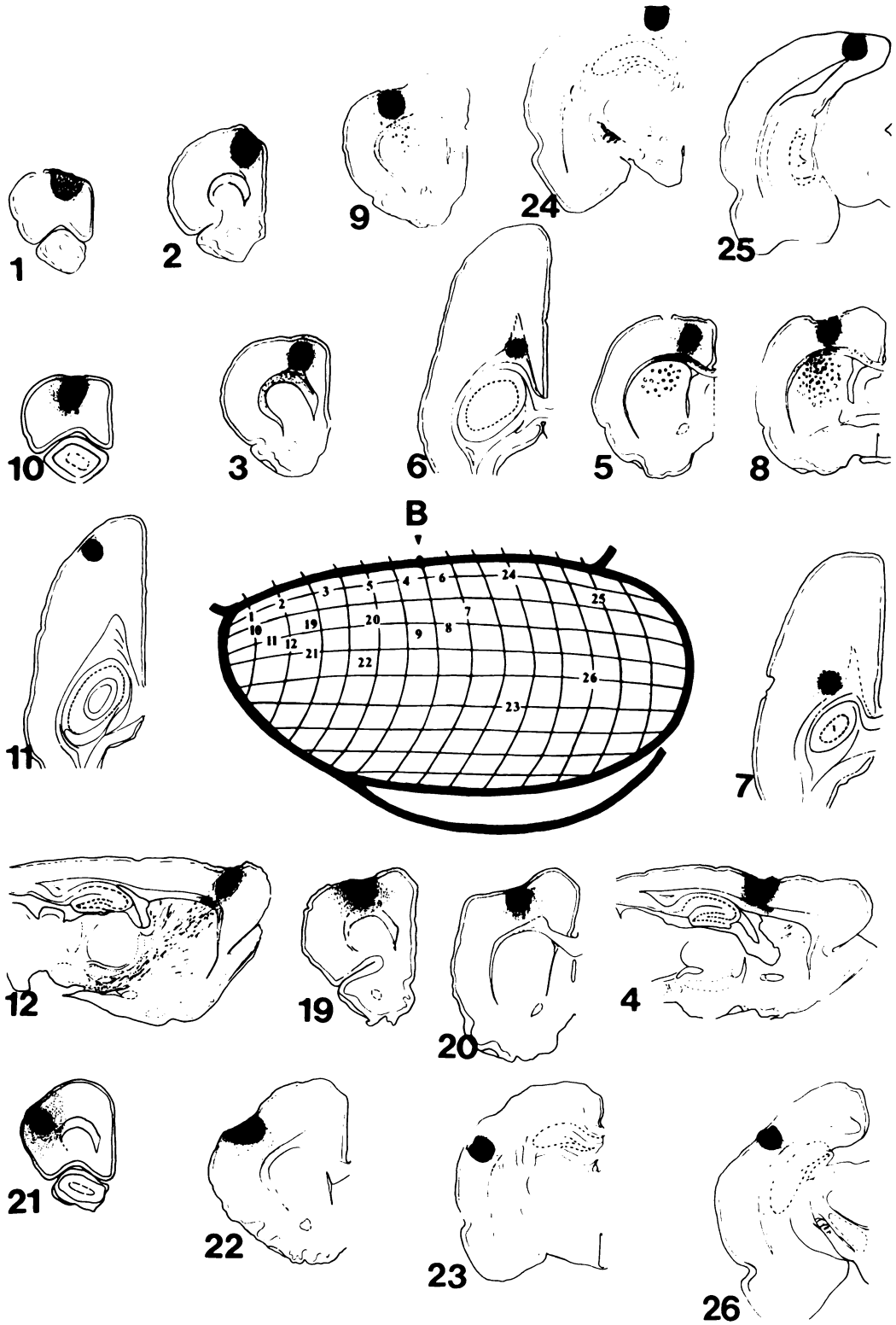
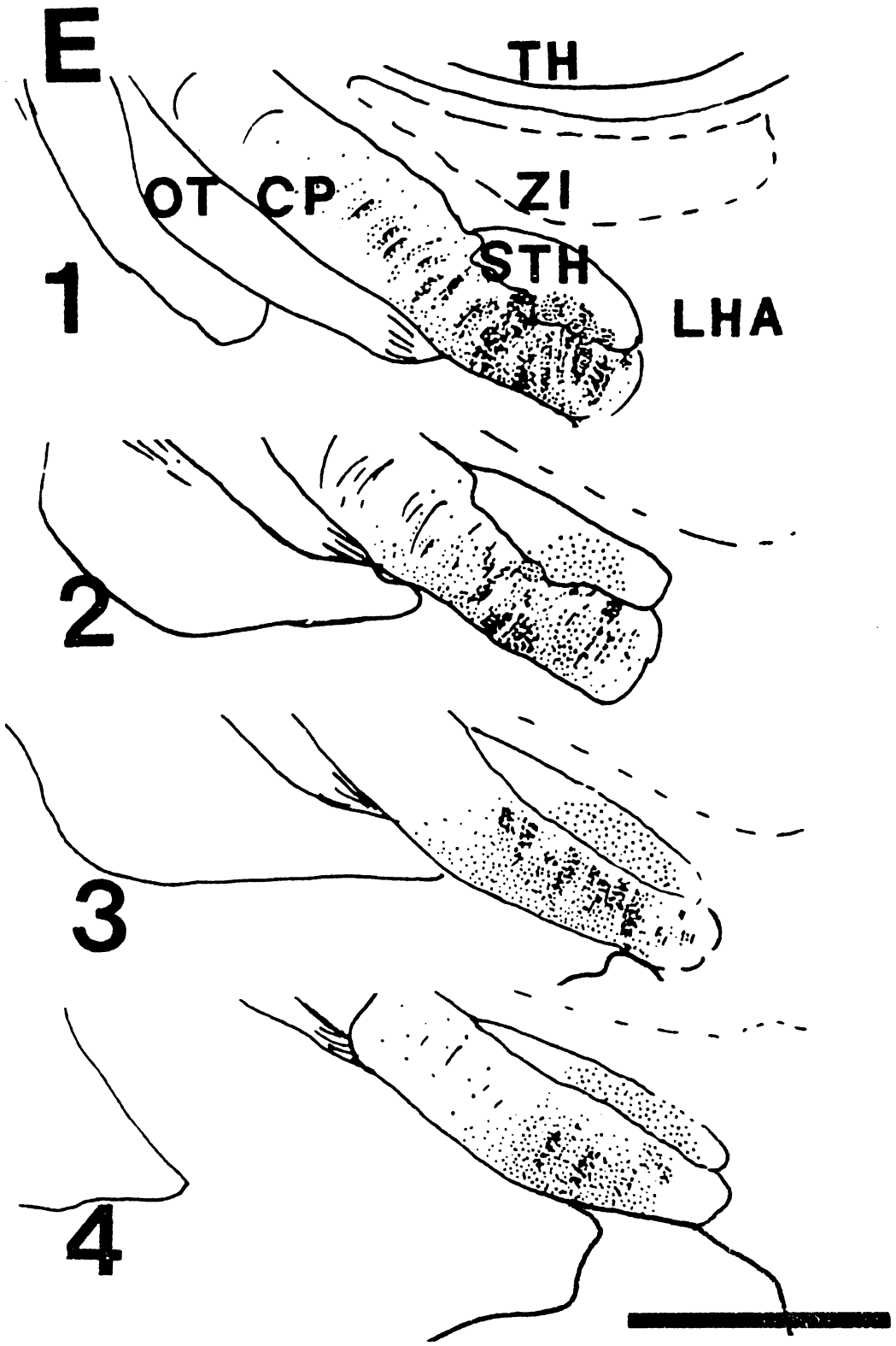

1
2
9
24
25
10
3
6
5
8
B
11
7
12
19
20
4
21
22
23
26

Figure 14.

Illustration of case #3 (representative of the rostral part of the medial agranular cortical region injections). A & B, Dark and light-field photomicrographs of the injection site. The center of the injection site and its extent is shown in B. Note the diffuse pattern of silver grains surrounding the center of the injection site shown in A. Scale bar = 2mm. C, Dark-field photomicrograph showing the labeling in STH. Note the light and diffuse labeling within the medial half of STH. D, light-field photomicrograph shows the cytoarchitectural borders of STH. Scale bar = 0.5mm. E, Drawing of the projection pattern in frontal sections. Labelings are observed in the entire rostral caudal extent of the nucleus. Fiber-like silver grain accumulations in CP are indicated by heavy stipples. Fibers which were cut in cross section in CP or STH are indicated by stippled circles indicated by arrow heads. The uneven but diffuselly distributed silver grains (stipples) within STH probably represent terminal fields. E1, rostral and E4, caudal. Scale bar = 1mm.

A
B
C
D


E
TH
OT
CP
ZI
STH
1
LHA
2
3
4

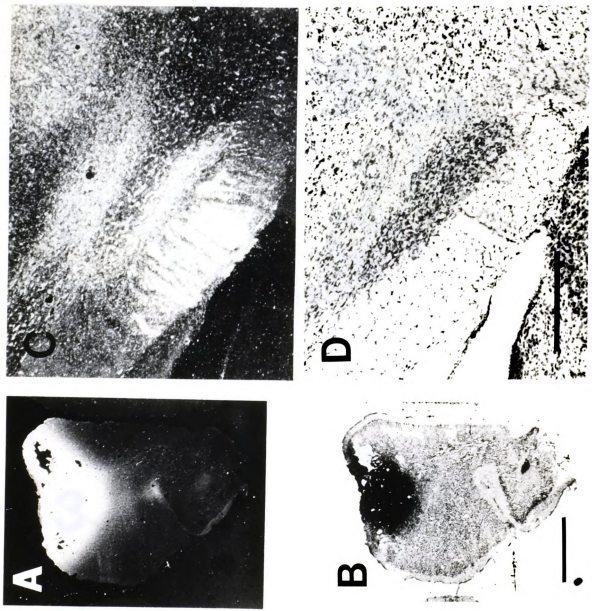
cortex, (M1) (Donophue, 1982), corresponding to the mouth, jaw and the tongue representations area (Hall and Lindholm, 1974) were considerably more dense than that of the medial agranular area (Fig.16C and E). A comma shaped accumulation of silver grains was found in the lateral two-thirds of the rostral two-thirds of STH (Fig.19C). The rostrocaudal parts of this cortical region displayed some superimposed labeling in STH such that the rostral parts of the cortex projections were confined to the lateral two-thirds of the rostral two-thirds with more concentration at the dorsolateral tip of STH, and the caudal parts of the cortex projections were mostly restricted to the dorsolateral tip and the dorsal aspect of the lateral half of STH (Fig.19C CXSTH 11 & 12 and 21 & 22 respectively).

2b. Medial part of the lateral agranular cortex

Generally, the projections from this region occupied almost the same part of STH as that of the medial granular cortex. Although overlapping between adjacent projections are seen, the pattern of labeling appears different than those of the other cortical regions (Fig. 13, 15 and 19B). As seen from these figures, the amino acid injections in this cortical area resulted in a band-shaped labeling of STH. The band-shaped labeling was confined to the ventral aspect of rostral STH, and tended to shift dorsally at the caudal part of STH. The projections from the rostrocaudal extent of the cortex which correspond to the rhinarium, vibrissa and forelimb representations areas respectively

Figure 15.

Illustration of case #19 (representative of the medial part of the lateral agranular cortical region injections). A & B, Dark and light field photomicrographs of the injection site Scale bar = 2mm. C, Dark-field photomicrograph showing the labeling within STH. Note the dense and relatively wide band of labeling extends from the dorsolateral to the ventromedial aspect of STH. D, light-field photomicrograph showing the cytoarchitectural borders of STH. Scale bar = 0.5mm. E, Drawing of the projection in frontal sections. Labelings are seen in rostral through caudal sections. Note the shifting of the terminals from the ventral STH in the rostral sections to the dorsal aspect in the caudal sections. E1, rostral and E4, caudal. Scale bar = 1mm.


A
B
C
D

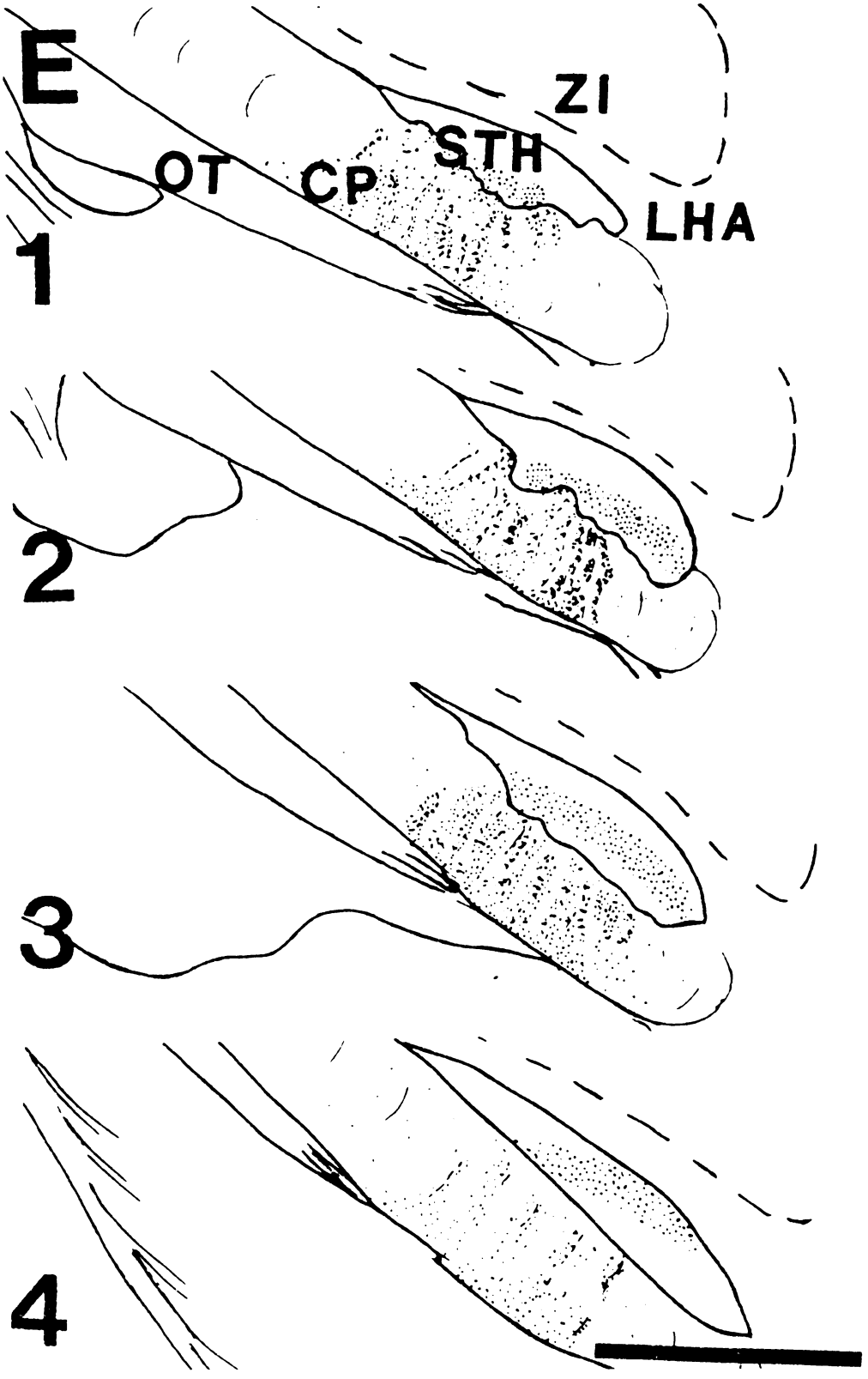
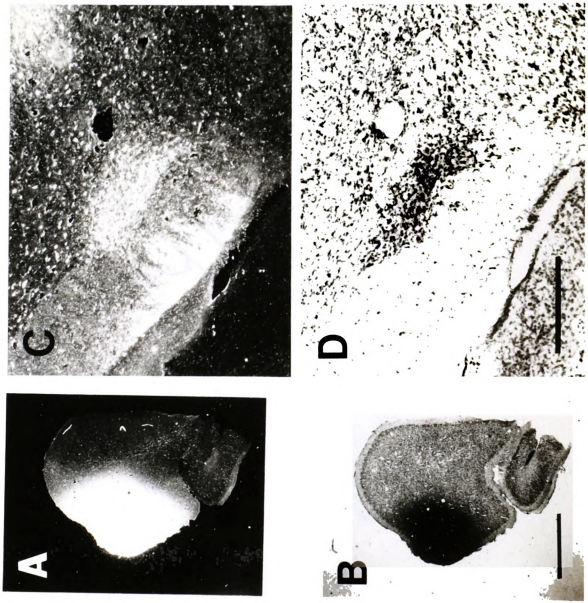
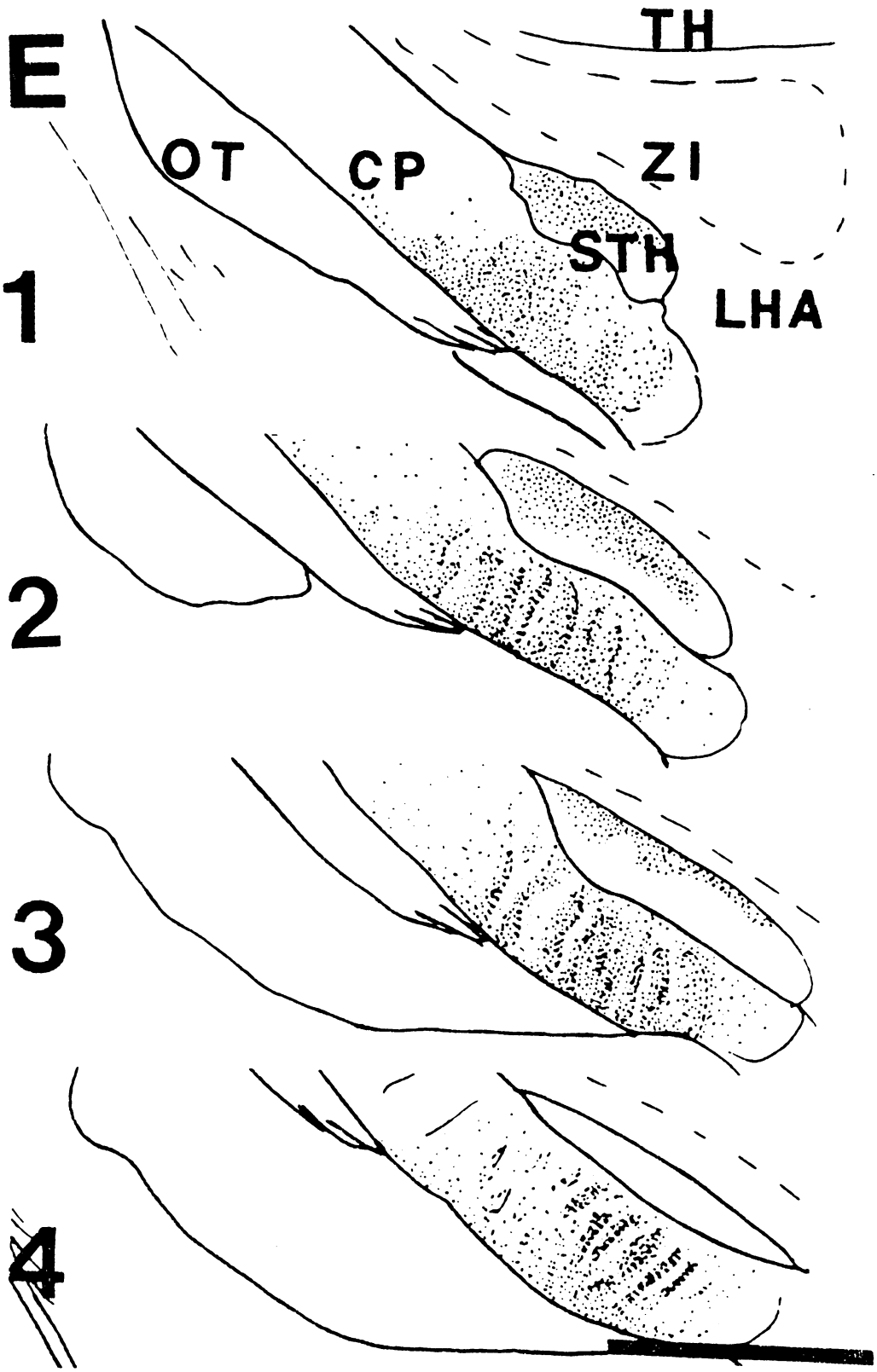

E
ZI
STH
OT
CP
LHA
1
2
3
4

Figure 16.

Illustration of case #21 (representative of the rostral part of the lateral agranular cortical region injections). A & B, Dark and light-field photomicrographs of the injection site Scale bar = 2mm. C, Dark-field photomicrograph showing the labeling within STH. Note the heavy projection in the dorsal aspect of the nucleus. D, light field photomicrograph shows the cytoarchitectural borders of STH. Scale bar = 0.5mm. E, Drawing of projection in frontal sections. Labelings are seen in the rostral two-thirds of STH. Note the widespread labeling in the rostral part while the labeling becomes restricted to the more dorsal aspect of STH toward the middle third of the nucleus. No labeling is seen in the caudal third of the nucleus. E1, rostral and E4, caudal. Scale bar = 1mm.


A
B
C
D


E
TH
OT
CP
ZI
STH
LHA
1
2
3
4

(Hall and Lindholm, 1974) were confined to the medial half of rostral STH, rostrally, and extended to the medial part of the lateral half of STH caudally.

3. Caudal part of frontal medial agranular cortex

Figure 17C and E show the faint projections of this region, corresponding to the caudal portion of eye & eyelid and the trunk representations areas (Hall and Lindholm, 1974). Silver grains were confined to the dorsolateral portion of the caudal two-thirds of STH. The overlapping pattern in these projections can be contrasted with the projections from the lateral agranular region, in which the superimposed labeling occurs rostrally and the partial overlap occurs caudally. Whereas the superimposed labeling in this case was seen caudally and the partial overlap occurred rostrally.

4. Caudal part of the lateral agranular cortex cortex

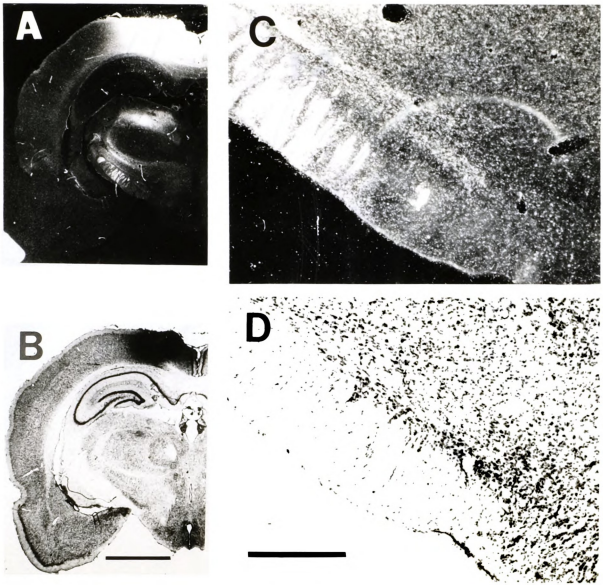
Figure 19E shows the labeling from the caudal part of the lateral agranular cortex, which corresponds to the hindlimb representations area (Hall and Lindholm, 1974). Labeling was observed in the ventral aspect of the middle third of STH (see also table 1 and Fig.13 for injection site).

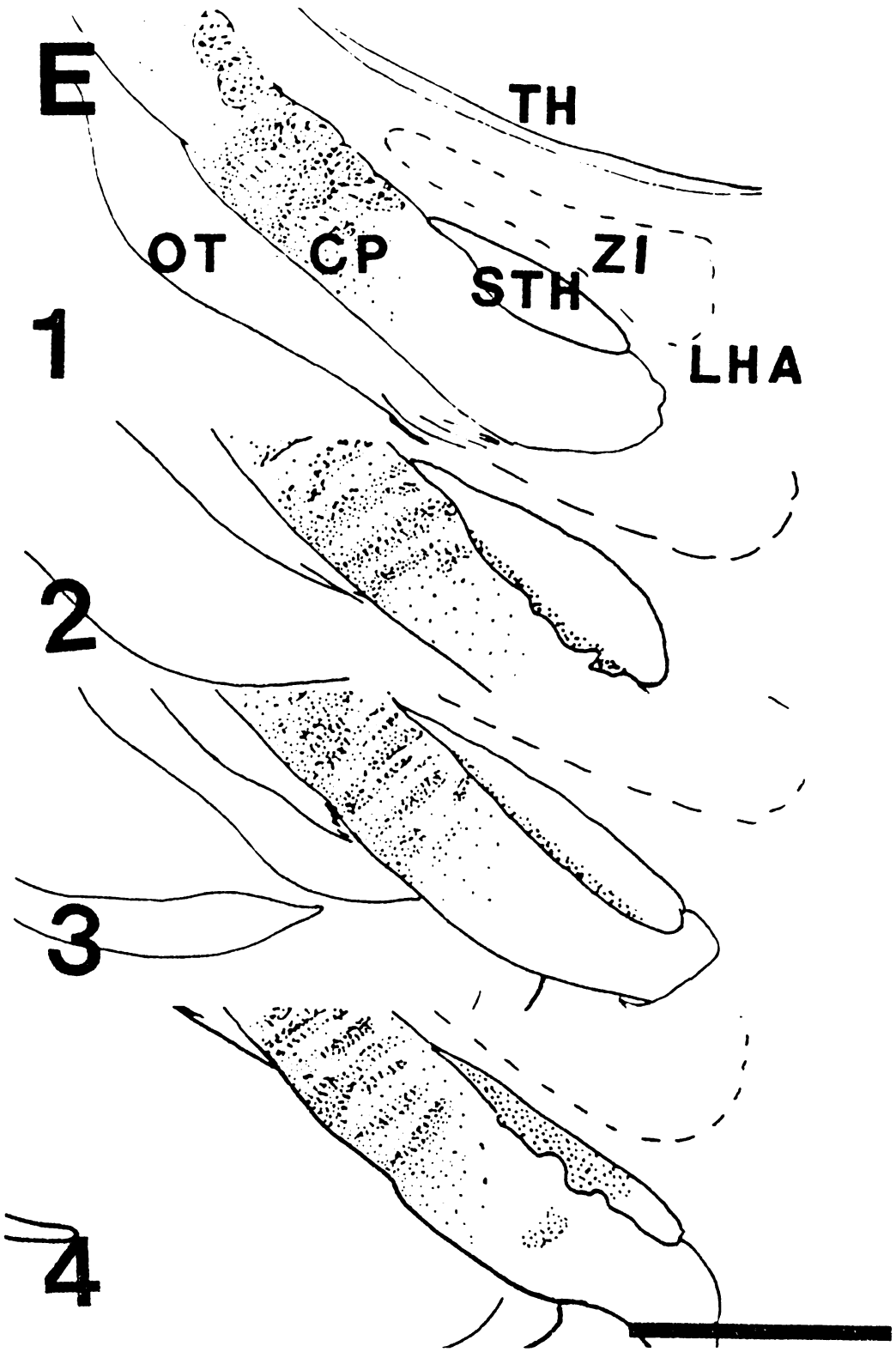
5. Other cerebral cortical areas

When the injections were confined to the frontal granular (CXSTH 23), occipital (CXSTH 25) and parietal

Figure 17.

Illustration of case #24 (representative of caudal part of the frontal medial agranular cortical region injections). A & B, Dark and light-field photomicrographs of the injection site Scale bar = 2mm. C, Dark-feld photomicrograph showing the labeling within STH. Note the diffused labelings in the lateral two-thirds of STH. D, light field photomicrograph shows the cytoarchitectural borders of STH. Scale bar = 0.5mm. E, Drawing of projection pattern in frontal sections. Labeling covers almost the entire nucleus in the caudal sections and becomes restricted to the ventral portion in the middle sections. Labeling is not seen in the rostral third of STH. E1, rostral and E4, caudal. Scale bar =1mm.


A
C
B
D

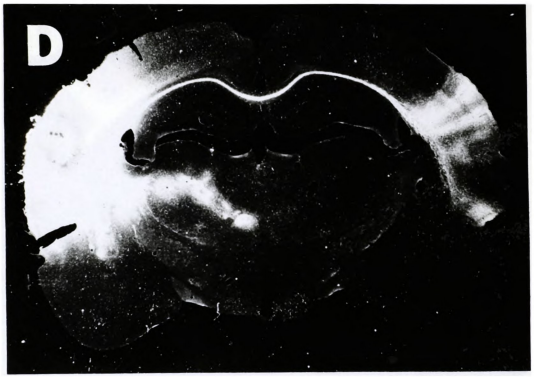

E
TH
OT
CP
STH
ZI
1
LHA
2
3
4

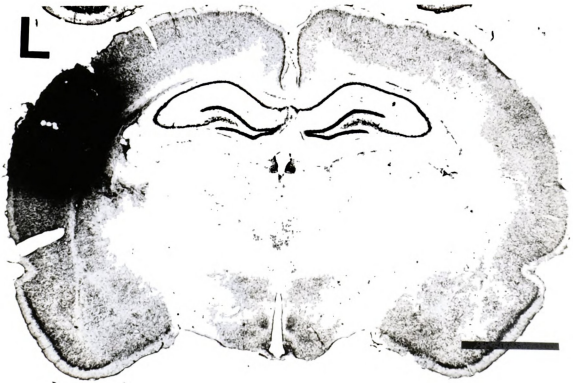
(CXSTH 26) areas (see Fig.18 and table 1), no labeling was found within STH.

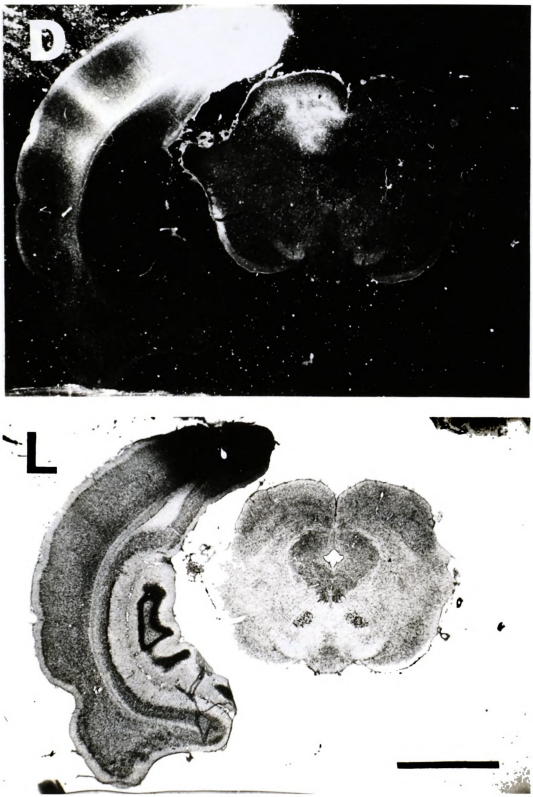
In summary, the medial frontal agranular cortex projections to STH were faint and less in density than the more pronounced lateral frontal agranular projections. Caudally spaced injections of each region produced orderly overlapped or superimposed projections to STH. Each cortical region showed some topographically arranged projections in STH. The projection from the whole frontal agranular cortex fills all parts of STH. Injections in the frontal granular, parietal and occipital cortex did not result in labeling within STH.

Figure 18.

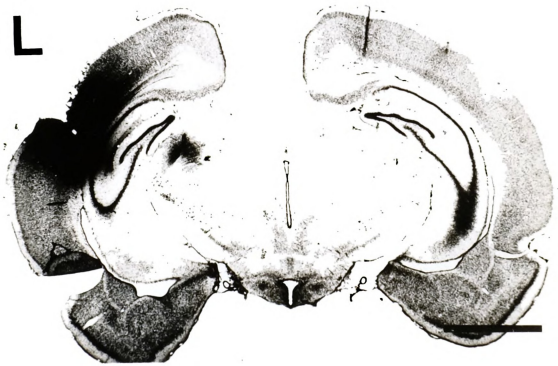
Dark-field (D) and light-field (L) photomicrographs illustrating the injection sites and the extent of the amino acid injections into the somatosensory, visual and parietal cortex (cases #23, #25 and #26). No labelings were seen in STH in these cases. Scale bar = 2mm.


D


L


D
L

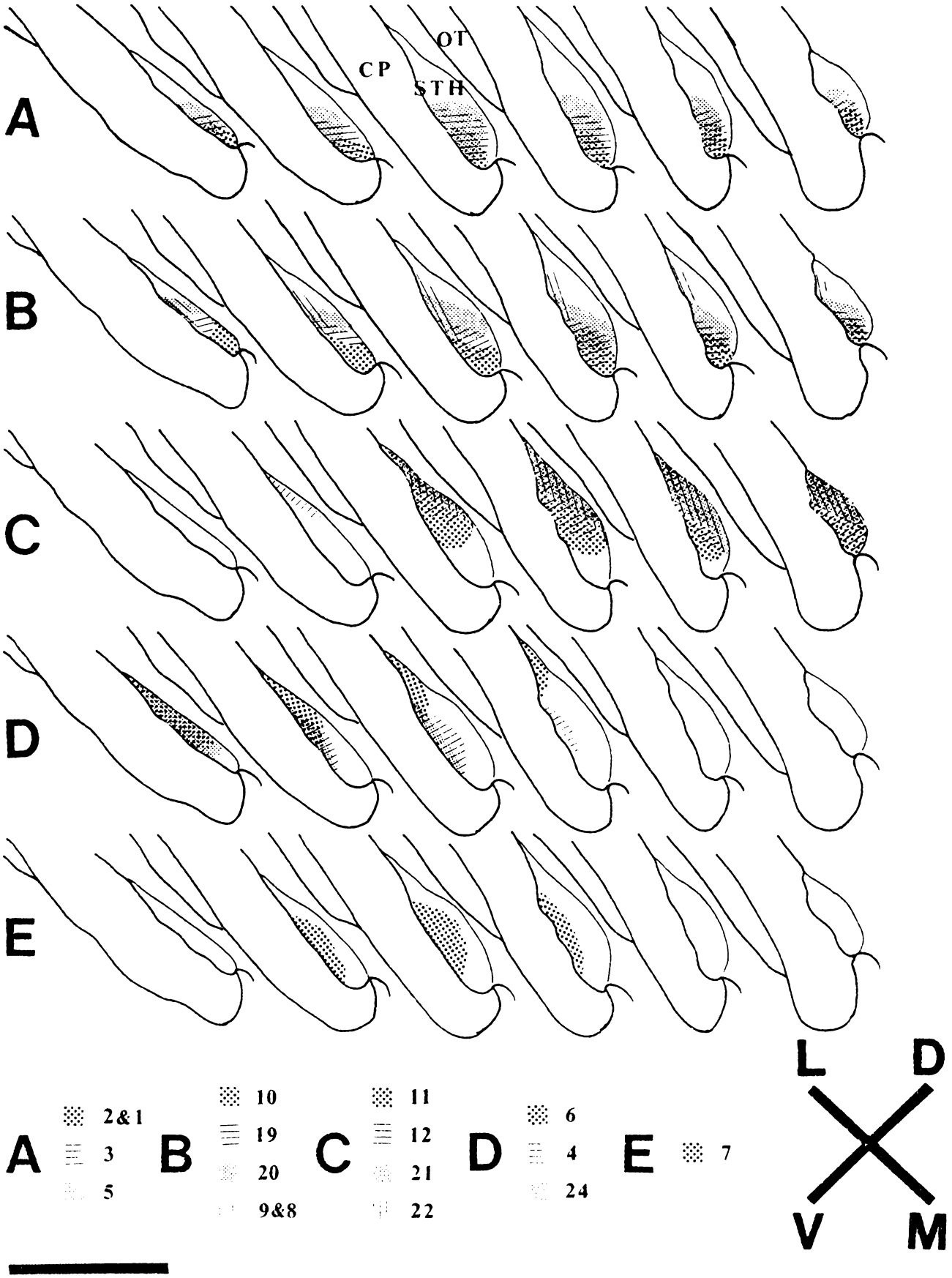

D


L

(case #7 in Fig.13). Labeling is in the ventral aspect of the middle third of STH. No labeling is seen in the rostral or the caudal sections. Scale bar for all groups = 1mm.

Figure 19.

Summary diagram of the projections from the agranular cerebral cortical areas to STH shown in frontal sections. Rostrocaudal extent of the nucleus is indicated from right to left in each row. A, Projections from the rostral part of the medial agranular cortical areas (cases #1, 2, 3 and 5 in Fig.13). Labeling is in the medial half of the nucleus except at the rostral portion of STH. The dorsal portion of the medial half of STH is void of labeling. B, Projections from the medial part of the lateral agranular cortical areas (cases #10, 19, 20, 9 and 8 in Fig.13). Labeling is in the medial half of STH, and extends to the ventral portion of the lateral half of the nucleus. C, Projections from the rostral part of the lateral agranular cortical areas (cases #11, 12, 21 and 22 in Fig.13). Labeling occupies the entire nucleus at the rostral portion of STH. In the middle sections, the labeling becomes sparse in the medial half of the nucleus. In the caudal sections, the labeling becomes restricted to the lateral half of STH and eventually at the most caudal sections there is no labeling. D, Projections from the caudal part of the frontal medial agranular cortical areas (cases #4, 6 and 24 in Fig.13). The pattern of labeling is almost mirror image of that in C. Almost entire nucleus is labeled in the caudal sections and no labeling in the rostral sections. In the middle sections, labeling is predominantly in the lateral half and the ventral portion of the medial half of STH. E, Projections from the caudal part of the lateral agranular cortical areas


OT
CP
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2&1
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19
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9&8
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CHAPTER 4. DISCUSSION

A. Golgi Study

General Comments On Neuronal Types:

Many of the previous Golgi studies on STH neurons have either not shown illustrated figures (Kolliker, 1896; Mirto, 1896), or have presented only drawings (Ramon y Cajal, 1910-1911; Ramon Moliner, 1962; Leontovich & Zhukova, 1963; Iwahori, 1978; Yelnik & Percheron, 1979). Rafols & Fox (1976) were the only ones to illustrate their data with both drawings and photomicrographs. In the present study we attempted to illustrate cell morphology with both drawings and photomicrographs of STH neurons, so that more adequate comparisons could be made concerning the varieties of cell morphology described in this study.

In the few recent Golgi studies a number of STH neuronal types have been described. Rafols & Fox (1976) demonstrated two varieties of principal neurons, radiating and elongated fusiform, in addition to a local Golgi type II interneuron in the monkey. Iwahori (1978) distinguished three neuronal types in the kitten: Type I are the main constituent of the nucleus, having medium sized oval or polygonal cell bodies. Type II are large with multipolar or polygonal cell bodies. And type III are small with polygonal cell bodies. However, Yelnik & Percheron (1979) emphasized that STH consists of only one type of neurons (Golgi type I) in cat, monkey and man. And in a most recent intracellular labeling study by Kita et al., (1983), two

types of STH neurons were distinguished, STH neurons with and without intranuclear collaterals.

In our Golgi material axons were rarely and only partially impregnated. So we have classified rat STH neurons on the basis of their somatic and dendritic morphological characteristics. The results of the statistical analysis of somatic size and the number of proximal dendrites suggested that rat STH consisted of only one type of neuron. This type can be further subdivided into three morphologically different varieties.

1. Subthalamic Neurons With 3 to 5 Proximal Dendrites:

Neurons which are morphologically similar to this variety have been illustrated by Kita et al. (1983) in the rat STH. Moreover, they have provided more axonal information on the distribution and morphology of axons in their intracellular HRP labeling of STH neurons. These cells are also similar to those illustrated by Leontovich & Zhukova (1963) in their figure 12, in the dog; Rafols & Fox (1976) in their figures 2 and 9 as one of the principal neurons of the adult monkey STH; Iwahori (1978) in his figures 2C, 4A,I in the cat, and Yelnik & Percheron (1979) in their figures 6E, 7D and 8B,C in the cat, monkey and man. In the present study this variety of rat STH neuron was the most frequently impregnated and has been found to be usually medium in somatic size, with an elongated ovoid shaped soma. The quantity of this type of neuron however, was less than the other types demonstrated by Rafols & Fox (1976) and

Iwahori (1978). The latter authors have observed more radiating STH Golgi impregnated neurons than the elongated ovoid ones. This type of STH neuron was not even mentioned in earlier studies (Kolliker, 1896; Mirto, 1896; Ramon y Cajal, 1910-1911).

2. Subthalamic Neurons With 4 to 6 Proximal Dendrites:

This variety of rat STH neuron appears to correspond to the radiating principal neurons of Rafols & Fox (1976) in their figures 1C, D; 10; 13 in the monkey; type I neurons of Iwahori (1978) in his figures 2A; 4B, C, D, E, F, G, H, J, K, L in the kitten; some of the Golgi type I neurons of Yelnik & Percheron (1979) in their figures 6A, B, C, D in the cat; 6F, G, H, I in the monkey; 6K, L, M in the baboon and 7C, 8D in the human, and also to some neurons illustrated by Kita et al. (1983) in the rat. In the present study the feature which characterized the morphology of this variety of neurons is the origin and number of proximal dendrites that reflects its radiating dendritic field. This variety of rat STH neuron was less frequently impregnated than the elongated ovoid ones mentioned above. On the other hand, in previous Golgi studies, radiating neurons were either the only ones illustrated (Ramon y Cajal, 1910-1911; Ramon Moliner, 1962; Leontovich & Zhukova, 1963) or considered to be the predominent neuron in STH (Rafols & Fox, 1976; Iwahori, 1978; Yelnik & Percheron, 1979).

3. Subthalamic Neurons With Only Two Proximal Dendrites:

This variety of rat STH neurons were characterized by a fusiform shaped soma and only two proximal dendrites. Like the radiating variety, these neurons were infrequently impregnated. This variety has not been previously observed in other species or in the rat using the intracellular HRP labeling technique (Kita et al., 1983; Hammond et al., 1981), with the exception of one cell illustrated in figure 7 of Yelnik & Percheron (1979).

Golgi type II local interneurons described in detail by Rafols & Fox (1976) have not been observed in any other Golgi or intracellular labeling study of STH. In the present study, despite the use of several Golgi impregnating techniques and a large number of rats, none of the observed neurons were seen to have the morphological characteristic features of Golgi type II neurons.

Dendritic Appendages Of Rat Subthalamic Neurons:

In the present study, in addition to dendritic spines, we have observed on a majority of rat STH neurons thin dendritic appendages or filiform processes which are variable in length (3 to 60μm long) and end with a round head. These processes have not been described on principal neurons in previous Golgi studies in other species. Yelnik & Percheron (1979) in their figure 5H, illustrated one small human STH neuron in which the dendrites were characterized by thin processes with intermittent swellings. Rafols & Fox (1976) in their figure 3, have also shown a Golgi type II

neuron characterized by bulbous dendritic appendages and beaded axon-like processes. The presence of this type of dendritic appendages was observed in intracellularly HRP labeled rat STH neurons (Kita et al., 1983).

The question of whether STH is an open or closed nucleus has been discussed by Yelnik & Percheron (1979). An open nucleus is is defined as one where dendrites of neurons within the nucleus intermingle with dendrites of neurons in adjacent structures after they have crossed beyond the cytoarchitectonic limits of the nucleus (Mannen, 1960). In our observations we have found dendrites of some centrally located STH neurons to extend beyond the confines of the nucleus and terminate in ZI and LHA. None of the previous Golgi studies in different species (Kolliker, 1896 in rat and mouse; Mirto, 1896 in man; Ramon y Cajal, 1910-1911 in mice and cat; Ramon Moliner, 1962 in cat; Leontovich & Zhukova, 1963 in dog; Rafols & Fox, 1976 in monkey; Iwahori, 1978 in kitten; Yelnik & Percheron, 1979 in cat, monkey and man) have indicated any such STH neurons' dendrites spreading out of the dorsal, dosomedial or the ventromedial borders of the nucleus. However, in recent studies by Hammond et al. (1981) and Kita et al. (1983) using the intracellular HRP labeling technique, some rat STH neurons' dendrites were shown to extend beyond the borders of the nucleus and terminate in ZI, IC and CP. Since our Golgi-impregnation confirm the findings of Hammond et al. (1981) and Kitai et al. (1983) concerning dendritic extension beyond the confines of the rat STH, we suggest

that the rat STH be considered as an open nucleus.

Afferent Fibers Of Subthalamic Nucleus:

One of the main afferent fibers to STH arises from the ipsilateral globus pallidus (the homolog of the lateral pallidal segment in primate). This has been demonstrated in a variety of species using different techniques. Cerebral cortical afferent to STH have also been demonstrated. A few afferents from the pedunculopontine nucleus have been reported as well as a nigrosubthalamic projection.

Kolliker (1896), Mirto (1896), Ramon y Cajal (1910-1911), and Iwahori (1978) have mentioned that STH afferents were mainly axon-collaterals of fibers that descended within CP. In the present study axon-collaterals were also observed to arise mainly within CP coursing toward STH. Thy were of two directions: 1) axon-collaterals from fibers directed rostrocaudally in CP and 2) axon-collaterals of fibers directed caudorostrally in CP. In addition, a number of axon-collaterals from IC fibers were seen to enter the nucleus after crossing IC rostrally. The latter fibers were found to arise from the large cells of the globus pallidus (Shanner, 1936). Some fiber bundles passing STH without emiting any axon-collateral were also seen.

B. Nissl Study

The cytoarchitecture of STH has been described in man (Foix & Nicolesco, 1925) to have STH neurons that

appeared larger in the dorsal and internal parts of the nucleus than in ventral and external parts. However opposite observations were reported by Kodama (1928) in man, and by Whittier & Mettler (1949) in the monkey. The latter authors observed large STH somata in the lateral parts and the smallar ones in the medial parts of the nucleus. Rafols & Fox (1976) have also localized the smaller somata of the monkey to a narrow strip along the ventral border at the medial end of the nucleus. Yelnik & Percheron (1979) have revealed that different shapes of somata were intermingled so that no part of the nucleus contained any particular shape of neuron. In spite of the unimodal distribution of STH neurons, the latter authors found more fusiform somata in sagittal sections and more round or polygonal somata in frontal sections of samples taken from monkey and man.

Our results generally confirmed the findings of Yelnik & Percheron (1979). However, the rat STH somata were observed to be more fusiform specifically in dorsolateral parts of the nucleus when observed in frontal sections, and more polygonal or round when observed in sagittal or horizontal sections. Thus the results of the present studies (Golgi and Nissl) suggest that the size or shape of STH neurons seem to be linked most likely to the plane of section.

C. Autoradiographic Study

Two amino-acid injections were confined to the rostral part of the medial frontal agranular cortex, and the other caudally placed two injections extended into the lateral agranular (Agl) regions. These injections resulted in only faint labeling in STH. This may indicate a minor projection from this particular part of the frontal cortex to STH. However, the projections from the lateral and intermediate agranular regions to STH were more dense than that of the medial agranular part. This indicated that a large number of projection neurons to STH are located in these regions of the agranular cortex. Interestingly, Donoghue & Wise (1982) have shown that the Agl of the frontal cortex contains more cortico-spinal projection neurons than that of the medial agranular (Agm) of the frontal cortex. The similarity of the results in these two studies and previous STH Golgi studies (Cajal, 1910-1911; Iwahori, 1978) as well as the present Golgi study of rat STH demonstrating some axon collaterals entering STH from IC and CP fibers, suggests that the cortico- STH fibers are axon collaterals of cortico-spinal fibers.

Hartmann-Von Monakow et al. (1978) have shown that the monkey precentral and frontal motor cortex (area 4) contain more projection neurons to STH compared to areas 6, 8 and 9 of the prefrontal cortex. These projections were topographically organized within the lateral half of monkey STH. In the present study we have also demonstrated that the rat cortico-STH projections are topographically organized. However, in the rat the arrangement of the face, forelimb and hindlimb representation area projections were

different from that of the monkey. In the rat, the jaw, lip and the tongue representation areas project to the dorsal aspect of STH, the forelimb area projects to the ventral aspect of the lateral half of STH, and the hindlimb area projects to the ventral aspect of the middle third of STH. On the other hand, in the monkey, the face area projects to the lateral, the leg area to the medial aspect of the lateral part of STH, and the arm area projection was found in between the face and the leg areas (Hartmann-Von Monakow et al., 1978). Another interesting point which was revealed from this study was that the projection from the eye and eyelid representation area to STH in the rat was found to have similar characterestics to the monkey area 8 (prefrontal eye field)projection to STH. The similarities included 1) Light and diffuse labeling; 2) The labeling is confined to the ventral part of the medial half of STH (Kunzle & Akert, 1977 and Hartmann -Von Monakow et al., 1978). Our observations have also shown that the projections from rat frontal agranular cortex filled the entire STH. However, in a study in the monkey, the cumulative plot of labelings of all injection cases in frontal and prefrontal cortex failed to cover STH completely(Hartmann-Von Monakow et al., 1978). The difference in results may be due to either a species difference or that the amino-acids injections in the various frontal and prefrontal cortices in the monkey did not cover the entire projection areas. The present study confirmed a point suggested by all of the previous studies that the

somatosensory or the postcentral cortex has no projections to STH (Petras, 1965, 1969; Kunzle, 1977 and Hartmann-Von Monakow et al., 1978). In addition to the somatosensory area, we have shown that the rat parietal and occipital cortices also have no projections to STH.

In conclusion, the cortico-STH projections in the rat are somatotopically organized with some overlapping. These projections, (with moderate labeling from the lateral agranular cortex and light labeling from the medial agranular cortex),fills the entire ipsilateral STH.

CHAPTER 5. CONCLUSION

Nissl and Golgi studies on rat STH revealed the following: Rat STH is densely packed with neurons of variably shaped somata. Statistical analysis of somatic size and the number of proximal dendrites, suggested that STH consists of a single type of neuron with some varieties. Generally two to six proximal dendrites originated from the soma and gave rise to sparsely-spined tapering secondary and tertiary dendritic branches extending up to 500um in length. The dendritic fields of individual STH neurons were usually large. Some neurons located in the central part of STH had some of their dendrites crossing the borders of the nucleus into the zona incerta, the lateral hypothalamic area or the cerebral peduncle. This suggested that STH was an open nucleus, and that some STH neurons may recieve inputs not only from afferents which terminate within the nucleus, but also from those terminating in the surrounding areas. The cortico-STH projections were studied by an autoradiographic technique in the rat. The cortico-STH projections were found to be ipsilateral and arose from the frontal agranular cerebral cortex in which heavier projections were from lateral and relatively lighter from medial frontal areas. The overall cortical projections filled the entire STH, however these projections exhibit a fine topographical organization. The above findings suggested that a single STH neuron with a large dendritic field may receive

overlapped cortical afferents. For example, a centrally located STH neuron which had dendrites directed dorsomedially and ventrolaterally may receive cortical terminals originating from both lateral and medial agranular regions on respective dendrites, or a neuron located at the cerebral peduncle may receive cortical terminals originating from both rostral and caudal parts of the medial agranular region.

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