MORPHOLOGICAL ANALYSIS OF RAT NEOSTRIATAL NEURONS

By

Howard Tien-Haw Chang

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ABSTRACT

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The aim of the present study was to investigate the morphological characteristics and the synaptic relationships of individual neurons of the rat neostriatum. Both light and electron microscopy were employed. Neurons were impregnated with the rapid Golgi technique and subsequently processed by the Golgi gold-toning method. Additional material included some efferent neurons labeled retrogradely with extracellulary injected horseradish peroxidase (HRP). Some neurons labeled intracellularly with HRP were also studied.

At least two types of large neurons (somatic cross-sectional areas, SA > 300 square μm) and five types of medium neurons (SA between 100 and 300 square μm) were distinguished in Golgi preparations of the adult rat neostriatum. Type I large cells have aspinous somata with long radiating, sparsely-spined dendrites which may be varicose distally, whereas type II large cells have spines on both somatic and dendritic surfaces. Medium type I cells have aspinous somata and proximal dendrites, but their distal dendrites are densely covered with spines. Medium type II cells have somatic spines, and their radiating dendrites are sparsely-spined. Other medium cells have no somatic spines: Type III cells have poorly-branched and sparsely-spined dendrites. Type IV cells have profusely-branched, sparsely-spined dendrites. Type V cells have

radiating and varicose dendrites which may also be sparsely-spined.

At the electron microscopic level, the presence of many axosomatic synapses on the type II large neurons distinguishes their somata from those of type I large cells. Deeply indented nuclei, dense bodies, well developed rough endoplasmic reticulum, and a perinuclear Golgi complex are found in both types of large neurons. For the medium neurons, only type I neurons have round, unindented nuclei, with the other types having varying degrees of nuclear indentations. Intranuclear rods are found in the type II medium neurons. Axosomatic synapses are found on all types of medium neurons, but are most numerous on the type II cells. Numerous ribosomes but only a few stacks of rough endoplasmic reticulum are present in the cytoplasm of the type I medium neurons. Other medium neurons have fewer free ribosomes, but have more stacks of rough endoplasmic reticulum.

The dendrites of the type I medium neurons receive asymmetrical axospinous synapses with terminals which contain small to medium vesicles. The few synapses found on their dendritic shafts are mostly symmetrical and the presynaptic terminals contain large vesicles. Dendrites of other neurons form synapses with terminals which contain mainly small to medium vesicles. No serial synapses have been observed.

Although the main axons of both types of large cells and one type IV medium neuron are myelinated as are those of type I medium cells, only the latter have been demonstrated unequivocally to be efferent neurons. Their axons have been traced into the globus pallidus. The distribution patterns of the intrinsic axon collaterals

as well as the efferent axons of individual type I medium cells vary greatly, indicating that these neurons may have heterogenous functions. The axon terminals of medium type I cells contain large vesicles and make mainly symmetrical axodendritic and axosomatic synapses with their target neurons.

Except for the type II large cells, intrinsic axon collaterals have been observed for every type of neuron. The unmyelinated axon of a type V medium neuron arborizes extensively near the parent dendritic field and make symmetrical synapses with somata and dendrites of neighboring neurons, most of which have the characteristics of type I medium neurons.

Several small neurons (SA mostly less than 100 square μm) are also found in the rat neostriatum: some have aspinous soma with sparsely-spined dendrites, others have somatic spines. Rarely, medium neurons with dendrites exceeding 500 μm in length are found, but it is premature to classify them as separate types of neurons.

To my parents, who made everything possible.

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FOREWORD

Owing to the complex nature of some of the experimental procedures employed in the intracellular labeling studies, these investigations were carried out in collaboration with Dr. G.A. Bishop and Dr. S.T. Kitai in 1979, and with Dr. C.J. Wilson and Dr. S.T. Kitai in 1980. Without their patient directions and skillful assistance, these studies would have been impossible. Portions of this study have been reported in the following papers and communications:

Bishop, G.A., H.T. Chang and S.T. Kitai (1979) Light and electron microscopic analysis of various neostriatal neurons intracellularly labeled with HRP: II. Patterns of axonal distribution. Soc. Neurosci. Abstr. 5, 67.

Chang, H.T., G.A. Bishop and S.T. Kitai (1979) Light and electron microscopic analysis of various neostriatal neurons intracellularly labeled with HRP: I. Soma-dendritic morphology. Soc. Neurosci. Abstr. 5, 69.

Chang, H.T., C.J. Wilson and S.T. Kitai (1980) Collateral arborization of axons of rat striatal neurons in the globus pallidus studied by intracellular horseradish peroxidase labeling. Soc. Neurosci. Abstr. 6, 269.

Chang, H.T. and S.T. Kitai (1981) Rat neostriatal neurons: A Golgi study. Anat. Rec. 199, 48A.

Bishop, G.A., H.T. Chang and S.T. Kitai (1981) Morphological and physiological properties of neostriatal neurons: An intracellular HRP Study in the rat. Neurosci. (in press).

Chang, H.T., C.J. Wilson and S.T. Kitai (1981) Single neostriatal efferent axons in the globus pallidus: A light and electron microsopic study. Science (in press).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1. INTRODUCTION

Although previous anatomical studies have described a number of neostriatal neuronal types in various species, the classification and the morphological descriptions of these neurons have remained controversial. The aim of the present study was to investigate the morphological characteristics of various types neostriatal neurons in the rat at both the light and electron microscopic levels. The rapid Golgi method was used to establish a classification system for the neostriatal neurons based on their light microscopic morphology. The Golgi gold-toning procedures were used to analyze the ultrastructural features of these different types of neurons. Attemps were also made to identify the morphological characteristics of the neostriatal projection neurons which were labeled retrogradely with horseradish peroxidase (HRP) following extracellular injection in the neostriatal target areas. Additionally, both light and electron microscopic analysis were carried out on physiologically characterized neostriatal neurons which have been intracellularly labeled with HRP.

Background:

A. Gross Anatomy of Neostriatum

The neostriatum is a large telencephalic structure entirely covered by the cerebral cortex and the subcortical white matter dorsally and laterally. Mediodorsally it borders the lateral

ventricles and rostroventrally it is contiguous with the nucleus accumbens septi (the fundus striati). Medioventrally and caudally it is contiguous with the external segment of the globus pallidus. Bundles of thinly myelinated fibers within this gray matter converge radially toward the paleostriatum as the "spokes of a wheel" (Papez, 1942) and are responsible for its striated appearance. The neostriatum together with the paleostriatum constitute the corpus striatum, generally regarded as the principal component of the so-called "extrapyramidal system" (Wilson, 1912). Frequently, the neostriatum is simply refered to as the striatum, whereas the paleostriatum, consisting of both the external and internal segments of the globus pallidus, is often refered to as the pallidum. The globus pallidus of the rodents is homologous to the external segment of the globus pallidus of the primates, and their entopeduncular nucleus is homologous to the internal segment of the globus pallidus of the primates.

The corpus striatum arises from the basal plate of the telencephalon as a single gray mass at the level of the interventricular foramen (Hamilton & Mossman, 1972). As the cerebral cortex expands over the diencephalon of primates and carnivores during development, part of the striatal ridge is carried in the wall of lateral ventricle into the temporal lobe. Developing corticopedal and corticofugal fibers course through this gray matter to join the internal capsule and the cerebral cortex. Thus, the two parts of the neostriatum, caudate nucleus medially and putamen laterally, are continuous rostrally and incompletely separated caudally by the internal capsule in primates and carnivores, but are essentially

indistinguishable in rodents in which the thickely myelinated fiber bundles of the internal capsule fan out within the mass of the neostriatum before reaching the subcortical white matter. Since the putamen and the globus pallidus lie adjacent to each other and together they have a lens-shape appearance, they form the lentiform nucleus described in many older literatures (see review by Nauta & Mehler, 1966).

The corpus striatum is sometimes refered to as the basal ganglia by clinicians (see review by Graybiel & Ragsdale, '79). However, "basal ganglia" has also been used to designate most or all of the non-cortical telencephalic gray matter, including the claustrum, the amygdaloid complex (archistriatum) and other gray matter ventral to the neostriatum and paleostriatum (e.g. the nucleus accumbens septi, the olfactory tubercle and the substantia innominata) which has been suggested to be the ventral extension of the striatum and pallidum (Heimer & Van Hoesen, 1979). More recently, the substantia nigra and the subthalamus have been included in the basal ganglia due to their intimate anatomical connections with the corpus striatum (Graybiel & Ragsdale, 1979).

B. Historical Background

Prior to the development of modern empirical scientific research methods in the 19th century, speculations of neostriatal functions were based primarily on its proximity to other brain structures. By virtue of its position near the cerebral ventricles approximately midway between the cerebral cortex and brain stem, the corpus striatum was described as "internode by which the cerebrum

coheres with the medulla oblongata" by Willis in 1667, who believed that all incoming sensations were received by the striatum, which was therefore called "sensorium commune" (cited by Wilson, 1914). This sensory relaying role was also promoted by Swedenborg who declared, in 1740, "The royal road of the sensations of the body to the soul is thorugh the corpora striata, and all determinations of the will also descend by that road.", and that it was "the Mercury of the Olympus, it announces to the soul what is happening to the body, and it bears the mandate of the soul to the body." (cited by Wilson, 1914).

Speculations of striatal functions found certain restriction as new experimental brain research methods were developed to test these theories. In 1820, Flourens failed to elicit movements in the rabbit by mechanical stimulation of either the striatum, or the cortex (cited by Wilson, 1914). Magendie, in 1841, found similar negative results with mechanical stimulation of the striatum with a needle, but he found that rabbits with large bilateral striatal lesions lept forward as if under an irresistible impulse. Magendie, therefore, concluded that the striatum controlled backward movements which normally would be in balance with the cerebellum which controlled the forward movement. Thus, if the striatum were destroyed, the animal would run forward due to unchecked cerebellar actions. However, Longet in 1842, failing to reproduce similar results, declared that Magendie's theory was "purement imaginaire" (cited by Wilson, 1914). Nevertheless, the possible motor function of the striatum and the belief that it was a station between the cerebral cortex and the spinal cord stimulated numerous experiments in which the striatum was pinched, pricked, pierced, electrified or injected with various

chemicals to see whether or not such treatments would produce movements (see review by Wilson, 1914). As both positive and negative results were obtained in these rather crude experiments, the role of the striatum in motor functions remained controversial.

Moreover, as anatomical studies demonstrated that the fibers of the pyramidal system were uninterrupted on their way from the cortex through the striatum, various functions previously assigned to the striatum were shown to be the properties of the adjacent cortico-spinal paths (i.e. the internal capsule), the striatum "almost at once fell from its high estate and depreciated in physiological significance" (Wilson, 1914).

The motor role of the striatum received a major boost from the clinico-pathological and experimental lesion studies by Wilson (1912, 1914) who was also one of the earliest to use stereotaxic methods in the experimental investigation of striatal connections with the help of Sir Victor Horsley (Wilson, 1914). Behavioral changes manifested mainly in terms of motor functions were observed in patients with intact pyramidal tracts but with either hepato-lenticular degeneration (Wilson, 1912) or other lesions involving the corpus striatum (Vogt & Vogt, 1920; McLardy, 1948). Using the classical but not very sensitive Marchi method of staining the degenerating axons, Wilson did not observe cortico-striatal connections. This result together with the assumption that the corpus striatum gave rise to an independent descending pathway to the spinal cord (his lenticulo-rubro-spinal projecting system) led him to believe that corpus striatum was completely independent of the cerebral cortex, hence independent of the pyramidal tracts. Although this view

is no longer valid in light of later studies using more sensitive methods which enabled the visualization of the massive cortico-striate projections, Wilson's terminology, the "extrapyramidal system" (Wilson, 1912), is still in continuous use. Perhaps this is for the lack of a better term describing the functions and/or the clinical manifestation of the dysfuncitons of the basal ganglia and its related nuclei.

In addition to the classical clinico-pathological and experimental lesion methods used by Wilson (1912, 1914), other experimental approaches have been used in the investigation of neostriatal functions. These methods include various versions of electrical stimulation, neurochemical tests and electrophysiological single unit recording. Often, more than one approach was pursued simultaneously. From the results of these experiments, the neostriatum has been ascribed a number of functions such as motor, sensory, inhibitory and cognitive (see review by Divac & Öberg, 1979). It is clear that interpretations of data obtained in these experiments have been based on the contemporary knowledge of the anatomical and physiological characteristics of the neostraital neurons and their intrinsic as well as extrinsic connections. Since these theories have remarkably broad definitions, they are not mutually exclusive. Thus, the functional role of the neostriatum remains as enigmatic as the definition of "sensorimotor integration" (Ungerstedt, Ljungberg & Ranje, 1977).

C. Neostriatal Afferent Connections

The majority of the afferent inputs to the corpus striatum have been demonstrated to terminate in the neostraitum. These afferents arise from the cerebral cortex, the intralaminar thalamic nuclei, the substantia nigra and the dorsal raphe nucleus. (For more detailed review on the neostriatal connections, see Graybiel & Ragsdale, 1979; Grofová, 1979; Dray, 1979; and Carpenter, 1976).

a) The Cerebral Cortex

Although Ramon y Cajal (1911) observed the corticostriate connections by the axon collaterals of pyramidal cells which "transmit voluntary motor excitation" in his Golgi preparations, neither he nor his contemporaries could demonstrate any direct corticostriate connections (i.e. cortical axons which terminate mainly or exclusively in the striatum). With the development of the sensitive silver staining technique for degenerating axons (Nauta & Gygax, 1954), however, the corticostriate projection was conclusively demonstrated (Webster, 1961; Carman, Cowan & Powell, 1963; Carman, Cowan, Powell & Webster, 1965; Webster, 1965; Kemp & Powell, 1970). In all species studied, the neostriatum receives axons from all parts of the cerebral cortex in a topographical pattern. The corticostriate fibers, mainly ipsilateral but also some contralateral projections (Carman et al., 1965), are orderly arranged, showing distinct mediolateral, dorsoventral and anteroposterior organization with some degrees of overlap.

Studies with recently developed axon pathway tracing techniques (i.e. HRP and autoradiography) revealed further details

about the corticostriate connections. The cortical terminal fields in the striatum have a patchy or strip appearance suggesting clustering of terminals (Künzle, 1975). The primary motor and sensory areas project almost exclusively to the entire rostrocaudal extent of the putamen (Künzle, 1975, 1977; Jones et al., 1977) whereas the prefrontal cortex project almost exclusively to the caudate nulceus (Goldman & Nauta, 1977). On the other hand, premotor area (Künzle , 1978) and temporal cortex (Yeterian, 1978) project extensively to both caudate and putamen. After analyzing the intricate patterns of the corticostriate projections from their autoradiographic material in the monkey, Yeterian and van Hoesen (1978) suggested that a given region of the striatum receives input not only from a particular area of cortex, but also from all other cortical areas reciprocally interconnected with that area. Whether this is also true for all cortical areas or for other species remains unclear.

The cells of origin of corticostriate fibers have been labeled retrogradely with HRP extracellularly injected in the striatum and are found in the layer III and layer VI of the cat (Kitai et al., 1976; Oka, 1980) or superficial part of the cortical layer V in the monkey (Jones et al., 1977) and the rat (Hedreen, 1977, Wise & Jones, 1977). Based on the soma size and the location of these neurons, most of these authors suggest that the corticostriate pathway is an independent cortical projection system different from those of corticospinal or corticobulbar fiber systems. This is in apparent contrast to the observations by both Ramón y Cajal (1911) and Webster (1961) in their Golgi preparations in which many collaterals arising from the fibers of the internal capsule terminated within the

neostriatum. Ramón y Cajal (1911) also observed that "In the rat and mouse, pyramidal cell axons have occasionally been observed to emit collaterals at intervals in the passage through the corpus striatum ... and to all appearances transmit voluntary motor excitation".

Recent electrophysiological studies indicate that at least some cortical neurons project to both the striatum and the brain stem (Endo, Araki & Yagi, 1973; Oka & Jinnai, 1978; Jinnai & Matsuda, 1979). Additionally, several electrophysiologically identified pyramidal-tract cortical neurons in the rat were labeled intracellularly with HRP and the distribution of their axons analyzed with the light microscope (Donoghue & Kitai, 1980). Collaterals arising from the HRP labeled main axons in the internal capsule were observed to branch within the neostriatum and appeared to have terminal boutons. Therefore, the corticostriate pathway is likely composed of fibers both independent from those of corticospinal or corticobulbar pathways as well as collaterals from these fiber systems.

The cortical afferent boutons contain small to medium round vesicles and make asymmetrical synapses mainly with dendritic spines of neostriatal neurons (Kemp & Powell, 1971c; Hassler et al., 1978; Hattori et al., 1979; Frotscher et al., 1981; Somogyi et al., 1981).

b) The Thalamus

The thalamostriatal connection was first detected in human neuropathological material (Vogt & Vogt, 1941; McLardy, 1948). Subsequently, this pathway was investigated in experiments in which

lesions were placed in the striatum in order to produce retrograde cellular degenerations in the thalamus (Droogleever-Fortuyn & Stefens, 1951; Powell & Cowan, 1954; Cowan & Powell, 1955; Powell & Cowan, 1956, 1967). Lesions were also placed in various regions of the thalamus in order to study the degenerating axons, some of which were followed to the striatum (Nauta & Whitlock, 1954; Jones, 1961; Mehler, 1966). Retrograde axonal transport of HRP extracellularly injected in the striatum (Jones & Leavitt, 1974; Nauta et al., 1974; Royce, 1978b; Sato et al., 1979) demonstrated that this pathway is strictly ipsilateral and organized topographically in the anteroposterior direction. These studies showed that the intralaminar thalamic neuclei, mainly the centromedian-parafascicular complex and the medial part of the centrolateral nucleus were the principle sources of this pathway. Additionally, some neurons in the ventroanterior, mediodorsal, anteromedial and rhomboid thalamic nuclei also contribute to this projection (Royce, 1978b; Sato et al., 1979).

Studies with the autoradiographic techniques (i.e. anterograde axoplasmic transport of radioactively labeled proteins) showed that intralaminar nuclei project densely to the striatum and diffusely to the cerebral cortex (Herkenham, 1978; Kalil, 1978; Royce, 1978a). It is interesting to note that intralaminar nuclei project mainly to the putamen in the monkey (Kalil, 1978) but mainly to the caudate nucleus in the cat (Royce, 1978a). Patchy distribution of thalamostriatal terminal fields are noted by these authors, but their relationships to those of corticostriatal terminal fields remain unclear. It is also not clear whether there are populations of thalamic neurons projecting exclusively to the striatum or groups of

neurons which project to both striatum and cortex. The thalamostriatal terminal boutons contain small round vesicles and make asymmetrical synapses mainly with dendritic spines of striatal neurons (Kemp & Powell, 1971c; Chung et al., 1977).

c) The Substantia Nigra

The nigrostriatal connection was demonstrated before either the the corticostriatal or the thalamostriatal pathways by the observations that striatal lesions caused retrograde cellular degenerations in the substantia nigra (von Monakow, 1895; Holms, 1901; Fox & Schmitz, 1944; Rosegay, 1944). However, this pathway could not be demonstrated by either the Marchi method (Ranson, Ranson & Ranson, 1941; Rosegay, 1944) or the Nauta technique (Afifi & Kaelberg, 1964; Carpenter & McMasters, 1964; Carpenter & Strominger, 1967; Cole, Nauta & Mehler, 1964; Faull & Carman, 1968). The degenerating nigral axons were traced to the pallidum but none could be traced into the striatum.

The nigrostriatal projection, however, was convincingly demonstrated from biochemical and histochemical (i.e. catecholamine histofluorescence) techniques in the early 1960's (Andén et al., 1964; 1965; 1966; Bédard, Larochelle & Parent, 1969; Fuxe, 1965; Hökfelt & Ungerstedt, 1969; Poirier & Sourkes, 1967; Ungerstedt, 1971; Lindvall & Bjorklund, 1974). Thereafter, the nigrostriatal pathway was revealed by the Fink-Heimer and Wiitanen silver techniques (Carpenter & Peter, 1972; Ibata, Nojyo, Matsuura & Sano, 1973; Maler, Fibiger & McGeer, 1973; Usunoff, Hassler, Romansky, Usunova & Wagner, 1976) and by retrograde transport of HRP extracellularly injected into the

striatum (Carter & Fibiger, 1977; Fallon & Moore, 1978; Faull & Mehler, 1978; VanderMaelen, Kocsis & Kitai, 1978; Kuyper, Kievit & Groen-Klevant, 1974; Miller, Richardson, Fibiger & McLennan, 1975; Nauta, Pritz & Lasek, 1974; Royce, 1978b; Sotelo & Riche, 1974; Sato et al., 1979). The nigrostriatal fibers ascend via the medial forebrain bundle (MFB) and project topographically to various parts of the neostriatum with a distinct mediolateral, rostrocaudal and reversed dorsoventral organization (Fallon & Moore, 1978). Although this pathway is considered to be ipsilateral, a few contralateral nigral neurons have been labeled from extracellular injections of the neostriatum in the cat (Royce, 1978b).

The nigrostriate fibers arise largely from the pars compacta of the substantia nigra (SNpc) where a concentration of dopamine containing neurons are located. Additionally, neurons in the pars reticulata of the substantia nigra (SNpr) and midbrain tegmental areas (e.g. retrorubral area) also project to the neostriatum (Fallon & Moore, 1978; Royce, 1978; VanderMaelen et al., 1978). On the other hand, the existence of a minor non-catecholaminergic projection from the substantia nigra to the neostriatum is suggested from studies with retrograde labeling combined with either selective lesion (Fibiger, Pudritz, McGeer & McGeer, 1977) or histofluorescence techniques (Guyenet & Craine, 1980; van der Kooy, Coscina & Hattori, 1981).

Electron microscopic studies indicate that the nigral inputs make mainly asymmetrical axodendritic and axospinous synapses on striatal neurons (Kemp & Powell, 1971; Hattori et al., 1973; Bak, Choi, Hassler, Usunoff & Wagner, 1975). Labeling with 5-hydroxydopamine (Arluison, Agid & Javoy, 1978a; Groves, 1980) or

tritiated dopamine (Arluison et al., 1978b) or tritiated norepinephrine (Aghajanian & Bloom, 1967) indicate that dopaminergic terminals make mainly asymmetrical synapses. These results agree with those derived from lesions with 6-hydroxydopamine which have additionally demonstrated that the terminals of non-catecholaminergic nigrostriatal afferents contain small pleomorphic vesicles and form symmetrical axodendritic synapses (Hattori, Fibiger, McGeer & Malwr, 1973).

d) The Dorsal Raphe Nucleus

Projection from dorsal raphe nucleus to the neostriatum has been demonstrated recently with retrograde HRP transport technique (Couch & Goldstein, 1977; Miller, Richardson, Fibiger & McLennaan, 1975; Nauta, Pritz & Lasek, 1974; Jacob, Foote & Bloom, 1978) and the autoradiographic axonal tracing technique (Azimita & Segal, 1978; Bobillier, Petitjean, Salvert, Ligier & Seguin, 1975; Bobillier, Seguin, Petitjean, Salvert, Touret & Jouvet, 1976; Conrad, Leonard & Pfaff, 1974). Both biochemical and histofluorescence studies indicate that the raphe-striatal fibers convey serotonin (5-hydroxytryptamine, or 5-HT) to the neostriatum (Kotowski, Giacalone, Garattim & Valzelli, 1968; Kuhar, Aghajanian & Roth, 1972; Kuhar, Roth, Aghajaninan, 1971; Poirier, McGeer, Larochelle, McGeer, Bedard, & Boucher, 1969; Poirier,Singh, Boucher, Bouvier, Olivier & Larochelle, 1967; Geyer, Puerto, Dawsey, Knapp, Bullard & Mandell, 1976; Holman and Vogt, 1972). Using microdissection and microassay techniques, it is found that the 5-HT terminals are more concentrated in the ventrocaudal neostriatum (Ternaux et al., 1977) which is consistent with the data

obtained from autoradiographic axoplasmic transport studies (Bobillier et al., 1975; Conrad et al, 1974). Recent immunocytochemical studies suggest that 5-HT terminals make asymmetrical axospinous synapses in the cat and monkey neostriatum (Pasik, Pasik, Saavedra & Holstein, 1981).

e) Other Minor Afferents

Several studies based on retrograde HRP axonal tracing techniques have described various other neostriatal afferent areas, including the locus coeruleus (Couch & Goldstein, 1977) and the globus pallidus (Staines, Atamadja & Fibiger, 1981). However, since these pathways have not been confirmed by independent studies, their true nature remains unclear.

f) Physiology of Neostriatal Afferents

The available data agrees that the afferents from the cerebral cortex and the thalamus exert predominantly excitatory synaptic actions on neostriatal neurons (Purpura & Malliani, 1967; Buchwald, Price, Vernon, & Hull, 1973; Kitai, Kocsis, Preston & Sugimori, 1976; Kocsis, Sugimori & Kitai, 1977; VanderMaelen & Kitai, 1980). The transmitters of the corticostriatal pathways appear to be glutamate (Spencer, 1976; Divac, Fonnum & Storm-Mathisen, 1977; McGeer, McGeer, Sherer & Singh, 1977; Fonnum & Walaas, 1979; Walaas, 1981), whereas those of the thalamostriatal fibers remain unclear (Simke & Saelens, 1977; Fonnum & Walaas, 1979).

The physiological effect of the substantia nigra as well as the action of dopamine on the neostriatal neurons have remained

controversial. Studies with extracellular recordings and microiontophoresis techniques have shown that nigral stimulation or dopamine iontophoresis produced either inhibitiory (Ben-ARi & Kelly, 1976; Bloom, Costa & Salmoiraghi, 1965; Connor, 1970; Gonzalez-Vegas, 1974; Herz & Zieglgansberger, 1968; Siggins, 1978) or exitatory actions (Norcross & Sphelmann, 1977 & 1978; Richardson, Miller & McLennan, 1977; Spehlmann & Norcross, 1978). Studies with intracellular recording techniques, however, demonstrate that nigral stimulation produces excitatory postsynaptic potentials (EPSPs) in neostriatal neurons and that iontophoretically applied dopamine has a depolarizing effect on the neostriatal neurons (Bernardi, Marciani, Morocutti & Giacomini, 1978; Herring & Hull, 1980; Kitai, Sugimori & Kocsis, 1976; Kitai & Kocsis, 1978; Kocsis & Kitai, 1977). The nature of a possible non-catecholaminergic nigrostriatal projection (Fibiger et al., 1972; Feltz & deChamplain, 1972; Hattori et al, 1973) remains unclear.

Extracellular recording investigations have reported that dorsal raphe stimulation has an inhibitory effect on neostriatal neurons (Miller et al., 1975; Olpe & Koella, 1976). However, iontophoretically applied 5-HT is reported to have either an excitatory (Bevan, Bradshaw & Szabadi, 1975) or inhibitory effect (Herz & Zieglganzberger, 1968) on neostriatal neurons. Recently, studies employing intracellular recording techniques have shown that stimulation of dorsal raphe results in mainly excitation of neostriatal neurons (VanderMaelen et al., 1980) and that this excitation is positively correlated to the levels of available 5-HT (Park et al., 1980).

D. Neostriatal Efferent Connections

The major neostriatal efferent targets are both segments of the globus pallidus and the substantia nigra.

a) The Pallidum

The striato-pallidal fibers are topographically organized in the mediolateral and dorsoventral directions (Cowan and Powell, 1966; Nauta & Mehler, 1966; Szabo, 1967, 1970; see review in Grofová, 1979). The striatal efferent terminals contain large vesicles and form symmetrical axodendritic synapses with their target cells (Kemp, 1970; Fox et al., 1974a). The striato-pallidal axons are thinly myelinated such that groups of these axons converge radially toward the globus pallidus with the "spokes of wheel" appearance in myelin-stained sections, and are also called "Wilson's pencils" (Wilson, 1914; Fox & Rafols, 1976). The striatal efferent fibers, after traversing both segments of the globus pallidus, pierce the cerebral peduncle forming the "comb system" (Edinger, 1911, cited in Fox & Rafols, 1976) to reach the substantia nigra. Most of the myelinated striatal axons become unmyelinated before reaching the substantia nigra (Fox et al., 1975).

Electrophysiological studies (Yoshida, Rabin & Anderson, 1972) and Golgi studies (Fox et al., 1975) have suggested that the striato-pallidal and striato-nigral pathways are collateral fiber system. Recent studies with intracellular injection of HRP in the rat (Preston et al., 1980) have presented evidence supporting this view: HRP labeled axons are observed to give rise to collaterals in the globus pallidus before fading from view in the internal capsule,

presumably coursing toward either the entopeduncular nucleus or the substantia nigra or both.

b) The Substantia Nigra

The striato-nigral fibers are also topographically organized in the mediolateral, rostrocaudal and reversed dorsoventral directions (see review in Grofova, 1979), with most of the fibers terminating in the pars reticulata of the substantia nigra (SNpr) (Kemp, 1970; Grofová & Rinvik, 1970; Schwyn & Fox, 1974; Bunny & Aghajanian, 1976). Since neurons in the SNpc have dendrites that extend into the SNpr (Schwyn & Fox, 1974; Juraska et al., 1976), this pattern of strio-nigral projection is significant in that it may indicate the existence of a direct feedback loop between the neostriatal projection cells and the dopaminergic neurons of the SNpc, without an intervening interneuron. The striato-nigral terminals, like the striato-pallidal terminals, also contain large vesicles and make mainly symmetrical axodendritic synapses (Grofová & Rinvik, 1970; Kemp, 1970; Hajdu, Hassler & Bak, 1973).

c) Physiology of the Neostriatal Efferents

Studies do not fully agree on the nature of the synatic influence of neostriatal projection cells on their target cells in the globus pallidus (GP) and the substantia nigra (SN). Mainly inhibitory but some excitatory responses are observed in GP neurons following stimulation of the neostriatum (Malliani & Purpura, 1967; Yoshida, Rabin & Anderson, 1972; Levine, Hull & Buchwald, 1974). Similarly, mainly inhibitory and some excitatory responses were observed in the

SN neurons following stimulation of the neostriatum (Frigyesi & Purpura, 1967; Yoshida & Precht, 1971; Precht & Yoshida, 1971; McNair et al, 1972; Frigyesi & Purpura, 1967; Preston et al., 1981).

The neostriatal efferent fibers appear to convey both enkephalin and gamma-aminobutyric acid (GABA) to the pallidum (Obata & Yoshida, 1973; Cuello & Paxinos, 1978; Brann & Emson, 1980; Emson, Arregui, Clement-Jones, Sandberg & Rossor, 1980; Fonnum & Walaas, 1979) whereas those fibers terminating in SN contain both GABA and substance P (Precht & Yoshida, 1971; Brownstein, Mroz, Tappaz & Leeman, 1977; Gale, Bird, Spokes, Iversen & Jessel, 1978; Hong, Yang, Racagni & Costa, 1977; Jessell, Emson, Paxinos & Cuello, 1978; Hattori et al., 1973a; Kanazawa, Bird, O'Connell & Powell, 1977; Emson et al., 1980; Pickle, Joh, Reis, Leeman & Miller, 1979; Ribak, Vaughn & Roberts, 1979, 1980; Ljungdahl, Hokfelt, Goldstein & Park, 1975;). At least some of the striopallidal and strionigral axons are thought to be collaterals of the same neostriatal neuron (Yoshida et al., 1972; Fox & Rafols, 1976; Szabo, 1981), however it is unclear whether the same individual neostriatal efferent axon contain both GABA and enkephalin and/or substance P.

E. Neostriatal Intrinsic Organization

a) Light Microscopic Studies of Nissl Preparations

Light microscopic analysis of Nissl stained sections have recognized that the neostriatal neurons are composed of numerous medium sized neurons (somatic diameters range from 10 to 20 μm) and a few large (somatic diameters range from 20 to 50 μm) and small (somatic diameters generally less than 10 μm) neurons distributed in a seemingly random and homogeneous pattern. Most of the medium cells have large, pale nuclei surrounded by a narrow rim of cytoplasm with little or no Nissl substance (i.e. they are achromatic or hypochromatic). In contrast, the large neurons and a very few medium neurons have a lot of cytoplasm and large number of Nissl bodies (Ramón y Cajal, 1911; Kemp & Powell, 1971a; also see reviews by Fox & Rafols, 1976; Pasik, Pasik & DiFiglia, 1979). Although clustering of somata of neostriatal neurons have been reported (Mensah, 1977), the significance of this observation remains unclear since neither the dendritic nor the axonal morphology of the neurons in a cluster are revealed in Nissl preparations.

b) Golgi Studies

Studies of neostriatal neurons using the Golgi techniques have described a number of neuronal types in several species. However, the description and the classification of these neurons have remained controversial despite attempts at classifying them according to a unifying system (Pasik et al., 1979).

Ramón y Cajal (1911) placed considerable emphasis on the distribution of the axons of neostriatal neurons in his Golgi study of rabbit and human infant and divided the neostriatal neurons into long- and short-axon cells. The short-axon cells included large, small and dwarf cells whereas the long-axon cells included medium and large neurons. With the exception of the dwarf cell, most of these neurons have various amount of dendritic spines. Since no magnification scales has been provided for the drawings of Ramón y Cajal, it has been difficult to evaluate the sizes of these neurons. For instance, his dwarf or neurogliform neuron (his Figure 325D) has somatic and dendritic size comparable to those of the "small" short axon neurons (his figure 325A), which are considered to be equivalent to the medium spiny neurons by many modern investigators (Kemp & Powell, 1971a; Pasik et al., 1976; 1979). Considerable controversies have thus been generated with respect to the identification of similar neurogliform cells in later Gogli studies in other species (Fox & Rafols, 1971/1972; Fox, Lu Qui & Rafols, 1974; DiFiglia, Pasik & Pasik, 1976).

Recent investigations of the neostriatum by the Golgi techniques began with the study of Leontovich (1954). Her extensive study, based on materials from hedgehogs, rabbits, mice, dog, monkey and human demonstrated the presence of numerous long-axon medium spiny neurons. In addition, both long- and short-axon large neurons as well as short-axon medium neurons with sparsely-spined dendrites have been described. However, since the illustrations in her report were of very low magnification, it has been difficult for the present author to discern their detailed morphology. Although both intrinsic

and projection neurons are shown in her semi-diagramatic summary illustration (her Figure 7), the lack of high magnification camera-lucida or photomicrographic documentation of the efferent axons arising from the presumed projection neurons may have contributed to the skepticism held by some investigators about her conclusion (Kemp & Powell, 1971a).

Other modern Golgi investigations include the extensive and influential studies on the cat caudate nucleus by Kemp and Powell (1971a) and the monkey neostriatum by Fox and his colleagues (Fox et al., 1971/1972a, b). Both of these groups described the presence of large number of short-axon medium spiny neurons which have extensive local collateral arborizations. Additionally, these cells are distinguished by their aspinous somata and proximal dendrites and densely spined distal dendrites. The long-axon neurons were proposed to be the large neurons with either aspinous or sparsely-spined long dendrites and a myelinated axon (Fox et al., 1971/1972b) and the medium neurons with sparsely-spined dendrites (Kemp & Powell, 1971a). In addition to the medium spiny neurons, other short-axon neurons included sparsely-spined medium neurons (i.e. medium smooth cell of Kemp & Powell, and spidery neuron of Fox et al.). These authors thus proposed that the neostriatal neurons consisted of large numbers of medium sized intrinsic neurons which received direct afferent inputs, and a small number of large efferent neurons responsible for most or all of neostriatal outputs.

This theory was challenged by the Pasiks and DiFiglia in their Golgi studies of the monkey neostriatum (DiFiglia et al., 1976; Pasik et al., 1976; Pasik et al., 1977; Pasik et al., 1979). Drawing

support from the study of Leontovich (1954) and from new retrograde HRP transport studies (Grofová, 1975; Bunny & Aghajanian, 1976) which indicated that a majority of neostriatal neurons are medium efferent neurons, the Pasiks and DiFiglia concluded that the most frequently impregnated medium spiny neurons were projections neurons (their Spiny I cells). Their Spiny II cells, consisted of both large and medium neurons with somatic spines and sparsely-spined dendrites, were also considered to be long-axon cells. These authors further described the morphology of the short-axon neurons as consisting of large aspinous neurons with varicose dendrites (Aspiny II cell), and medium aspinous neurons with either varicose (Aspiny I cells) or straight dendrites (Aspiny III cells). Additionally, a neurogliform neuron without identifiable axon was described. A classification scheme similar to those of the Pasiks and DiFiglia has been used for the dog neostriatal neurons (Tanaka, 1980). However, both the large and medium Spiny II cells in the dog have no somatic spines. The Aspiny III cells in the dog have very long dendrites whereas those in the monkey have shorter dendrites.

Recent Golgi studies in the rat have also described a number of neostriatal neuronal types (Mensah & Deadwyler, 1974; Lu & Brown, 1977; Danner & Pfister, 1979a, b). However, there are many discrepancies with respect to the morphological descriptions and the classification of these neurons: from 5 to 7 types of neurons have been illustrated in either free-hand drawings or partial photomicrographs. All of these studies described the presence of numerous medium spiny neurons which they considered to be a type of intrinsic neurons based on their dense local axon arborizations.

Several other types of neurons included the so-called "medium long-axon" cells which have either few (Mensah & Deadwyler, 1974; Danner & Pfister, 1979a, b) or many dendritic spines (Lu & Brown, 1977). Medium neurons with either smooth dendrites or varicose dendrites were considered to be short-axon neurons whereas large aspinous neurons were considered to be long-axon neurons despite the fact that long axons have never been illustrated.

A recent study has provided camera-lucida tracings of 5 types of rat neostriatal neurons (Dimova et al., 1980). The medium spiny neurons are classified as the type I neurons. Their type II neurons are medium neurons with sparsely-spined dendrites, including some spines on the primary dendrites. Type III cells are medium neurons with varicose dendrites and type IV cells are medium neurons with sparsely-spined long dendrites. Type V neurons are giant neurons with varicose and sparsely-spined dendrites. However, neither photomicrographs of the neurons nor descriptions of their axonal distribution patterns have been provided in this recent study. Thus, the morphological characteristics of the rat neostriatal neurons as well as their classification relationships to those described in other species remain unclear.

Although no definitive evidence have been obtained in the monkey, studies in the rat have produced unequivocal proof that the medium spiny neuron (i.e. the Spiny I of the monkey) projects to the substantia nigra (Somogyi & Smith, 1979) and the globus pallidus (Preston et al., 1980). However, comparable evidence is lacking for other types of neurons which may be projection neurons in the rat or other species.

In summary, most of previous Golgi studies have attempted to classify the neostriatal neurons into projection neurons and intrinsic neurons and then subdivide these categories into several types according to their somatic and dendritic morphology. However, since in Golgi material, the axons are usually only partially impregnated, the identifications of projection neurons (i.e. long-axon cells) have not been accompanied by micrographic evidence. Consequently, naming certain cells as long-axon neurons have been educated guesses at best. Confusion in comparing the results of these various Golgi studies also arises from the fact that either very restrictive or very broad naming systems have been used without clear and consistent definitions. The descriptive terms such as "large", "medium", "spiny", "aspiny", "varicose", "smooth", "stellate", "spidery", "slender", "spindle" and so on have been used loosely and in different combinations by different authors. Thus, considerable controversy has arissen from attempts to correlate the results of these studies. For example, in a recent review (Pasik et al., 1979), the Spiny II neurons of the monkey (DiFiglia et al., 1976) have been correlated to the medium and large long-axon cells of the cat (Kemp & Powell, 1971a), the medium spindle and large cells of the rat (Lu & Brown, 1977), the large long-axon cell of human (Ramón y Cajal, 1911), the large aspiny long-axon cell of the monkey (Fox et al., 1971/1972b) and so on. The critical similarities among these various neurons are their sparsely-spined dendrites and their putatively long axons. However, the presence of long axons has not been convincingly demonstrated for any of these neurons, and none of these cells have somatic spines, a critical characteristic of the Spiny II neurons in

the original study of DiFiglia et al., (1976).

c) Electron Microscopic Studies of Normal Material

Electron microscopic studies of the neostriatum have revealed several types of neurons in the rat (Mori, 1966; Hattori et al., 1973a; Bak et al., 1975), the cat (Adinolfi & Pappas, 1968; Adinolfi, 1971; Kemp & Powell, 1971a) and the monkey (Fox et al., 1971/1972a, b; Pasik, Pasik & DiFiglia, 1976). These studies generally agree that the most frequently encountered somatic profiles are medium sized (or small, as called by Mori, 1966) neurons with large and pale, unindented nuclei with sparse cytoplasmic organelles. These somata have been correlated with the achromatic medium (or small) neurons observed in Nissl preparations (Kemp & Powell, 1971a, Fox, Andrade, Hillman & Schwyn, 1971/1972a). The large neostriatal neurons are less frequently found, and usually have indented nuclei surrounded by a large amount of well organized rough endopalsmic reticulum, Golgi complexe, and numerous dense bodies. Various rarely-found small to medium neurons have also been described in several studies as to have indented nuclei surrounded by varying amount of cytoplasmic organelles. Since only limited portions of any individual neuron could be analyzed in these studies, and distal neuronal processes usually could not be traced back to the parent somata, correlation of these ultrastructural observations with the light microscopic findings (i.e. Golgi studies) has remained tentative until recently (see below).

Analysis of the neostriatal neuropil has also resulted in controversial classifications of various types of axon terminals and

synaptic connections based on sets of different criteria (Mori, 1966; Adinolfi & Pappas, 1968; Adinolfi, 1971; Kemp & Powell, 1971b; Rafols & Fox, 1971/1972; Kawana, Akert & Bruppacher, 1971; Bak et al., 1975; Pasik et al., 1976; Chung et al., 1977; Hassler et al., 1978). In general, the most prevalent synaptic profiles are axon terminals containing small round vesicles making asymmetrical axospinous synapses. These are considered to have arised mainly from the cerebral cortex, the thalamus and the midbrain areas (Kemp & Powell, 1971b, c; Chung et al., 1977; Hassler et al., 1978; Bak et al., 1975). Other axon terminals contain either large or small pleomorphic vesicles and make mainly symmetrical synapses with somata and dendritic shafts, and are considered to be terminals of intrinsic collaterals (Hassler et al., 1977; Tennyson & Marco, 1973).

d) Golgi Gold-toned Neostriatal Neurons

Recently, direct electron microscopic analysis of cytoplasmic details of Golgi impregnated neurons has become possible through gold-toning procedures (Fairén et al., 1977). With this technique, the ultrastructural morphology of two types of monkey neostriatal neurons, the Spiny I and the Aspiny I cells, have been analyzed (DiFiglia et al., 1980). The somatic and dendritic morphology of several types of rat neostriatal neurons has also been studied (Dimova et al., 1980). In general, the Spiny I cells of the monkey and the medium type I cells of the rat are characterized by round, unindented nuclei whereas the other cells have indented nuclei (Dimova et al., 1980). The Golgi gold-toning technique has also been combined with extracellular uptake of HRP to demonstrate that the medium spiny

neurons project to the substantia nigra in the rat (Somogyi & Smith, 1979) and that these cells receive direct cerebral cortical afferents which have been labeled by anterograde degeneration (Somogyi, Bolam & Smith, 1981).

e) Labeling with Extracellularly Injected Markers

The properties of axon terminal uptake of extracellularly injected markers such as HRP molecules and retrograde transport of these markers back to the soma have been widely used in recent investigations of connections in the nervous system. HRP injected extracellularly into the substantia nigra has resulted in the labeling of a large number of medium sized neostriatal neurons (Grofova, 1975; Bunny & Aghajanian, 1976). Recent studies combining the technique of retrograde transport of HRP with acetylcholinesterase histochemistry have shown that the neostriatal efferent neurons appear to be grouped in geometrically complex compartments which partially overlap the cholinesterase-rich areas (Graybiel & Ragsdale, 1979; Graybiel, Ragsdale & Moon-Edley, 1979). It is unclear how these patchy arrangements of the efferent neurons are related to the patchy distribution of the neostriatal afferent terminal fields formed by the cerebral cortex and the thalamus observed in autoradiographic studies (Künzle, 1975, 1977, 1978; Kalil, 1978).

Identification of large neostriatal projection neurons have been described in studies in which the HRP has been injected into the midbrain (Grofová, 1979) or the auditory cortex (Jayaraman, 1980). Herpes virus injected into the midbrain has labeled both large and medium neurons (Bak et al., 1977; 1978). However, since only partial

morphology of the somata and proximal dendrites is revealed by these retrograde labelings techniques, the morphological identification of these neostriatal projection neurons has remained tentative. Recently, combining the technique of retrograde transport of HRP with Golgi impregnation and Golgi gold-toning procedures, some of the rat neostriatal projection neurons have been demonstrated to be medium spiny neurons (Somogyi & Smith, 1979).

f) Labeling with Intracllularly Injected Markers

The recently developed technique of intracellular labeling with either fluorescent or enzyme (i.e. HRP) markers have enabled direct analysis of the morphology of neurons which have been physiologically characterized through intracellular recording techniques (Kitai, Kocsis, Preston & Sugimori, 1976). Studies in the cat (Kitai et al., 1976; Kitai, KOcsis & Preston, 1978; Kocsis & Kitai, 1977; Kocsis, Sugimori & Kitai, 1977) and the rat (Preston, Bishop & Kitai, 1980; VanderMaelen & Kitai, 1980) have shown that these cells receive convergent excitatory inputs from the cerebral cortex, the thalamus and the substantia nigra. Additionally, they receive excitatory inputs from the dorsal raphe nucleus (VanderMaelen, Bonduki & Kitai, 1979).

Light microscopic analysis has furtherly confirmed and extended the Golgi studies in revealing the full extent of the elaborate intrinsic axonal collateral arborizations of the medium spiny neurons (Preston et al., 1980; Wilson & Groves, 1980). The HRP labeled main axons have been traced into the globus pallidus and the internal capsule and a few collaterals have been observed to terminate

in the globus pallidus (Preston et al., 1980). It is interesting to note that only medium spiny neurons have been labeled intracellularly in either the cat or the rat, suggesting that the predominant neostriatal neuron type is the medium spiny neuron. Electron microscopic analysis has revealed that the intrinsic collaterals of the HRP labeled medium spiny neurons synpase with the spine necks, dendritic shafts, somata and axonal initial segments of neighboring neurons (Wilson & Groves, 1980).

g) Biochemical, Histochemical & Immunocytochemical Studies

Biochemical studies have shown that the putative neurotransmitters in the neostriatum include glutamate, gamma-aminobytric acid (GABA), acetylcholine, dopamine, serotonin (5-HT), substance P and enkephalin. Of these, afferent transmitters are considered to be glutamate from the cerebral cortex, dopamine from the substantia nigra, and 5-HT from the dorsal raphe nucleus. The transmitter of the thalamostriatal pathway remains unclear. Acetylcholine and GABA have been considered to be the transmitters of neostriatal intrinsic neurons. At the same time, both GABA and substance P are involved in the striato-nigral projections (Fonnum, Grofova, Rinvik & Storm-Mathisen, 1974; Brownstein, Mroz, Tappaz & Leeman, 1977; Gale, Hong & Guidotti, 1977; Hong, Yang, Racagni & Costa, 1977; Jessel, Emson, Paxinos & Cuello, 1978; Kanazawa, Bird, O'Connel & Powell, 1977; Fonnum, Gottsfeld & Grofová, 1978) whereas both GABA and enkephalin are involved in the striato-pallidal pathway (Fonnum et al., 1978; Cuello & Paxinos,1978; Brann & Emson, 1980; Ribak et al., 1980; also see review by Fonnum & Walaas, 1979).

Histochemical studies indicate that the large neostriatal neurons have the highest acetylcholinesterase (AChE) synthesizing activity (Butcher & Bilezikjian, 1975). Combined AChE histochemistry with HRP retrograde transport studies indicated that neostriatal projection cells are mostly AChE-negative medium neurons (Henderson, 1981), however AChE-positive medium neurons have been found to project to the substantia nigra by another laboratory (Kaiya et al., 1979). Since AChE is considered to be a necessary but not a sufficient marker for cholinergic neurons, it is unclear how these cells are related to the true cholinergic neurons which would contain the acetylcholine synthesizing enzyme-choline acetyltransferase (CAT). A recent study reported that AChE is involved in hydrolyzing substance P molecules in the central nervous system (Chubb, Hodgson & White, 1980). Since the neostriatum has a very high content of substance P, the AChE neurons are probably involved in interactions with both the cholinergic interneurons and the substance P projection neurons.

Immunocytochemical studies with anti-choline acetyltransferase (anti-CAT), however, resulted in labeling of either medium (Hattori, Singh, McGeer & McGeer, 1976) or large neurons (Kimura, McGeer, Peng & McGeer, 1980). One study at the electron microscopic level shows that the CAT-containing medium neurons have unindented nuclei and have dendritic spines (Hattori et al., 1976). These discrepancies arising from the same laboratory have contributed much to the unabated controversies with respect to the identity of cholinergic neostriatal neurons.

The GABA-synthesizing enzyme, glutamic acid decarboxylase (GAD), has been used as a marker for GABAergic neurons. Studies with

anti-GAD antibodies have indicated that the GABAergic neostriatal neurons are medium neurons and have dendritic spines (Ribak et al., 1979). Similar findings have been obtained from studies with anti-enkephalin antibodies (DiFiglia, Aronin, Liotta & Martin, 1980; Pickel, Sumal, Beckley, Miller & Reis, 1980). The results from these immunocytochemical studies, therefore, indicate that medium neurons with dendritic spines may contain three or four different types of putative neurotransmitters: acetylcholine, GABA, enkephalin and possibly substance P. Whether these transmitters are contained in the same neuron or in biochemically distinct neurons which are morphologically similar remain unclear. This difficulty is furtherly compounded by the fact that virtually all of these studies have been conducted on the rat neostriatum, in which the morphological descriptions of different types of neurons have many discrepancies, as has been mentioned above.

F. Summary of Background

The neostriatum receives topographical projections from the cerebral cortex, intralaminar thalamic nuclei, substantia nigra and the dorsal raphe nucleus. The neostriatum projects topographically to the paleostriatum and the substantia nigra. The putative neurotransmitters of the afferents are glutamate, dopamine and serotonin (5-HT) arising from the cerebral cortex, substantia nigra and dorsal raphe nucleus respectively. The transmitter involved in thalamostriatal projection remains unclear. Electron microscopic studies indicate that the majority of the afferent terminals contain small to medium sized round vesicles and make asymmetrical axospinous synapses in the neostriatum. The neostriatal efferent terminals contain large synaptic vesicles and make symmetrical axodendritic and axosomatic synapses with targets cells in the globus pallidus and substantia nigra.

The transmitters of neostriatal efferents are considered to consist of gamma-aminobutyric acid (GABA) and enkephalin in the striatopallidal fibers, and substance P and GABA in the striatonigral fibers. It is not clear whether individual neostriatal efferent neurons contain more than one putative transmitter. Acetylcholine is considered to be the transmitters of the neostriatal interneurons. Immunocytochemical studies in the rat indicate that glutamate decarboxylase (GAD) containing neurons are medium neurons with dendritic spines, and are similar to the enkephalin containing neurons as well as substance P containing cells. Choline acetyltransferase containing neurons have been reported to be either large or medium

neurons, with the large neurons having the highest acetylcholinesterase synthetic activity.

Evidence derived from retrograde transport of extracellularly injected markers indicates that the overwhelming majority of neostriatal efferent neurons are medium neurons, and rarely, a few large neurons. However, more than six types of neostriatal neurons have been described in Golgi and electron microscopic studies in several species. Of these cells, only the medium spiny neuron has been demonstrated conclusively to be a projection neuron. This neuron, in addition, has intrinsic integration functions through its elablorate intrinsic axonal collateral arborization. The medium spiny neuron receives convergent excitatory afferent inputs and exert recurrent inhibitory actions which are mediated by GABA.

The morphological characteristics and the classification of other neurons, both large and medium, remain controversial. Some of these neurons have sparsely-spined dendrites, others have varicose dendrites. Although myelinated axons have been observed to arise from some of these cells, no photomicrographs or camera lucida tracings are available demonstrating that these axons have been traced out of the neostriatum. Since the main axons frequently are not impregnated in Golgi preparations, dividing these neurons into Golgi type I (i.e. efferent cells) or Golgi type II (i.e. intrinsic interneurons) remains controversial.

G. Research Objectives

Since the rat has been the most widely used subject in recent experimental investigations of neostriatal functions, elucidation of the morphology of various types of rat neostriatal neurons at both light and electron microscopic levels should serve to clarify many of the controversies with respect to the intrinsic anatomical organization of the neostriatum, and should enable better understanding of the structural basis of various neostriatal functions.

The specific aims of this study are:

1) To determine the detailed light microscopic morphological characteristics of various types of rat neostriatal neurons by the classical Golgi technique.

2) To directly analyze the ultrastructural morphology of Golgi impregnated neurons by the Golgi gold-toning procedure.

3) To investigate the light and electron microscopic morphology of the neostriatal projection neurons which have been labeled following the extracellular injection of HRP into the globus pallidus, and to compare with that from the Golgi preparations.

4) To investigate the light and electron microscopic morphology of physiologically characterized neostriatal neurons which have been labeled intracellulary with HRP, and to compare with that from the Golgi preparations.

Chapter 2. METHODS

A. Golgi Studies

Male adult (250-400 gm) Long-Evans hooded rats and Sprague-Dawley albino rats were used in this study. After anesthetizing the animals with i.p. injections of either Nembutal (50 mg/kg body weight) or Urethane (2 gm/kg), they were perfused through the aorta with 300 ml of 0.9% saline solution followed by 1 liter of fixtive containing 2% glutaraldehyde and 2% formaldehyde (prepared freshly from paraformaldehyde powder) in a 0.1 M phosphate buffer (pH=7.3). The temperature of all perfusing solutions was maintained at 4 degrees Celsius.

After perfusion, the brains were removed and kept overnight in a refregerator in the fixative solution. Brains were then cut into 3 to 4 mm thick blocks in either coronal or sagittal planes, rinsed briefly with 0.1 M phosphate buffer (pH=7.3, 353 mOsm.) and immersed in the first solution of the rapid Golgi technique (Valverde, 1970) consisting of 2.4% potassium dichromate and 0.2% osmium tetroxide (OsO_4). Vials containing at least 20 ml of this solution per brain block were stored at room temperature and away from direct light for 3 to 7 days. The brain blocks were then briefly rinsed with 0.75% silver nitrate solution and immersed in fresh 0.75% silver nitrate solution, 20 ml per block, for 2 to 7 days at room temperature and away from direct light. The vials were occassionally agitated to promote even exposure of tissue surfaces to the solutions during both of these periods.

At the completion of the rapid Golgi reaction, the blocks were either sectioned immediatedly or temporarily stored in 100% glycerine in the refregerator. The longest storage was four months. The blocks were cut with an Oxford Vibratome into 100 um thick sections. The sections were either dehydrated through ethanol and cleared in xylene for light microscopic analysis only, or cleared in graded series of glycerine solutions (20%-100%) for both light microscopic analysis and possible further gold-toning treatments preparatory to electron microscopic analysis. The Golgi impregnated neurons were examined and photographed with a Leitz Orthoplan microscope and detailed tracings of neurons were made with the aid of a Leitz drawing tube.

Golgi gold-toning procedures:

After light microscopic analysis of desired neurons in glycerine cleared sections, the sections were rehydrated through a descending series of glycerine soultions (100%-20%) to distilled water. Sections were then immersed in a 0.05% solution of yellow gold chloride (hydrogen tetrachloroaurate (III), HAuCl4-4H20) for 15 minutes in an ice bath, with frequent agitation. The sections were rinsed briefly three times with ice cold distilled water before being reacted with 0.05% oxalic acid for 2 minutes in the ice bath to reduce the gold chloride to metallic gold. The sections were then rinsed again with distilled water and returned gradually to room temperature. The de-impregnation of Golgi reaction products was accomplished by reacting with freshly prepared 1% sodium thiosulfate solution for 30 to 60 minutes at room temperature with frequent agitation.

Upon completion of the above reactions, the tissue sections were rinsed in distilled water and postfixed in phosphate buffered 2% OsO4 (pH=7.3) for 1 hour. The sections were then dehydrated through a graded series of ethanol and propylene oxide and flat embedded in either Epon-Araldite or Spurr's plastic on Teflon-coated glass slides and coverslips (Wilson & Groves, 1979).

Light microscopic examination of polymerized plastic sections was conducted to select the optimally gold-toned neurons for electron microscopic analysis. These neurons were photographed again and their surrounding landmarks traced with a Leitz drawing tube to facilitate later search for the gold-toned profiles under the electron microscope. The Teflon-coated coverslips were removed and squares (0.5 mm per side) containing the desired neurons were cut off the slide and re-embedded on blank plastic blocks. Serial thin sections were cut with a Sorvall MT-2 Ultramicrotome. Blocks were often reexamined with a light microscope fitted with a metalurgical objective to check the progress of the thin sectioning. The thin sections were picked up on Formvar coated slot grids, stained with methanolic uranyl acetate and lead citrate, and examined with a Philips 201 transmission electron microscope.

B. Extracellular Horseradish Peroxidase Labeling

Adult male Long-Evans hooded rats (250-400 gm) were anesthetized with Nembutal (50 mg/kg body weight) admininstered intraperitoneally and placed in a stereotaxic head holder (David Kopf Instruments). The skull was opened and 0.1 to 0.2 microliters (μl) of a solution of 20 to 40 % horseradish peroxidase (HRP) in water was

pressure injected into the globus pallidus (GP) using a glass micropipette (tip diameter 50 μm) fitted on a Hamilton microsyringe. The micropipette was intoduced into the rat brain at an angle to avoid leakage of HRP solution into the neostriatum. The animals were sacrificed after two days by an overdose of Nembutal (60 mg/kg body weight) followed by perfusion through the aorta with 300 ml of 0.9% saline and 1 liter of fixative consisting of 2% glutaraldehyde and 2% formaldehyde (prepared freshly from paraformaldehyde) in a 0.1 M phosphate buffer (pH=7.3). Following the perfusion, the brains were removed and stored in the fixative overnight. Sagittal 100 or 50 um thick sections were cut on an Oxford Vibratome and reacted for demonstration of HRP activity (Graham & Karnovsky, 1966): Sections were immersed in 0.5% cobalt chloride in 0.1 Tris buffer (pH=7.3) for 10 minutes (Adams, 1977), rinsed briefly with the Tris buffer and placed in 0.1 M phosphate buffer (pH=7.3) to react with 0.05% 3,3-diaminobenzidene tetrahydrochloride (DAB) and 0.06% hydrogen peroxide for 30 minutes.

The sections were then cleared with either glycerine for immediate light microscopic analysis to be followed by postfixation with phosphate buffered 2% OsO_4 and plastic embedding (Christensen & Ebner, 1978), or were scanned directly in the phosphate buffer under a light microscope to locate the HRP labeled profiles and followed by postfixation and flat-emmbedding in Spurr's plastic on Teflon-coated glass slides and coverslips (Wilson & Groves, 1979). Light and electron microscopic analysis of HRP labeled neurons were identical to those of Golgi gold-toned neurons mentioned earlier.

C. Intracellular Horseradish Peroxidase Labeling

Adult male Long-Evans hooded rats (250-400 gm) were anesthetized with Urethane (1.5 gm/kg body weight) administered intraperitoneally and placed in a stereotaxic head holder (David Kopf Instruments). Following dorsal craniotomy, bipolar or monopolar stimulating electrodes (insulated stainless steel insect pins, size "00", bared at the tips) were stereotaxically positioned in the substantia nigra, thalamus or cortex for orthodromic and antidromic (from substantia nigra only) activation of neostriatal cells.

Glass micropipettes pulled to a fine tip on a Narishige electrode puller and beveled to tip diameters of 0.5 to 1.0 um under microscopic observation were used for recording intracellular potentials and injecting horseradish peroxidase (HRP) into the neurons being recorded. The electrolyte solution within the glass electrode consisted of 4% HRP in 0.05 M Tris buffer (pH=7.6) and 0.5 M potassium chloride, potassium acetate or potassium methylsulfate. After placement of the microelectrode below the cortical surface, the exposed brain surface was covered with soft paraffin wax to reduce brain pulsation. Recordings were made with a conventional active bridge amplifier. Neurons recorded intracellularly were labeled with HRP by passing positiive DC current pulses (3-15 nA, 100 to 300 msec in durations, 3 to 5 Hz) for 3 to 5 minutes through the recording microelectrode. Extracellular field responses were recorded to compare data obtained intracellularly.

Following intracellular HRP injections, the rats were perfused through the aorta with 0.9% saline followed by a fixative consisting of 2% formaldehyde and 2% glutaraldehyde in 0.1 M phosphate

buffer (pH=7.3). The brains were removed and stored in the fixative overnight. Sagittal 100 or 50 um thick sections were cut on an Oxford Vibratome and reacted for demonstration of HRP activity (Graham & Karnovsky, 1966): Sections were immersed in 0.5% cobalt chloride in 0.1 Tris buffer (pH=7.3) for 10 minutes (Adams, 1977), rinsed briefly with the Tris buffer and placed in 0.1 M phosphate buffer (pH=7.3) to react with 0.05% 3,3-diaminobenzidene tetrahydrochloride (DAB) and 0.06% hydrogen peroxide for 30 minutes.

The sections were then cleared with either glycerine for immediate light microscopic analysis, followed by postfixation with phosphate buffered 2% Os04 and plastic embedding (Christensen & Ebner, 1978), or were scanned directly in the phosphate buffer under a light microscope to locate the HRP labeled profiles and followed by postfixation and flat-emmbedding in Spurr's plastic on Teflon-coated glass slides and coverslips (Wilson & Groves, 1979). Light and electron microscopic analysis of HRP labeled neurons were identical to those of Golgi gold-toned neurons mentioned earlier.

D. Method of Analysis

Light Microscopy: The somatic cross-sectional areas were measured from the camera lucida (drawing tube) tracings with the aid of a graphic analyzer attached to a PDP-11 computer. The presence or absence of somatic spines and dendritic varicosities as well as the distribution of dendritic spines were noted. The dendritic branching characteristics as determined by the number of dendritic stems versus the number of dendritic tips were compared for neurons with similar somatic and dendritic surface morphology. Where possible, the distribution of the axons was also analyzed to determined the extent of its collateral arborizations.

Electron Microscopy: The somata of individual neurons previously characterized by light microscopic analysis were examined to compare the morphology of their nucleus and the distribution of various organelles. The somatic and dendritic synapses were analyzed to determined the symmetry of the synaptic contacts and the size and shape of the synaptic vesicles. The axons were also examined to determine the presence of synapses on the axon hillock or the initial segment, and whether the axons were myelinated. Where possible, the axon terminals were examined to determine the size and shape of the synaptic vesicles, the symmetry of the synaptic contacts, and the nature of the post-synaptic elements.

Chapter 3. RESULTS

A. Light Microscopic Analysis of Golgi Material

As have been commonly practiced in previous studies, neurons in the rat neostriatum are preliminarily divided into three groups on the basis of their somatic sizes. These groups are: a) Large cells, with somatic maximal cross-sectional areas (abbreviated as SA) larger than 300 square μm, approximately equivalent to somatic diameters larger than 20 μm. b) Medium cells, SA between 100 and 300 square μm, equivalent to diameters between 10 and 20 μm. c) Small cells, SA less than 100 square μm, or diameters less than 10 μm. After this initial arbitrary division, additional morphological characteristics such as somatic and dendritic surface appearance (presence or absence of spines, varicose vs. straight dendrites), dendritic length, dendritic branching patterns and axonal distribution patterns are analyzed.

LARGE NEURONS:

Large neurons in the rat neostriatum may be divided into at least two types, those with and those without somatic spines.

Type I Large Neurons: This type of large neuron (n = 12) has a smooth soma and 3 to 5 thick primary dendrites (i.e. the number of dendritic stem, S = 3 to 5) some of which branch near the soma into long secondary dendrites (Fig. 1A, 1B & 2A) while others extend over long distances before branching (Fig. 1C). The number of dendritic tips (F) ranges from 11 to 35.

Cells within this group have either polygonal (Fig. 1A, 1B) or fusiform shaped somata (Fig. 1C). The dendrites often arise from

Figure 1. Photomicrographs of type I large neurons.
A. Type I large neuron with polygonal soma. Somatic cross-sectional area (SA) = 344 square μm. Calibration scale (CAL) = 50 μm.
B. Higher magnification of A, small arrow points to a distal dendrite which has varicose appearance; blocked arrow points to the axonal branching point. CAL = 20 μm.
C. Type I large neuron with fusiform soma and radiating non-varicose, long dendrites; SA = 595 square μm. Same magnification as in A.

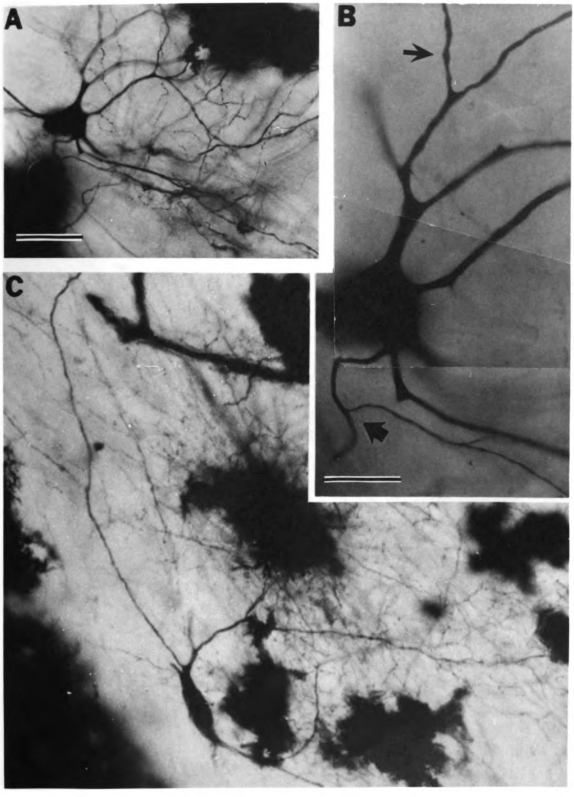

Figure 1

Figure 2. Microscopic tracings of type I large neuron. CAL = 50 μm.

A. Somato-dendritic morphology of a neuron shown also in Figure 1A & 1B. Note some of the sparsely-spined dendrites become varicose distally. Arrowhead points the axon which is illustrated separately in B.

B. Axonal distribution of the neuron shown in A. Note that the beaded collaterals overlapps extensively the caudal (to the right of the figure) parent dendritic field.

the poles of the soma radiating into a bipolar-shaped dendritic field which may be oriented along the course of the internal capsule fiber bundles (Fig. 1C, photomicrograph of fusiform large neuron). Other cells, however, have dendrites which are distributed without regard to the orientation of the fibers of passage, and give rise to multipolar-shaped dendritic fields. (Fig. 1A, 1B and 2A). The dendritic surface of the primary dendrites and the proximal secondary or tertiary dendrites are usually smooth and straight without varicose appearance, but a few dendritc spines are occasionally found (Fig. 2A).

Usually only a few of these long dendrites can be analyzed throughout their entire length because they often become unimpregnated or are cut into segments located within adjacent sections where they are frequently entangled with other Golgi impregnated profiles. Nevertheless, some dendrites have been reconstructed from serial sections and have been traced up to 400 μm away from the soma (Fig. 2A). Some dendrites become varicose or beaded in appearance distally (Fig. 1B, small arrowhead; Fig. 2A) and give rise to thin beaded processes or sparsely-spined processes. Other long dendrites, however, terminate with gradual tapering.

The axons of these large neurons are usually unimpregnated except for a portion of the initial segment. One type I large neuron, however, has an axon which gives rise to a local collateral near the soma (Fig. 1B, large arrowhead; Fig. 2B). The parent axon, showing no sign of tapering, becomes unimpregnated a short distance from the collateral branch point. The lone local collateral gives rise to many beaded processes (Fig. 2B) extending up to 400 μm away from the soma

and appears to arborize mainly within the caudal quadrant of the parent dendritic domain.

Type II Large Neurons: The somata of the second type of large neurons (n = 7) usually have at least 5 visible somatic spines (Fig. 3B) It is, however, impossible to evaluate accurately the number of somatic spines under the light microscope due to difficulties involved in visualizing spines hidden by the opaque soma. The somata may be polygonal (Fig. 3A, 3B & 4A), round (Fig. 3D) or fusiform (Fig. 4B) in shape and have cross-sectional areas ranging from 328 up to 624 square μm.

The dendrites (S from 3 to 9, F from 13 to 42) of these large neurons are multipolar in orientation and often appear to be shorter than those of type I large cells (compare Fig. 2A with Fig. 4A and 4B). Whether this reflects actual dendritic length difference or results from partial impregnation of dendrites of the type II cells remains unclear. The density of the dendritic spine distribution usually decreases with increasing distance from the soma. In comparison to the spiny dendrites of the medium spiny neurons (Fig. 3B, curved arrow), the dendrites of some of these large neurons are virtually aspinous. Distally, the dendrites are either smooth or sparsely studded with a few patches of spines, and occassionally, a few long and thin dendritic appendages. Varicose dendritic swellings or beaded dendritic appendages are not observed on these neurons. The axons of these cells are unimpregnated except for a portion of the initial segments (Fig. 4A & 4B, arrowhead).

Figure 3. Photomicrographs of type II large neurons and type I and type II medium neurons.

A. Type II large neuron, from a gold-toned Golgi section; SA = 542 square μm. CAL = 50 μm.

B. Higher magnification of A, straight arrow points to the somatic spines. Note that in comparison to a distal dendrite (curved arrow) of a type I medium neuron, the dendrites of the type II large neuron are virtually aspinous. CAL = 20 μm.

C. Medium type I neuron, from a gold-toned Gogli section; SA = 168 square μm, same magnification as in A.

D. Type II large neuron, rapid Golgi preparation; SA = 379 square μm. Note that its somatic size is intermediated between the large cell shown in A, and the medium cell shown in E. Same magnification as in A.

E. Type II medium neuron, rapid Golgi preparation; SA = 257 square μm, arrow points to the axon. Same magnification as in A.

F. Higher magnification of E, showing the presence of somatic spines; same magnification as in B.

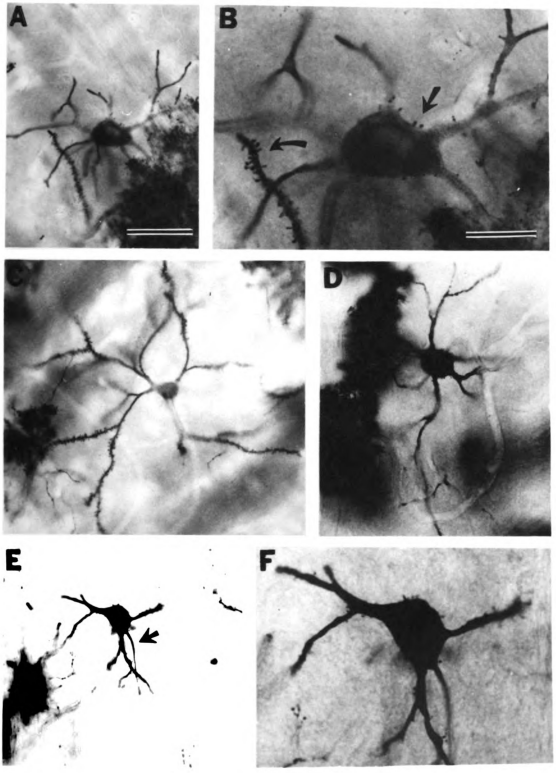

Figure 3

Figure 4. Microscopic tracings of type II large and type II medium neurons.

A & B: Type II large neurons, SA = 452 and 374 square μm respectively, arrowheads point to the axons.

C, D & E: Type II medium neuron, also in Figure 3E & 3F. Arrowhead point to the axon with a short segment of a collateral. Opened-block arrow points to the somatic region illustrated at higher magnification in D. Closed-block arrow point to the polymorphic terminal dendritic branches within the fiber bundles of the internal capsule, shown at higher magnification in E.

F: Another type II medium neuron with relatively even distribution of spines over both soma and dendrites. SA = 150 square μm, arrowhead points to the axon.

CAL = 50 μm for A, B, C and F; 25 μm for D and E.

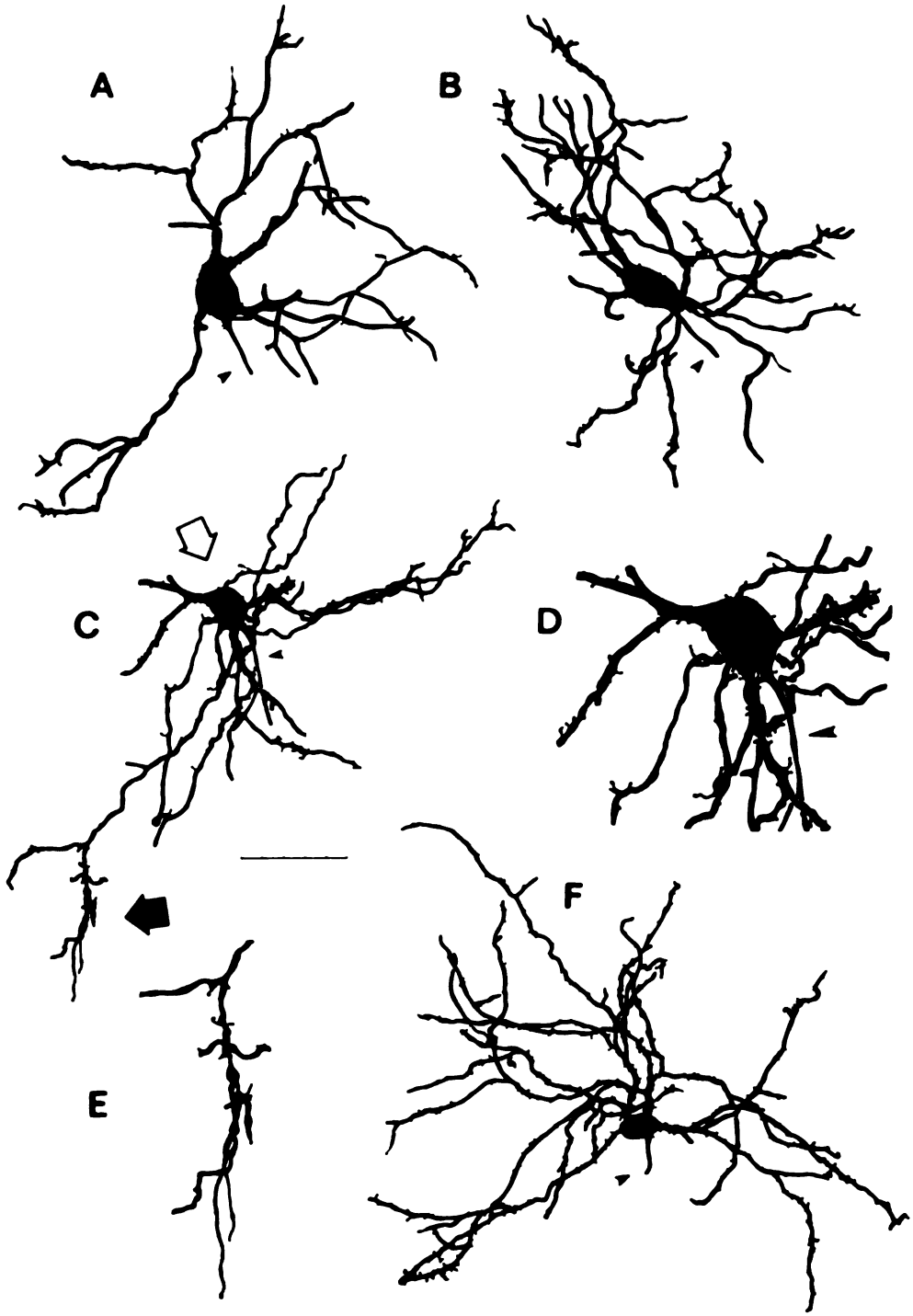

Figure 4

Figure 5. Microscopic tracings of type I medium neurons.
A. This cell examplifies the most frequently encountered neostriatal neurons in our Golgi preparations, having densely spine-studded distal dendrites, resembling bottle brushes; SA = 159 square μm.
B. A type I medium neuron with lower spine density on its dendrites; SA = 105 square μm.
C. A possible type I medium neuron with uneven dendritic spine distribution. This cell also has a smaller soma (SA = 88 square μm) than most of the other type I medium neurons.
Arrowheads point to the axons, CAL = 50 μm.

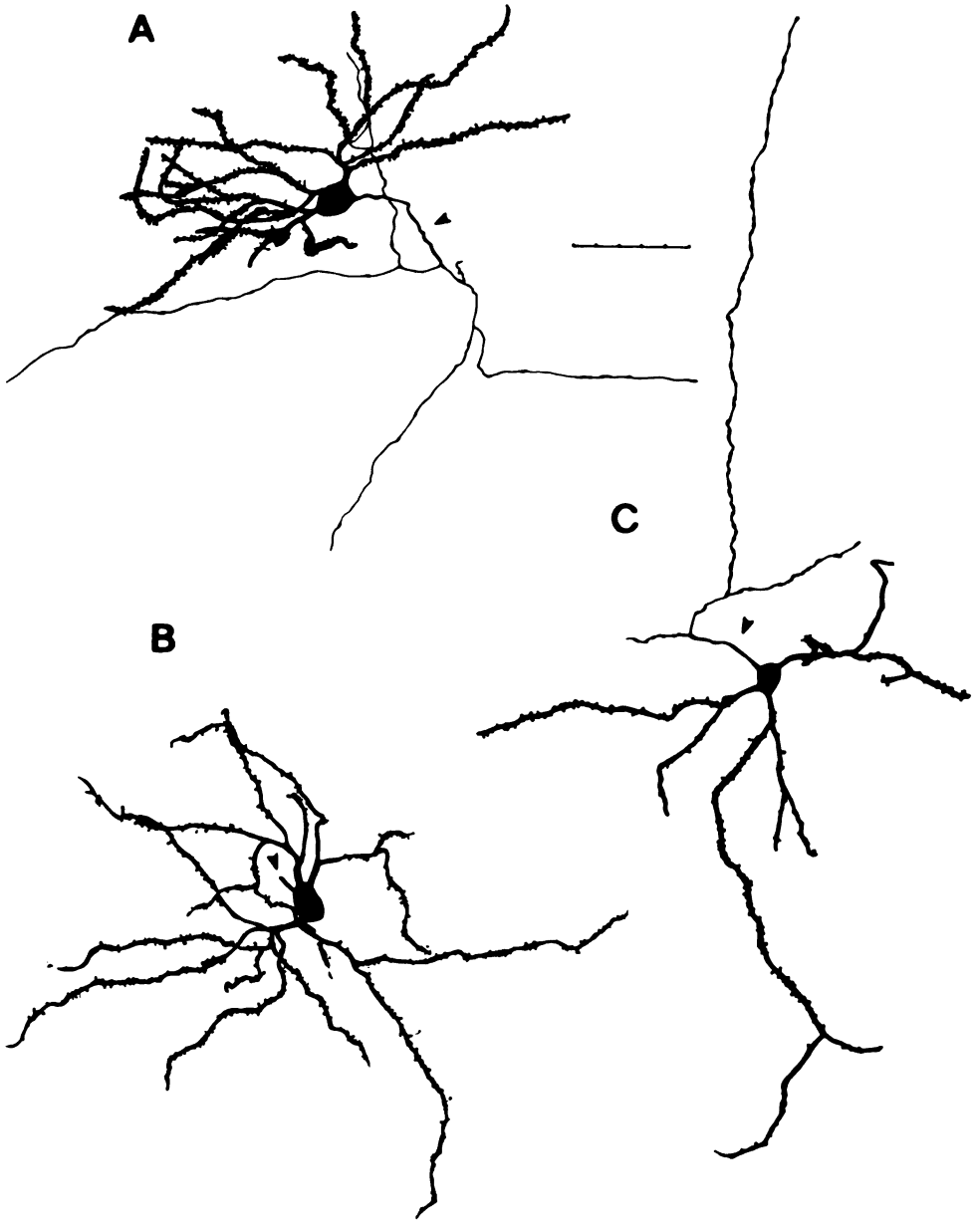

Figure 5

MEDIUM NEURONS: At least five types of medium neurons are found in the rat neostriatum.

Type I Medium Neurons: This is the most frequently impregnated neuronal type in our Golgi preparation (Fig. 3C; Fig. 5A). The morphology of this cell type appears to be identical to the medium spiny neurons previously described in the Golgi studies of the cat (Kemp & Powell, 1971), the monkey (Fox et al., 1971/1972; DiFiglia et al., 1976), the dog (Tanaka, 1980), and the rat (Mensah & Deadwyler, 1974; Lu & Brown, 1977; Danner & Pfister, 1979a & b; Dimova et al., 1980). These cells are characterized by their spine-free soma and proximal dendrites and densely spine-studded distal dendrites, which often resemble bottle brushes. Three to four local axon collaterals are emitted near the soma and ramify extensively within or near the parent dendritic domain. The main axons, some taking rather tortuous course, join the fiber bundles of the internal capsule.

In addition to these observations, we have also found several neurons which have similar but slightly different morphology as those of the more common medium spiny neurons. The distal dendrites of these neurons either do not have as many spines as the more commonly seen medium spiny neurons (compare Fig. 5A with 5B) or have a noticeably uneven distribution of spines on their dendrites (Fig. 5C). Yet, the characteristic distribution of many spines on the distal dendrites and the spine-free nature of the proximal dendrites and the soma indicate that these are neurons closely related to the medium spiny neurons. Since the sample size of these relatively spine-poor medium spiny neurons is much smaller than the spine-rich type, we do not feel that they constitute a completely separate cell type. It is

likely that they represent cells at the lower end of a continuous spectrum of spine density distribution of all the neostriatal medium spiny neurons.

Type II Medium Neurons: The second type of medium neostriatal neurons (n = 8) have somatic spines (Fig. 3E & 3F; Fig. 4C, 4D & 4F). The overall appearance of these neurons is similar to that of the type II large neurons mentioned previously. A variable number of spines are visible on the soma and the proximal dendrites as illustrated in the high magnification photomicrograph (Fig. 3F) and tracing (Fig. 4D). Frequently, the spine density on the dendrites appears to decrease with increasing distance away from the soma (Fig. 4C & 4D). Occasionally, the spines appear to be evenly distributed over the soma and the dendrites (Fig. 4F). However, none of the neurons of this type have as many dendritic spines as the type I medium spiny neurons (Fig. 5).

Some of the radiating and multipolarly distributed dendrites (S from 4 to 6, F from 19 to 36) extend into fascicles of internal capsule fibers of passage and terminate with elaborate branches of dendritic varicosities and appendages (Fig. 4C, closed arrow; Fig. 4E). The axons of these neurons have not been impregnated in our preparation beyond the initial segment (Fig. 3E, 4C, 4D & 4F, arrowheads), one of which gives rise to a branch which also quickly becomes unimpregnated (Fig. 4D, arrowhead).

Type III, IV & V Medium Neurons

The remaining types of medium neostriatal neurons share several similar morphological characteristics. Namely, these cells have aspinous somata and very sparsely-spined dendrites. However, visual inspections indicate that there are two populations of neurons with non-varicose dendrites, one has poorly-branched dendrites (type III) and the other has profusely-branched dendrites (type IV). The mean values of F and S for type III cells are, respectively, 19.6 (s.d.=5.2, n=10), 4.3 (s.d.=1.5) and those for type IV cells are 39.7 (s.d.=5.8, n=7) and 5.3 (s.d.=0.8). The difference in their F values is statistically significant ($t = 2.188$, $df = 15$, $p < 0.05$). Another group of medium neurons have varicose dendrites and is classified as type V medium neurons.

Type III Medium Neurons: These cells (n = 10) have relatively poorly branched dendrites (F from 10 to 24, S from 3 to 7) which may radiate up to 250 μm from the soma. The sparsely-spined dendrites are usually smooth without varicose appearance. Some dendrites have a few long and thin appendages. Many of these cells in frontal section have stellate dendritic fields (Fig. 6A, 7A, 7D) comparable to those of the type I cells (medium spiny projection cells) in appearance. Similarly, type III cells in sagittal section have either stellate (Fig. 6B) or slightly polarized dendritic fields without any apparant specific orientation (Fig. 6D). Since many type I cells are also fully impregnated in these same sections (Fig. 6C, arrowhead pointing to a spiny distal dendrite of a type I neuron), it is unlikely that the type III cells are results of partial

Figure 6. Photomicrographs of type III medium neurons and a medium neuron with long dendrites.

A, B & C: Examples of type III medium neurons with sparsely-spined, non-varicose dendrites. SA = 176, 137 and 177 square μm for A, B and C respectively. Arrow in A points to the axon of that cell. Arrow in C points to a distal dendrite of a neighboring type I medium neuron, illustrating that dendritic spines are well impregnated in these sections, therefore the type III medium neurons are probably not medium neurons with unimpregnated spines.

D & E: A rarely found medium neuron with a dendrite which extends up to 500 μm from the soma (curved arrow). Straight arrow points to the partially impregnated axon. SA = 150 square μm. Some of its dendrites form polymorphic terminal branches in the fiber bundles of the internal capsule, one of which is shown at higher magnification in E.

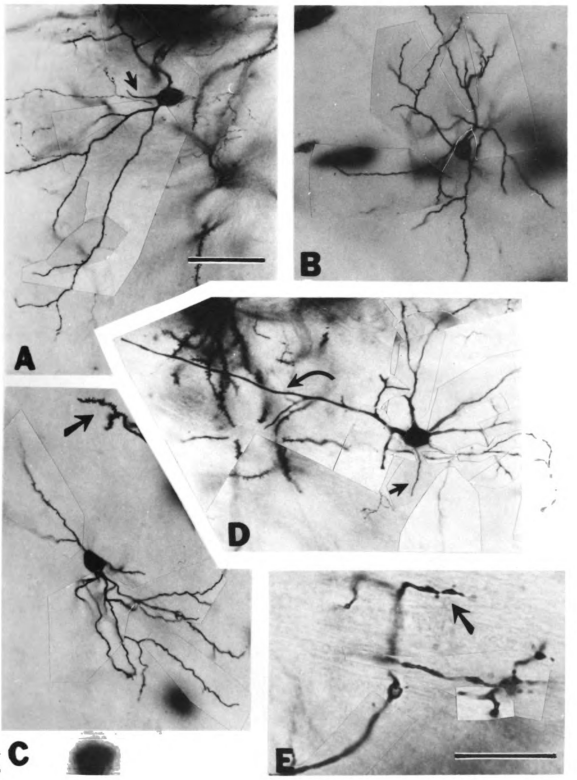

Figure 6

Figure 7. Microscopic tracings of type III medium neurons and a small neuron with intrinsic axons. CAL = 50 μm.

A: Somato-dendritic morphology of a type III medium neuron, also shown in Figure 6A; arrowhead points the axon.

B: A type III medium neuron, arrowhead points to a well-impregnated axon which arborize extensively near the parent dendritic field; SA = 160 square μm.

C: A small neuron, arrowhead points to its axon which has a similar distribution as that in B; SA = 91 square μm.

D: A type III medium neuron with one somatic spine, arrowhead points to the axon; SA = 138 square μm. Note its similarity to the type III neuron shown in A, and compare with the tyep II medium neurons shown in Figure 4C and 4F.

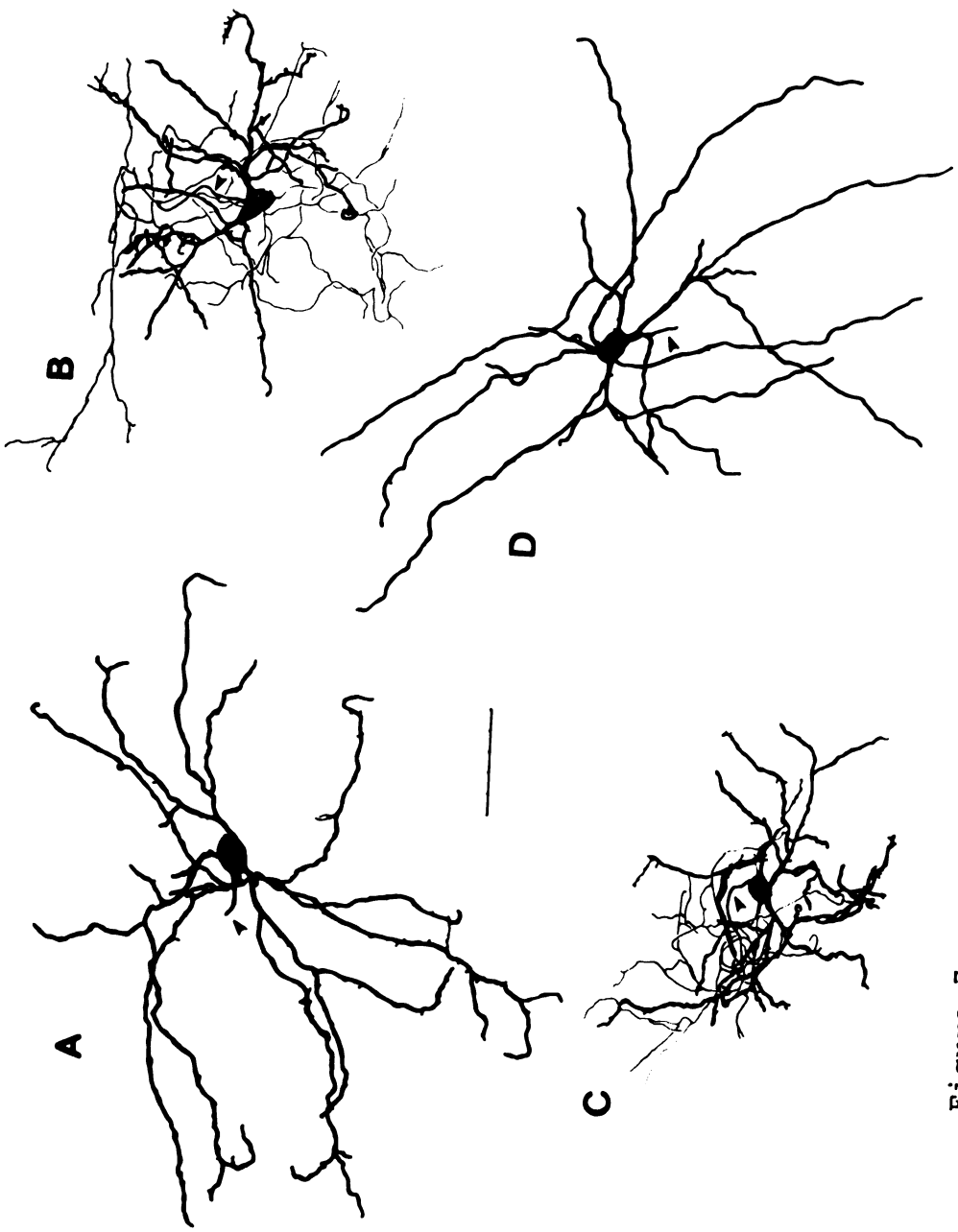

Figure 7

impregnation of type I cells.

Rarely, one or two somatic spines or appendages may be visible on the soma or the proximal dendrites of these neurons as shown in Figure 7D and 12G. However, since the overall appearance of these cells is closer to that of type III cells (Fig. 7A) than to that of type II cells (Fig. 4C, 4F), They are classified as type III cells in this study.

The axons of most of these neurons are unimpregnated except for the initial segments. One of the type III medium cells has a locally arborizing axon (Fig. 7B, arrowhead).

Type IV Medium Neurons: These cells (n = 7) have spine-free somata and smooth or very sparsely-spined dendrites which branch repeatly, forming an elaborate and interwoven dendritic field around the soma (F from 31 to 49, S from 4 to 6; dendritic range up to 250 um) (Fig. 8A, 9A, 9C & 9D). The smooth dendrites generally are not varicose. Occasionally, long and thin dendritic appendages (Fig. 9A, closed block arrow) or patches of dendritic spines or appendages (Fig. 9B) are seen dotting the bare dendrite. Some dendrites of this cell also extend into a fiber bundle of the internal capsule to form specialized endings consisting of branching polymorphic processes with varicosities and appendages (Fig. 8B & 8C and Fig. 9A & 9B)

The axons of these cells are generally unimpregnated except for a portion of their initial segments. One cell of this type has an axon whcih gives rise to a few beaded local collateral fibers arborizing near the dendritc domain of the parent cell (Fig. 9C, arrowhead). The main axon divides into two equal branches which

subsequently give rise to more collaterals. A bulb-like enlargement (Fig. 9D, block arrow) is seen on one of the main branchs near the axon bifurcation.

It is interesting to note that even though the neuron shown in Figures 8D has a soma size at the lower limit of the large size range (SA = 318 square μm), its profusely branching dendrites (F = 40 and S = 6) are shorter than the type I large cells (dendritic ranges of 200 μm vs. 400 μm of large type I cells). Therefore it appears to be more closely related to the type IV medium cells than other larger neostriatal neurons.

Type V Medium Neurons: These cells (n = 5) have smooth somata. The smooth primary dendrites divide near the soma into secondary dendrites which branch infrequently (F from 25 to 33, S from 3 to 5) and become varicose in appearance (Fig. 10, closed-block arrows; Fig. 11B, opened-block arrow) as they radiate into stellate dendritic field extending up to 250 μm away from the soma. A few spines or dendritic appendages (Fig. 11B, closed-block arrows) are found on the dendrites.

The most striking feature of this type of neuron is the distribution of its axon (Fig. 10, opened-block arrow). Arising from either the soma or the primary dendrite, the axon branches repeatly near the soma forming a dense network of axon collaterals consisting mainly of beaded thin fibers (Fig. 10, 11A & 11B, arrowhead points to the first branch point of the axon). There is a preferential orientation of the axon arborization toward one side of the dendritic field. There is no indication of a main axon leaving the domain of

Figure 8. Photomicrographs of type IV medium neurons.
A: A type IV medium neuron, SA = 222 square μm. The arrowhead points to the partially impregnated axon which becomes myelinated as showin in Figure 25C. Arrows point to the polymorphic terminal dendritic branches within the fiber bundles of the internal capsule, also shown in B and C. CAL = 50 μm.
B & C: Higher magnification of the terminal dendritic branches in the internal capsule fiber bundles. Note their spine-like appendages and varicose appearances. CAL = 10 μm.
D: A neuron with profusedly-branching dendrites (range about 200 μm in radial length) similar to other type IV neurons but its SA is slightly over 300 square μm (SA = 318 square μm). Same magnification as in A.

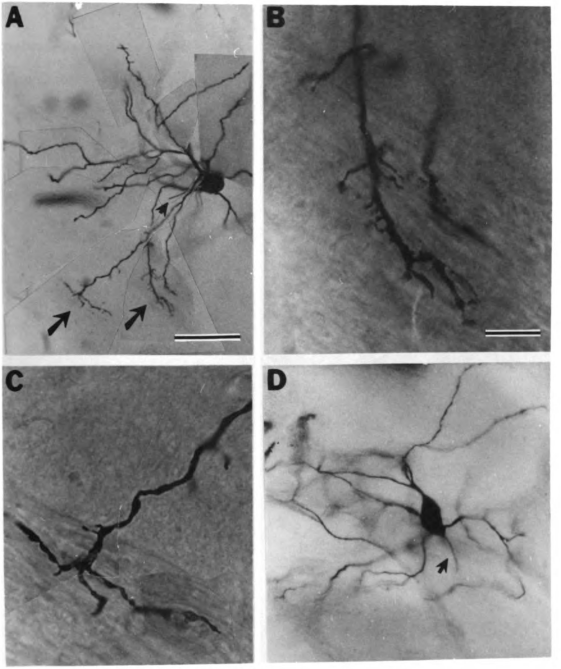

Figure 8

Figure 9. Microscopic tracings of type IV medium neuron.
A: Same neuron as shown in Figure 8A. Arrowhead points the axon. Closed-block arrow points to one of the long dendritic appendages of this cell. Opened-block arrow points to the terminal dendritic branches within the fiber bundles of the internal capsule, also illulstrated at higher magnification in B.
B: Terminal dendritic branches at higher magnification.
C: A type IV medium neuron, SA = 247 square μm. Arrowhead points to its partially impregnated axon which gives rise to several local collaterals.
D: Higher magnification of C, showing the axons in relation with withthe dendrites. The closed-block arrow points to an enlargement on one of the major axonal branches.
A & C are at the same mangnification, so are B & D.
CAL = 50 μm.

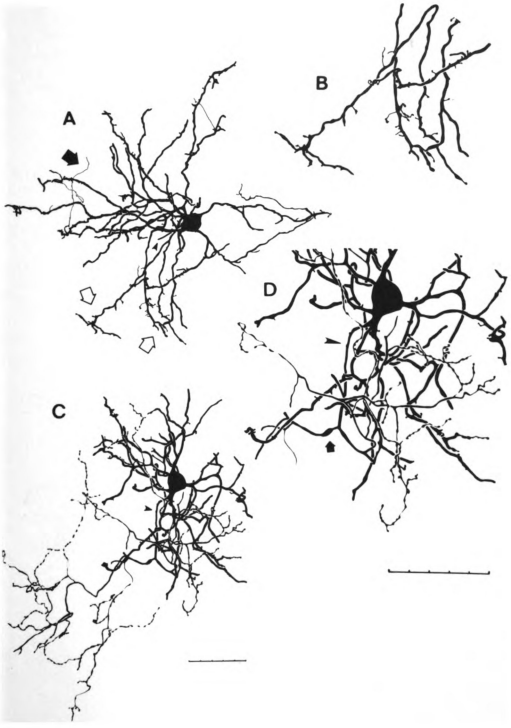

Figure 9

Figure 10. Photomicrograph of a type V medium neuron. Note that the main axon (opened-block arrow) branches first at the arrowhead, and then branches repeatly to form an elaborate collateral arborization near the parent dendrites, many of which have pronounced varicose varicose appearance (closed-block arrow). SA = 178 square µm. CAL = 20 µm.

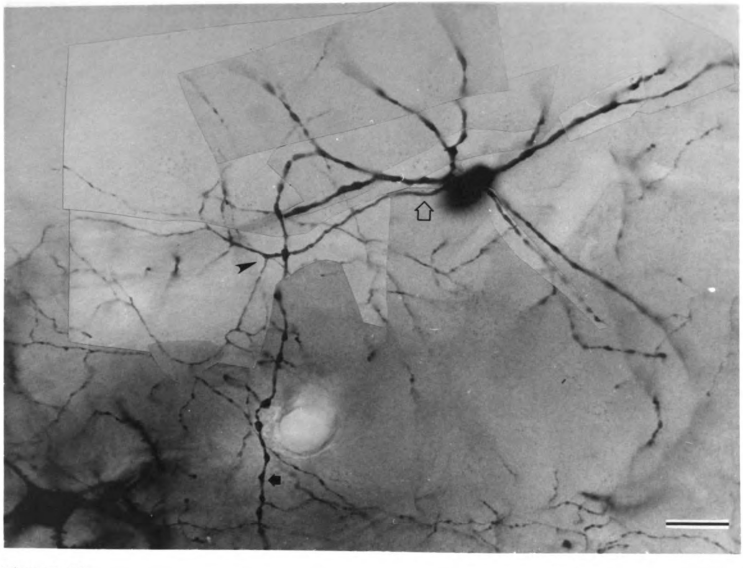

Figure 10

Figure 11. Microscopic tracings of a type V medium neuron.
A. Same neuron as that in Figure 10, shown at the same magnification as other types of neurons described previously. Arrowhead points to the first branch point of the axon. CAL = 50 μm.
B. Higher magnification of A, showing the presence of a few dendritic spine or appendages (closed-block arrows) and the varicose nature of the dendrites (opend-block) arrow. Additionally, the elaborate local axonal collateral arborization within the parent dendritic domain is illustrated in detail. CAL = 50 μm.

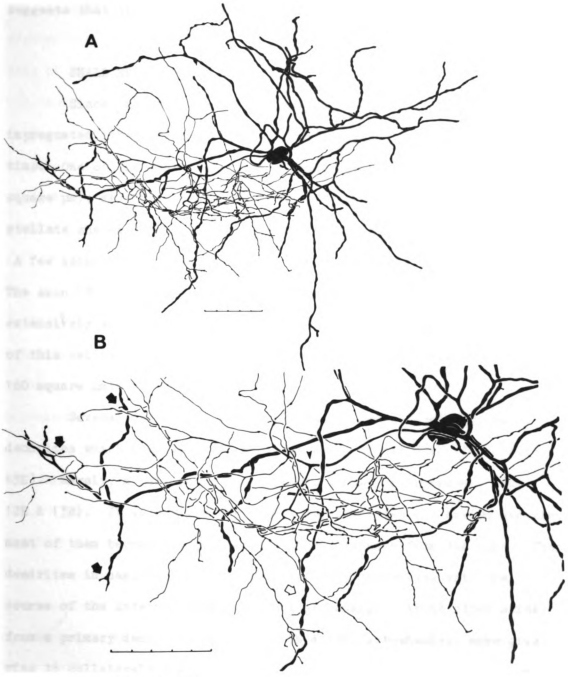

Figure 11

the local axon arborization. This pattern of axon arborization suggests that this is a type of neostriatal interneuron.

SMALL NEURONS:

Since the small neurons in the rat neostriatum are rarely impregnated, they are not subdivided into different types at this time. One of the small cells has a round soma (Fig. 7C, SA = 91 square μm), and three primary dendrites which branch into a small stellate shaped dendritic field, extending up to 150 μm from the soma. A few spines and beaded appendages are observed on the dendrites. The axon (Fig. 7C, arrowhead) arises from the soma and arborizes extensively within the parent dendritic field. The overall appearance of this cell is similar to the medium neuron shown in Figure 8B (SA = 160 square μm).

Several cells have smooth soma and a few sparsely-spined dendrites which usually arise from the poles of the soma (Fig. 13A & 13E). Rarely, a cell would have somatic spines or appendages (Fig. 12E & 13B). It is unclear how long their dendrites may extend because most of them become unimpregnated a short distance from the soma. The dendrites in sagittal sections usually align themselves with the course of the internal capsule fibers of passage. Axons often arise from a primary dendrite (Fig. 13A, 13B & 13E, arrowheads), some give rise to collaterals near the soma.

Two neurons with somatic sizes at the lower limit of the medium cells or the upper limit of the small cells (i.e. SA value is near 100 square μm) have no apparant axons. One of these cells has a maximal somatic cross-sectional area of 105 square μm (Fig. 12A, 12B &

13C). Its bipolarly distributed dendrites are sparsely-spined (Fig. 12A, arrowhead). The other cell has a maximal somatic cross-sectional area of 120 square μm (Fig. 12C & 13F), and it has aspinous dendrites some of which are varicose and have long and thin appendages (Fig. 12D, and 13H closed-block arrow). No axon is seen to arise from either soma or dendrites, but a thin process resembling a cilium is visible arising from the soma (Fig. 13H, opened-block arrow). This process has similar appearance to the cilia of the impregnated ependymal cells lining the ventricular surface in the same section.

RARELY-FOUND CELL TYPES

The neuron shown in Figure 13D (SA = 146 square μm) has profusely branching dendritic processes (S = 4, F = 33) which are sparsely covered with short and stubby appendages. One of the processes, however, has no surface appendages and appear to be extending away from other processes (Fig. 13D, arrowhead), indicating that this may be the axon of this cell.

Two medium cells have long dendrites. One (Fig.14A) has a fusiform aspinous soma (SA = 180 square μm) with four primary dendrites, one of which is cut off into an adjacent section. The dendrites are poorly branched and may radiate up to 450 μm in length. The dendritic surface is essentially aspinous with some varicose appearance. Only one or two spines or appendages are observed on the dendrites. The axon (Fig. 14A, arrowhead) of this cell gives rise to a few collaterals near the soma, but there is no indication that the main axon terminates locally near the soma.

The other unique medium neostriatal neuron with long dendrite (Fig. 14B, SA = 150 square μm) is also shown in photomicrogaph in Figure 6D. It has a few somatic spines resembling the type II medium neuron (Fig. 4F). Its radiating dendrites are sparsely-spined, resembling those of type III medium neurons (Fig. 6A, 7A, and 7D), but most of its dendrites terminate with elaborate polymorphic processes within the fiber bundles of the internal capsule (Fig. 6E; Fig. 14B, opened-block arrows) comparable to those of a type IV neuron mentioned earlier (Fig. 8B, 8C & 9B). On the other hand, it is similar to the cell shown in Figure 14A because it has a dendrite extending over 500 μm in length. The axon of this cell is unimpregnated except for its initial segment (Fig. 14B, arrowhead).

NEUROGLIAFORM CELLS

Although these cells are very rarely found in most of our preparations, they are well represented in some rat brains. They have small cell bodies (SA = 62 square μm, or equivalent to diameters less than 10 μm in diameter) and numerous short processes projecting radially from the soma, resembling a fuzz ball with radius of about 30 μm (Fig. 15). Many of the processes are varicose or beaded in appearance (Fig. 15B, arrow). However, none of the processes can be identified as either axon or dendrites. Similar cells have been found in both the cerebral cortex and the globus pallidus.

Figure 12. Photomicrographs of small neurons.

A & B: A neuron shown in B is at the same magnification as the other types of neurons described earlier. as well as that shown in Figure 12C, CAL = 50 μm. It is shown at higher magnification in A to demonstrate the presence of a few spines (arrow) on its dendrites. This cell is also shown in Figure 13C. CAL in A = 20 μm.

C & D: A neurons with SA = 120 square μm. This cell has sparsely-spined dendrites, some of which have long dendritic appendages. One of the long dendritic appendage (arrow) is shown at higher magnification in D. This cell is also shown in Figure 13F. CAL in C = 50 μm. CAL in D = 20 μm.

E: A fusiform neuron with a few somatic spines and a few sparsely-spined dendrites. This cell is also shown in Figure 13B. SA = 95 square μm. Same magnification as in D.

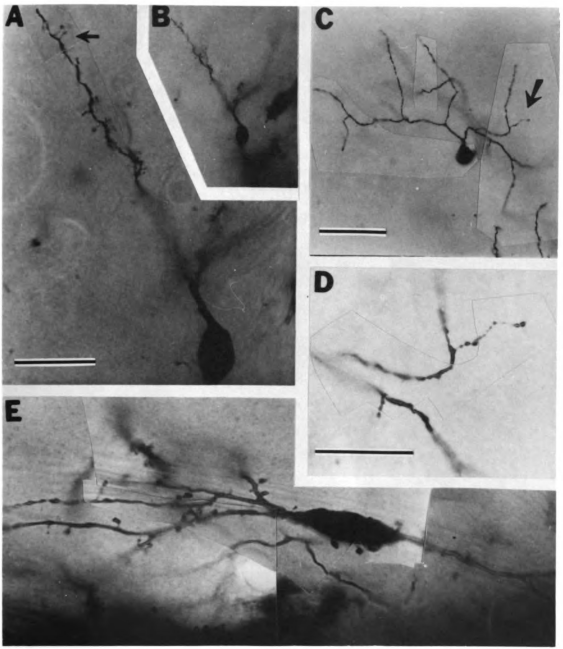

Figure 12

Figure 13. Microscopic tracings of small neurons. CAL = 50 μm. These are neurons with somatic cross-sectional areas near or below 100 square μm. Some of which are very rarely-found uniques cells which are not classified as separate types of neurons at this time.

A: A small neuron with smooth soma and sparsely-spined dendrites. Arrowhead points to the axon. SA = 66 square μm.

B: A small neuron with somatic spines and sparsely-spined dendrites. Arrowhead points to the axon. This is the same cell shown in Figure 12E.

C: A neuron with smooth soma and two primary dendrites which branch into sparsely-spined distal dendrites. No axon has been identified. This is the same cell shown in Figure 12A & 12B.

D: A neuron with many thin dendrites some of which have short appendages. Arrowhead points to a possible axon-like process. SA = 146 square μm.

E: Another small neuron with sparsely-spined dendrites and smooth soma. The axon (arrowhead) arises from a dendrite. SA = 94 square μm.

F & H: The same neuron as shown in Figure 12C. This cell has no identifiable axon. Opened-block arrow points to the somatic region shown at higher magnification in H. Small closed-block arrow points to the long dendritic appendage also shown in Figure 12D. Small opened-block arrow in H points to a cilium-like process on the soma. No axon has been identified for this cell.

G: A type III medium neuron with one somatic spine. Arrowhead points to the partially impregnated axon. SA = 129 square μm.

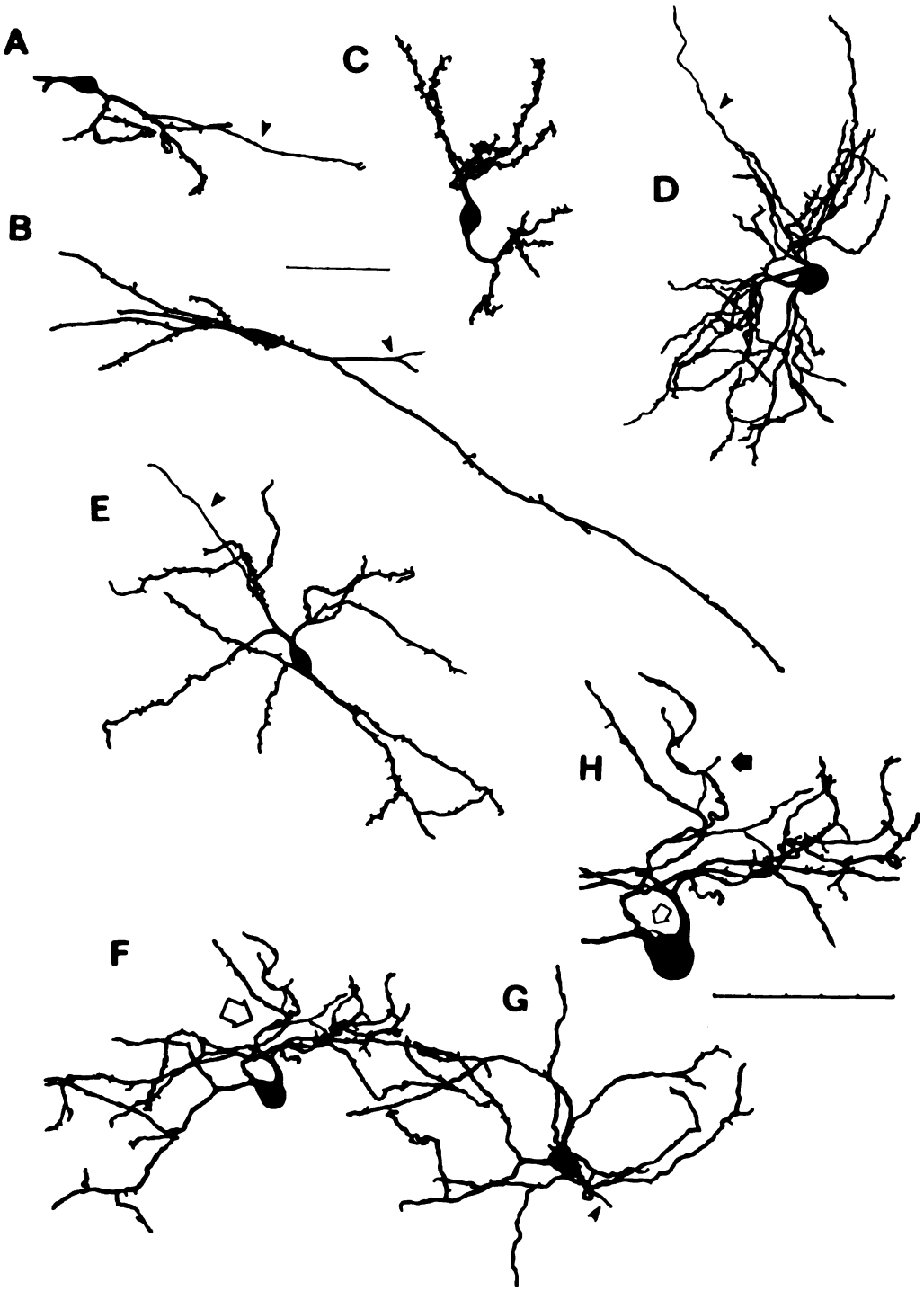

Figure 13

Figure 14. Microscopic tracings of medium neurons with long dendrites. CAL = 50 μm.

A: This neurons has virtually aspinous dendrites, some show slightly varicose appearance and extend up to 450 μm from soma (about twice the dendritic length of a type I medium neuron). Arrowhead points to its axon which gives rise to a few local collaterals before becoming unimpregnated. SA = 180 square μm.

B: This is the same neuron shown in Figure 6D. One of its dendrite extends up to 500 μm from the soma. Arrowhead points to the partially impregnated axon. Opened-block arrows points to the polymorphic terminal dendritic branches within the fiber bundles of the internal capsule. One of these terminal branches is also shown in Figure 6E.

Figure 15. Photomicrographs of a neurogliaform cell.
A: This small cell, SA = 50 square μm, is shown at the same magnification as those of other types of neurons described previously.
B: Higher magnification of A, showing its slender and varicose short processes (arrow) which extend no farther than 50 μm from the soma. CAL = 10 μm.

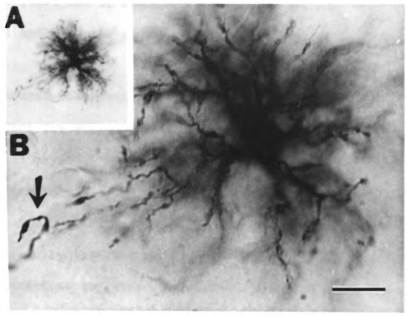

Figure 15

B. Electron Microscopic Analysis of Golgi Material

At least one of each type of rat neostriatal neurons has been gold-toned and analyzed at the electron microscopic level.

General Observations & Comments

Since the gold-toned Golgi materials have undergone much chemical and physical treatments before postfixation in OsO_4 solution and plastic embedding, the preservation of the tissue was often less than ideal for critical comparative analysis of samples from different rats. Frequently the gold particles would accumulate around the synaptic densities and would obscure the synaptic membrane specialization. Thus the symmetry of synaptic contacts in most Golgi gold-toned materials often remained unclear, and the analysis of synaptic relationships of gold-toned neurons was limited mostly to descriptions of the relative size of the synaptic vesicles in the presynaptic terminals. Occasionly, when the morphology of the presynaptic boutons were very poorly preserved, only indications of synaptic contacts could be resolved. Rarely, the opposing synaptic membranes are revealed as pale lines against the dark gold particles and the synaptic gap may be measured. In the case shown in Figure 17D, the synaptic gap measures 17 nm.

In general, the cytoplasm of the gold-toned neurons appears to be washed-out in comparison to the neighboring neurons which were not impregnated by Golgi reaction product. This appearance may have resulted from the dissolution of the Golgi reaction product by the sodium thiosulfate solution during the last step of the gold-toning

procedure, leaving empty spaces behind. In some cases, the deposition of gold particles was insufficient to provide an adequate light microscopic image after de-impregnation by the sodium thiosulfate solution. In these cases, locating the gold-toned profiles under the electron microsope depended on prior detailed tracings of surrounding landmarks (blood vessels or fiber bundles) and the recognition of this washed-out or extracted appearance of the de-impregnated cytoplasm.

Where feasible, the morphology of the axons was analysed to determine whether the initial segments receive synaptic inputs and whether the axons were myelinated. The morphology of the intrinsic collateral terminals and their postsynaptic targets were also studied.

Type I Large Neuron:

The light microscopic morphology of the type I large neuron selected for this electron microscopic analysis is shown in Figure 1C. The soma of this large neuron is characterized by the presence of a deeply indented nucleus surrounded by a large amount of organelles, including well organized perinuclear Golgi complexes and abundant Nissl substance (i.e. rough endoplasmic reticulum) (Fig. 16 & 17A). Numerous dense bodies resembling either lysosomes or lipofuscin granules are scattered in the cytoplasm. Despite the large size of its soma, very few axosomatic synapses are observed on the type I large neuron (Fig. 17A, white arrow). The presynaptic terminals contain either medium (40 to 45 nm in diameters) (Fig. 17B) or small vesicles (30 to 35 nm) (Fig. 17C & 17D).

Figure 16. Electronmicrograph of a type I larage neuron. This is the same neuron shown in Figure 1C after it has been gold-toned. Note that its nucleus is invaginated, and the cytoplasm contain large amount of Nissl substance (rough endopalsmic reticulum and free ribosomes), dense bodies, mitochondria and Golgi complex. The last organelle is better illustrated in Figure 17A. CAL = 5 μm.

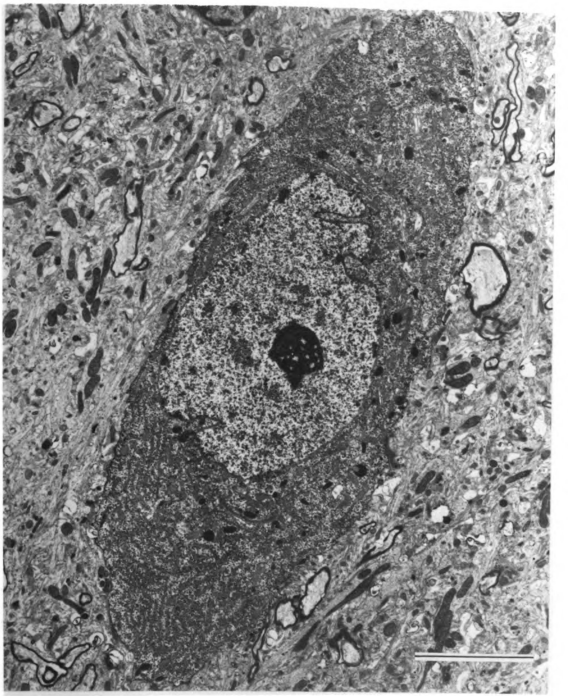

Figure 16

Figure 17. Electronmicrographs of soma and dendrites of a type I large neuron.

A: A tangential sections of the soma, illustrating the perinuclear Golgi complex (g). Only a small portion of the nucleus (n) is visible. Large amount of Nissl substance (rer) and dense bodies are also found throughout the cytoplasm. White arrow points to a axosomatic synapse also shown in C and D. CAL = 5 μm.

B: An axosomatic synapse (arrow), the presynaptic terminal contain medium vesicles. CAL = 0.5 μm.

C: Another axosomatic synapse (arrow), the presynaptic terminal contain small vesicles. Same magnification as in B.

D: Higher magnification of C, showing the opposing synaptic membranes contrasted by the dark gold particles. The synaptic gap = 17 nm. CAL = 0.5 μm.

E: A primary dendrite forms a synpase with two active sites (arrows) with a presynaptic terminal containing medium vesicles. Same magnification as in B.

F: A distal secondary dendrite is contacted by several terminals (arrows) containing medium vesicles. The dense deposit of gold particles prevents detail analysis of the synaptic junctions. Same magnification as in B.

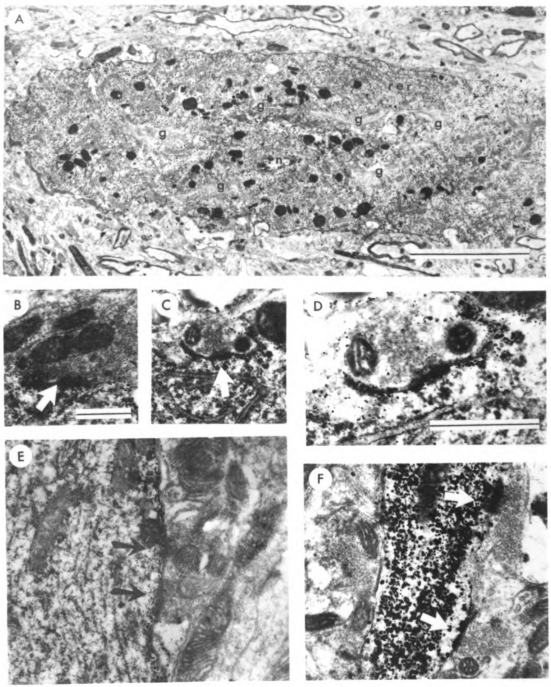

Figure 17

Figure 18. Electronmicrographs of soma and axon of a type II large neuron.

A: The soma of the type II large cell which is also shown in Figure 3A and 3B. Note that its nucleus is deeply invaginated, and the cytoplasm contains large number of mitochondria, microtubules and Golgi complexes (g). Although not apparent here, adjacent sections also contain abundant Nissl substance. CAL = 5 μm.

B: The axon of this neuron becomes unimpregnated at the point of myelination. CAL = 1 μm.

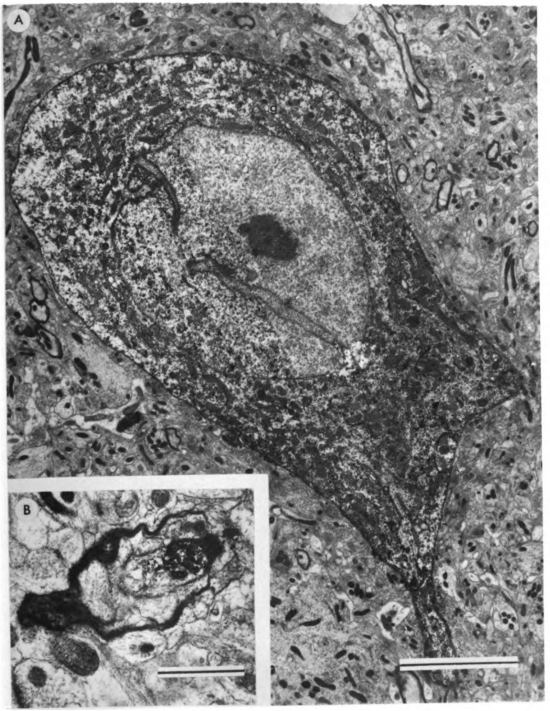

Figure 18

Figure 19. Electronmicrographs of a somatic spine and dendrites of a type II large neuron. CAL = 0.5 μm.

A: A somatic spine is contacted by terminal # 5. Also shown are numerous axosomatic synapses made by terminals #1, 2, 3 & 4. Terminal #6 also synapses with the somatic spine on an adjacent section.

B: A primary dendrite with a spine which is contacted by terminals #1 & 2. Terminals #4, 5 & 6 synapse with the dendritic shaft. Terminal #3 contain larger vesicles and form symmetrical contacts with an adjacent dendrite as well as the gold-toned dendrite on an adjacent section.

C: A distal secondary dendrite is contacted by terminals #1, 2 & 3 containing medium vesicles, and #4 containing small vesicles. Terminal #1 (curved arrow) also makes aymmetrical synapse with a neighboring spine (straight arrow).

D: A spine on a distal tertiary dendrite is contacted by terminal #1. Terminal #2 synapses on the dendritic shaft at the base of the spine. Terminal #4 synapses on the dendritic shaft opposite to the spine. All of these contain medium vesicles.

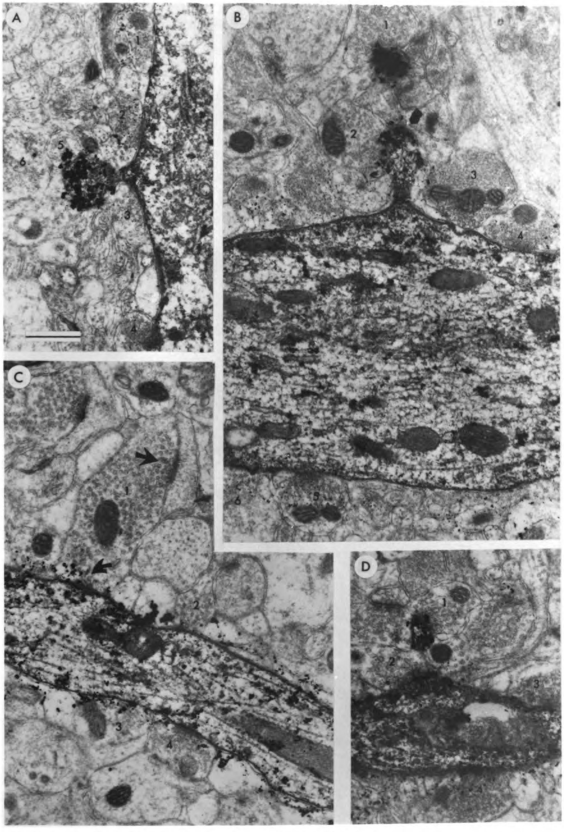

Figure 19

Figure 20. Electronmicrographs of dendrites of a type II large neuron, showing synapses and partial impregnations. CAL = 0.5 μm.

A: This proximal secondary dendrite is contacted by terminals #1, 2 & 3 containing medium vesicles but has different vesicle packing densities.

B: This tertiary dendrite becomes unimpregnated. The terminals #1, 2 & 3 synapses on the impregnated part, whereas #4 & 5 synapse on the unimpregnated part of the dendrite. These terminals contain medium to small vesicles. In comparison, a terminal containing large vesicles (arrow) synapses on an adjacent dendrite.

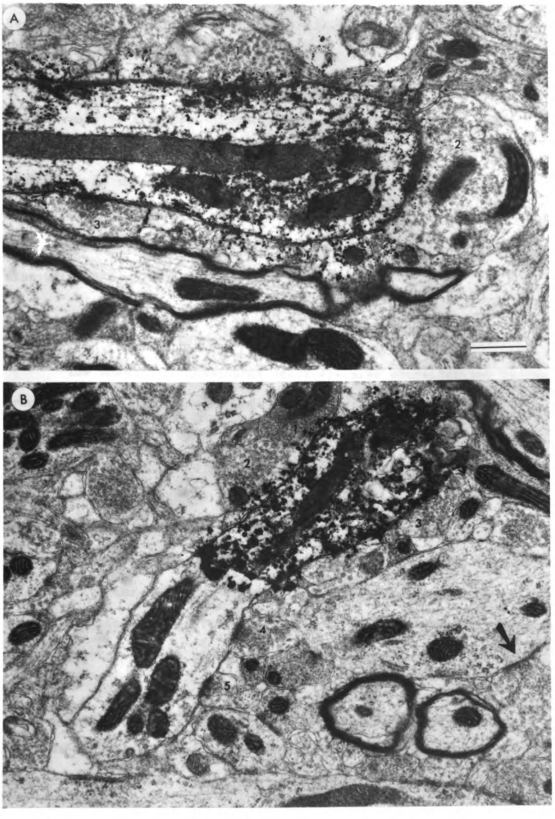

Figure 20

Synapses are also found on both proximal and distal dendrites. A presynaptic bouton containing medium vesicles is shown making a synpatic contact on a primary dendrite in Figure 17E with two active zones (arrows) and 22 nm synaptic gap. Terminals containing medium vesicles also make synaptic contacts with distal secondary dendrite (Fig. 17F, white arrows). The nature of the axonal morphology of the gold-toned type I large neurons at the electron microscopic level remains unclear. However, a type I large neuron has been labeled intracellularly with HRP, and it has a myelinated main axon (Fig. 32).

Type II Large Neurons:

The somata of these large cells are also characterized by the presence of deeply indented nuclei, some containing intranuclear filaments, surrounded by a large amount of cytoplasmic organelles (Fig. 18A). However, the presence of many axosomatic synapses and synapses on somatic spines (Fig. 19A) distinguishes this type of large cell from type I large cells. The primary dendrites (Fig. 19B) also appear to receive more synaptic contacts than those of type I large cells. Often more than one presynaptic terminal synapses with one somatic or primary dendritic spine and most of these terminals contain medium vesicles. It is unclear whether these spines contain a spine apparatus.

Many terminals containing medium vesicles make synaptic contacts with distal dendrites. Some of these terminals also make asymmetrical synapses with spines of neighboring neurons (Fig. 19C, terminal #1). Occasionally, terminals containing large vesicles

(diameter >50 nm) make synaptic contact with the dendritic shaft at the base of the dendritic spine (Fig. 19D, terminal #2). Although different sized presynaptic boutons containing varying amount of synaptic vesicles make synaptic contacts with the type II large cells (Fig. 20A), neither of these two parameters is reliable for classifying axon terminals. This is because the size of the presynaptic terminals and the packing density of the synaptic vesicles often vary according to the planes of sections. Some of the dendrites of the type II large neuron have been revealed to be only partially impregnated by the Golgi reaction products (Fig. 20B), illustrating the capriciousness of the Golgi technique and the need for critical interpretation of dendritic length based on Golgi data.

The main axon of the large type II neurons becomes myelinated at the point where the Golgi impregnation stopped (Fig. 18B). No collaterals arise from the unmyelinated initial segment.

Type I Medium Neurons:

The somata of these cells are characterized by a round, unindented nucleus surrounded by a rim of cytoplasm which contains many free ribosomes (in rosettes), mitochondria and microtubules (Fig. 21A). These cells appear to have the least amount of rough endoplasmic reticulum and the most poorly developed Golgi complexes of all the neostriatal neuronal types. The somatic profiles of the medium type I cells vary from small (7 or 8 μm in diameters) up to large (up to 20 μm or more in diameters) depending on the orientation of the plane of the section. Most of the microtubules appear to be aligned along the long axis of the neuron.

Axon terminals containing either large or medium vesicles have been found making synaptic contacts with the somata of the type I medium neurons (Fig. 21B). Similar boutons also make synaptic contacts with the primary dendrites. Although some axodendritic synapses have been observed on distal dendrites (Fig. 21C), these latter are mainly asymmetrical axospinous synapses with boutons containing medium vesicles. Further details relating to the ultrastructural morphology of the medium type I neurons are described in the intracellular HRP labeling studies in which the tissue is better fixed.

Type II Medium Neurons:

The somata of the type II medium neurons are characterized by the presence of a large nucleus which has indented nuclear membrane and often contains intranuclear rod (Fig. 22A). The intranuclear rod consists of aggregates of filaments aligned in the same direction (Fig. 22B). These neurons have synaptic contacts on both the somatic surface and the somatic spines (Fig. 22C). Often two or more boutons containing mainly medium vesicles terminate on the same somatic spine. Since the somatic spines occupy only a small portion of the somatic surface area, the somatic spines are not observed in most sections through the soma.

Terminals containing either medium or small vesicles make synaptic contacts with either dendritic shafts or dendritic spines of both the primary and distal dendrites (Fig. 22D, 23). It is unclear whether the spines of these cells have spine apparatus because the interior of the spinous processes have often been obscured by either

gold particles or dark, amorphous cytoplasmic derivatives (Fig. 23). Although the axon of one type II medium neuron has been traced through serial sections until the stopping point of Golgi impregnation, no indication of myelination has been observed.

Type III Medium Neuron:

The soma of the type III medium neuron contains a nucleus with relatively shallow nuclear membrane invaginations (Fig. 24A). Small stackes of rough endoplasmic reticulum and Golgi complexes are found in the surrounding cytoplasm. The axosomatic terminals contain large, medium or small vesicles (Fig. 24D, 24E, 24F). Primary as well as distal dendrites make synaptic contacts mainly with boutons containing medium vesicles (Fig. 24G, 24H, 24I). The rarely found dendritic spines or appendages are also postsynaptic (Fig. 24H).

It is interesting to note that an entire primary dendrite and its branches was not impregnated with the Golgi reaction product (Fig. 24B). The stopping point of impregnation at the base of the dendrite is marked by a gap less than 0.5 μm in width. Whether this gap was a cause of the failure of impregnation of this dendrite remains unclear.

Boutons containing large vesicles make synaptic contacts with the axon initial segment (Fig. 24C). However, this axon, like that of the type II medium neuron, became unimpregnated before any indication of myelination.

Figure 21. Electronmicrographs of a type I medium neuron.
A: The soma of type I medium neuron contains a round, unindented nucleus. The surrounding cytoplasm contains abundant free ribosomes, mitochondria and microtubules. CAL = 5 μm.
B: Axosomatic synapses on the type I medium neurons. Terminal #1 contains larger vesicles than terminal #2. CAL = 0.5 μm.
C: A distal dendrite with spines (s). Terminals #1 & 3 containing mediuum vesicles synapse with the spines, whereas terminal #2, containing medium to large vesicles, synapses on the dendritic shaft. CAL = 0.5 μm.

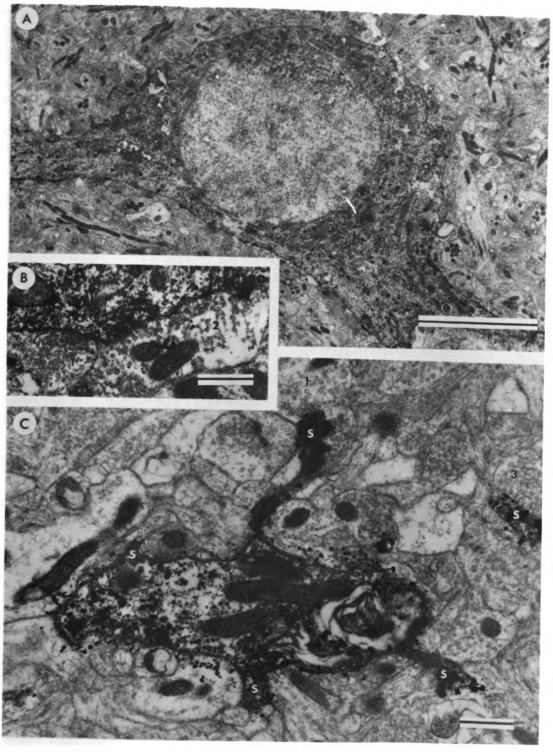

Figure 21

Figure 22. Electronmicrographs of a type II medium neuron.
A: The nucleus (n) of the type II medium neuron is invaginated and contains intranuclear rod. Arrow points to the head of a somatic spine also shown at higher magnification in C. CAL = 5 μm.
B: Higher magnification of the intranuclear rod showing its filamentous nature. Arrows points to the indented nuclear membrane. CAL = 0.5 μm.
C: Higher magnification of the somatic surface and the somatic spine. Terminals #1, 2 & 3 synapse on the head of the spine, and #4 & 5 synapse on the soma. Same magnification as in B.
D: The primary dendrite contains numerous microtubules and is contacted by terminals #1, 2, 3, 4, 5 & 6. Same magnification as in B.

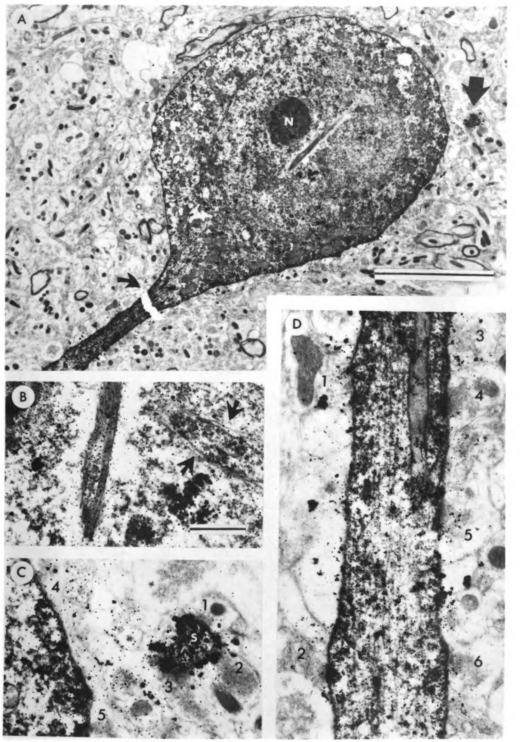

Figure 22

Figure 23. Electronmicrographs of dendrites of a type II medium neuron. CAL = 0.5 μm.

A: A cross-sectioned primary dendrite is contacted by terminals #3, 4, 5, 6 & 7. An emerging spine (s) is synapsed upon by terminals #1 & 2.

B: Terminal #1 synapses at the base of the spine (s) on a distal dendrite. Terminals #2 & 3 synapse on the shaft. The spine also make synapses on adjacent sections.

C: Another distal dendrite makes symmetrical synapse (arrow) with terminal #1 which contains small vesicles.

D: A thin distal dendrite is contacted by terminals #1 and #2, both contain medium vesicles.

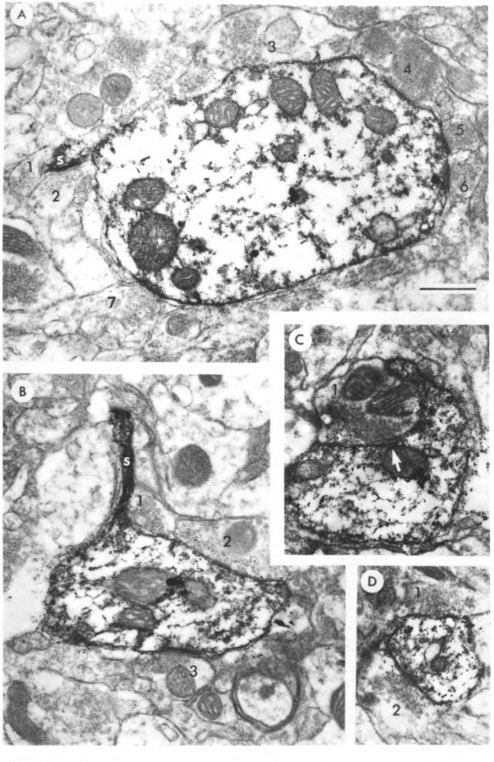

Figure 23

Figure 24. Electronmicrographs of a type III medium neuron.
A: The nucleus of this cell has shallow invaginations (arrows). The surrounding cytoplasm contains a few stacks of rough endoplasmic reticulum (rer) and Golgi apparatus (g). CAL = 5 μm.
B: One of the primary dendrites has not been impregnated by the Golgi reaction products. The cisterns of a Golgi apparatus (g) extend from the soma into the dendritic cytoplasm. n: nucleus. CAL = 1 μm.
C: A terminal synapsing on the axon initial segment (arrow) contains large vesicles.
D, E & F: Axosomatic terminals contain vesicles ranging from large (D), medium (E) to small (F).
G: A large terminal, containing medium vesicles, synapses on a primary dendrite.
H: Terminals #1 & 2 synapse on the dendritic shaft, and #3 on the spine (s) of a distal dendrite. All contain medium to small vesicles.
I: On a distal dendrite, the terminal #1 contains medium to small vesicles while #2 contains large vesicles.
C to I are at the same magnification, CAL = 0.5 μm.

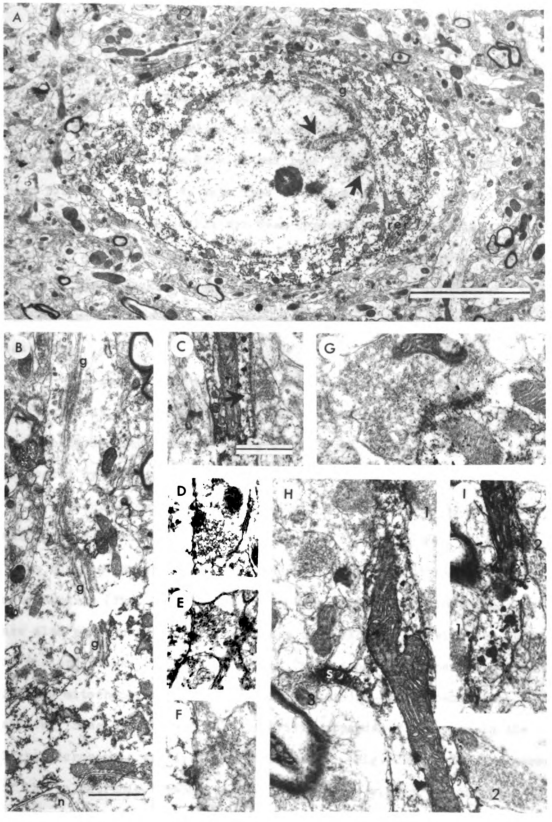

Figure 24

Type IV Medium Neuron:

The nucleus of the type IV neuron also has shallow indentations (Fig. 25A). This appearance together with the presence of small stacks of rough endoplasmic reticulum and Golgi complexes resemble the type III medium neuron described above. Somatic synapses similar to those on the type II and type III medium neurons have also been found. The soma of this type IV medium neuron is juxtaposed next to another neuron which has not been impregnated by the Golgi reaction product. No intervening glial processes have been observed at the point of apposition. This neighboring neuron has an unindented nucleus and little, if any, rough endoplasmic reticulum and Golgi complexes.

The axon of the type IV medium neuron has been found to be myelinated at 25 μm away from the soma (Fig. 25B, 25C). The Golgi impregnation stopped near the beginning point of myelination as indicated by the location of gold particles (Fig. 25C).

The proximal and the distal dendritic processes synapse mainly with boutons containing medium vesicles (Fig. 26), some of these boutons appear to be dark, and fully packed with vesicles and mitochondria (Fig. 26F, bouton #5). Spine-like dendritic appendages are also postsynaptic, with terminals synapsing mainly with the heads of the appendages (Fig. 26A, B, C). These dendritic appendages often contain mitochondria interconnecting with those of the dendritic shafts. Some of the very thin dendritic appendages observed in the light microscopic analysis are postsynaptic (Fig. 26D). The processes of the polymorphic terminal dendritic branches within the internal capsule fiber bundles also has been found to be postsynaptic (Fig. 26F).

Figure 25. Electronmicrographs of soma and axon of a type IV medium neuron.

A: This is the same cell shown in Figure 8A and 9A. The soma of this type IV neuron is apposed to another neuron (arrowheads) with no intervening glial processes. Its nucleus (N) has a few shallow indentations (arrow). The surrounding cytoplasm contains Golgi apparatus (g) and a few stacks of rough endoplasmic reticulum (rer). CAL = 5 μm.

B: A low magnification micrograph showing the soma of of the same type IV neuron (n) in relationship to its partially impregnated axon (white arrows). The long arrow indicates the trajectory of the axon initial segment which has been cut into adjacent sections. CAL = 10 μm.

C: A higher magnification of the partially impregnated axon. A mitochondria is seen crossing between the impregnated part of the initial segment and the unimpregnated myelinated axon (block arrow). CAL = 1 μm.

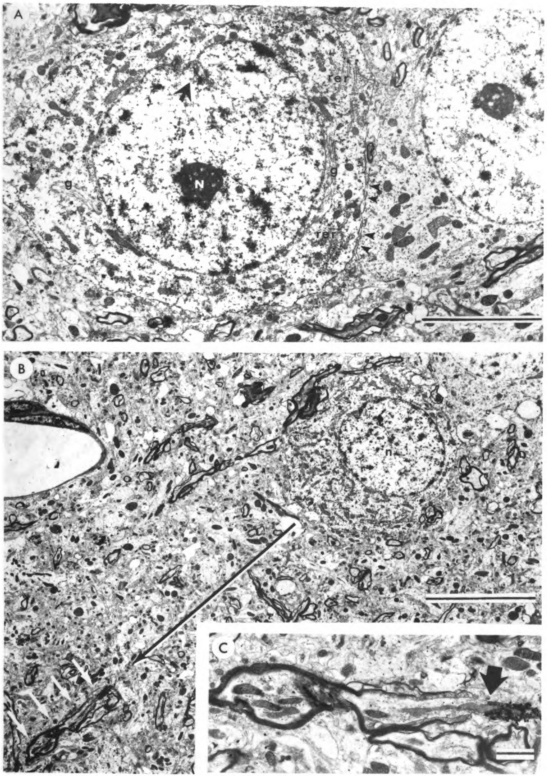

Figure 25

Figure 26. Electronmicrographs of dendrites of a type IV medium neuron. CAL = 0.5 μm.

A, B & C: These are adjacent sections through a spine-like appendage (s) on a distal dendrite. Terminal #1 contains medium vesicles and synapse on the head of the appendage, which appears to contain a mitochondrion. The thin spine-shaft is about 3 μm in length. Terminal #2 synapses on the dendritic shaft.

D: A thin dendritic appendage with a small bulbous head is encircled by an axon terminal (small white arrows).

E: One of the terminal dendritic branches within the fiber bundles of the internal capsule is shown to be postsyanptic (small white arrows).

F: The distal secondary dendrite is contacted by terminals #1, 2, 3, 4 & 5, containing mainly medium vesicles.

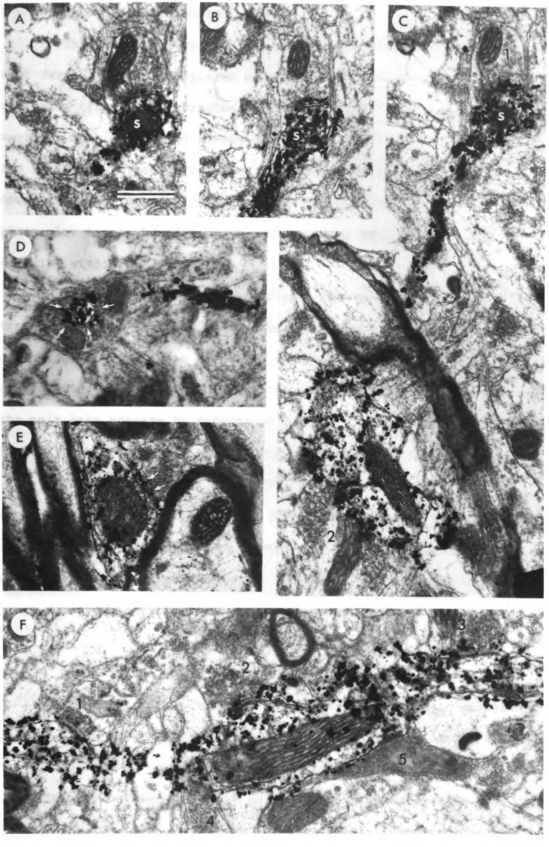

Figure 26

Type V Medium Neuron:

This neuron has a deeply invaginated nucleus surrounded by well developed Golgi complexes and several stacks of rough endoplasmic reticulum (Fig. 27A). Boutons containing either large or medium vesicles make synaptic contacts on both soma and dendrites, including the dendritic varicosities, have been observed (Fig. 27E, 27F). However, few cytoplasmic details could be resolved from this particular neuron because of the accumulation of electron dense material within the cytoplasm.

The axon previously identified light microscopically has been traced in serial sections to many branching points where the diameters of the daughter branches gradually decreased without any indication of myelination. The initial segment or rather, the proximal portion of the main axon appears to receive no synaptic contacts (Fig. 27D). Distally, the boutons of the axon collaterals make either axosomatic (Fig. 27B) or axodendritic (Fig. 27C) synapses. At high magnification, the boutons appear to contain elongated small vesicles (Fig. 27C) and make symmetrical synapses with a 24 nm synaptic gap. The postsynaptic somata often have unindented nuclei, very little rough endoplasmic reticulum but many free ribosomes.

Small Neuron

The small neuron selected for electron microscopic analysis is that shown in Figure 12A. This neuron has a large, deeply indented nucleus surrounded by a narrow rim of cytoplasm (Fig. 28A). The soma is juxtaposed between two other neurons with similar somatic morphology, and there is an area where no glial processes intervene

between this small cell and its adjacent neighbor (Fig. 28A, arrowheads). Some ribosomes and a few cisterns of rough endoplasmic reticulum are found within the perikarial cytoplasm. A few somatic synapses have been observed (Fig. 28B), the presynaptic boutons contain medium vesicles. Boutons containing small to medium vesicles make synaptic contacts on the primary dendrites (Fig. 28C), whereas the boutons contacting the distal dendrites and dendritic spines contain mainly medium or medium to large vesicles (Fig. 28D).

Figure 27. Electronmicrographs of a type V medium neuron.
A: The soma conatins a nucleus with deeply invaginated nuclear membrane (small white arrows). A very well developed Golgi apparatus (g) occupies a perinuclear position. A few stacks of rough endoplasmic reticulum (rer) are also found in the cytoplasm. CAL = 5 μm.
B: Two of the teminals (T) of the intrinsic collaterals of this type V neuron make symmetrical axosomatic synapses (arrows) on the same neighboring neuron which has unindented nucleus (n). CAL = 0.5 μm.
C: A higher magnification view of an intrinsic terminal (T) making a symmetrical axodendritic synapse (arrow) on a neighboring dendrite. Note the presence of small elongated vesicles within the amorphous dark terminal. CAL = 0.5 μm.
D, E & F are at the same magnification as in B.
D: The axon (AX) of this type V neuron is not myelinated.
E: The soma of this type V cell is contacted by a terminal containing large vesicles (arrow).
F: The dendrite is contacted by terminals #1, 2 & 3, containing mainly medium vesicles.

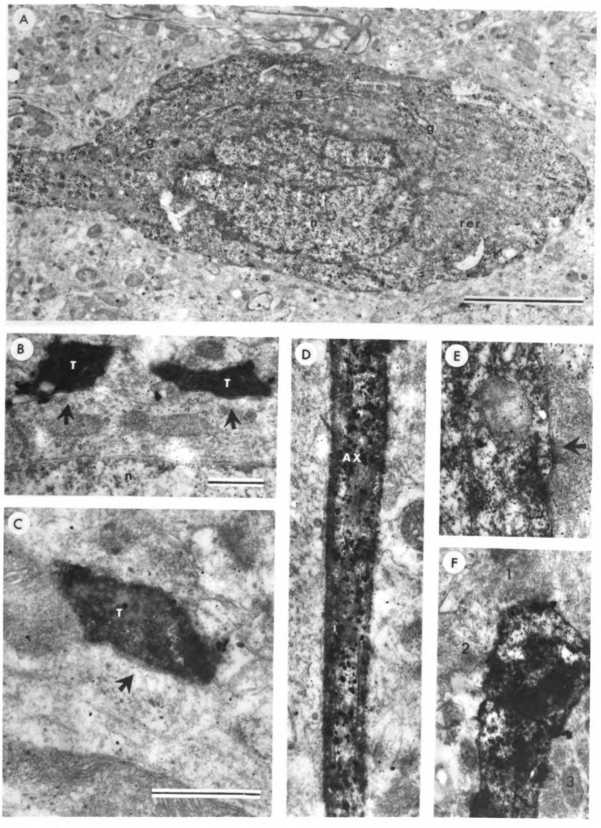

Figure 27

Figure 28. Electronmicrographs of a small neuron.
A: This is the same cell shown in Figure 12A, 12B and 13C. The soma of this small cell lies between the somata of two other neurons (1 & 2) both of which have indented nucleus. The nucleus (n) of the small cell is deeply invaginated. Few organelles are observed in the surrounding narrow rim of cytoplasm. Its soma is apposed to the soma of a neighbor cell without any intervening glial processes (arrowheads). CAL = 5 μm.
B, C & D are at the same magnification.
B: A terminal containing medium vesicles makes axosomatic synapse on this small cell (arrow). CAL = 0.5 μm.
C: A terminal containing small to medium vesicles synapses on the primary dendrite (arrow).
D: Terminals #1 synapses on a spine-like appendages of a distal dendrite (d). Terminal #2 synapses on the dendritic shaft. Both terminals contain medium vesicles.

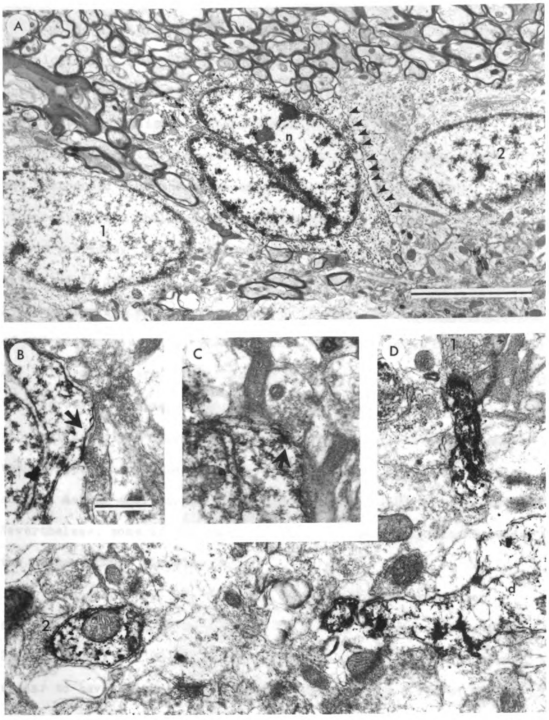

Figure 28

C. Extracellular HRP Studies

Light Microscopic Analysis

The site of extracellular HRP injections has been analyzed histologically, and only those cases where the HRP was restricted outside of the neostriatum were used for further light and electron microscopic analysis. A typical injection site placed in the globus pallidus is shown in Figure 29A. Note that the injection micropipette was introduced into the brain at an angle, penetrating the hippocampus, the thalamus and the internal capsule before reaching the globus pallidus. The neostriatal projection neurons labeled retrogradely from this type of injection are shown in Figure 29B and 29C. Typically, these neurons have cell body sizes at the lower range of the medium size (i.e. somatic cross-sectional areas near 150 square μm). The somata are often filled diffusedly with HRP reaction product together with some dark granules which may also be found in the proximal dendrites (Fig. 29B, 29C). Distally, the density of HRP reaction product gradually fades with increasing distance from the somata, making analysis of distal dendrites virtually impossible. Nevertheless, some of the dendritic spines were barely visible under the highest-power oil objectives.

In thick (100 μm) sections, the neostriatal projection neurons can be seen to have mainly multipolar dendritic trees (Fig. 29B). In semithin (2 μm) plastic section, the projection neurons appear to have a large round nucleus surrounded by a rim of HRP positive cytoplasm. In no case was a large neuron found to be labeled with HRP from extracellular injections placed outside of the

Figure 29. Photomicrographs of the site of extracellular horseradish peroxidase injection and the retrogradely labeled neostriatal projection neurons.

A: Horseradish peroxidase (HRP) injecxtion site is in the globus pallidus (G). Leakage of HRP into the neostriatum (caudate-putamen, or C-P), if any, is minimal.

B: A retrogradely filled neostriatal projection neuron. Both diffuse and granular HRP labeling are observed. SA = 113 square μm. The presence of dendritic spines on the distal dendrite (arrow) cannot be convincingly demonstrated by light microscopy. CAL = 20 μm.

C: A semi-thin plastic section through two neostriatal projection cells. Note that the upper cell has a round, unindented nucleus surrounded by a rim of HRP-positive cytoplasm with both diffuse and granular labelings. Dark HRP-positive granules are also clearly visible on the lower cell. Same magnification as in B.

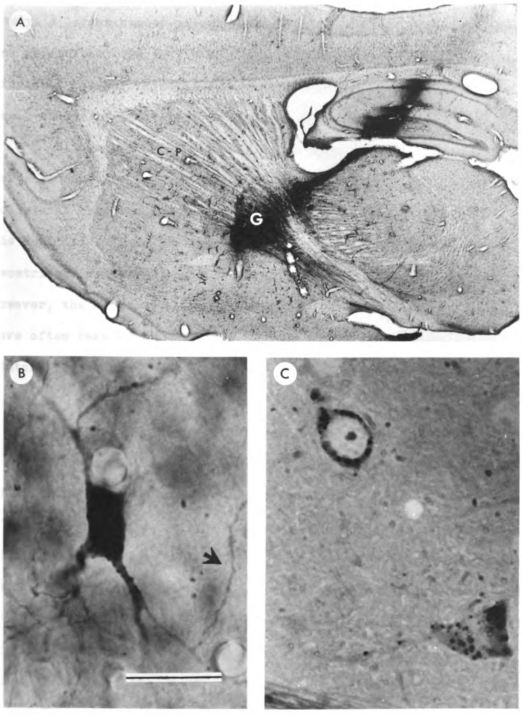

Figure 29

neostriatum. Although many medium neurons have been labeled by this study, a larger number of medium neostriatal neurons were not labeled, and may reflect the fact that the small doses of individual extracellular HRP injections in this study were able to reach only small number of neostriatal projection axons.

Although the axons of the neostriatal projection neurons serve as conduits for the HRP molecules, the axons and their collateral branches are the least labeled comparing to the somata and the dendrites. Thus the axonal distribution pattern of individual neostriatal projection neurons remains unclear with this method. However, the axons of passage, perhaps cortical or thalamic in origin, have often been well labeled in this material, giving rise to both boutons "en passant" and "terminaux" within the neostriatum.

Electron Microscopic Analysis

The soma of a neostriatal projection neuron contains a large round, unindented nucleus surrounded by a thin rim of cytoplasm which often appears darker than the surrounding neuropil due to its content of HRP reaction product (Fig. 30). A few dense bodies containing very dense HRP reaction product are also found in both the perikarial and primary dendritic cytoplasm. Many free ribosomes and some distended cisterns resembling damaged Golgi complexes have been found in the soma. Little, if any, rough endoplasmic reticulum is found in the soma. Boutons forming axosomatic synapses with the neostriatal projection neurons contain either medium or large vesicles, many of which are pleomorphic (Fig. 30B, 30C). Similar boutons also make synaptic contacts with the primary dendrites. Distally, the HRP

labeled dendrites give rise to spines which form asymmetrical synapses with boutons containing medium vesicles (Fig. 30D, 30E). The HRP reaction product within the spines is very faint, explaining the difficulties in visualizing them light microscopically.

Figure 30. Electronmicrographs of a neostriatal projection neuron retrogradely labeled with horseradish peroxidase.

A: The soma of a neostriatal projection neuron. Note that its nucleus is round and unindented. Surrounding rim of cytoplasm contains free ribosomes but few rough endoplasmic reticulum. CAL = 5 μm.

B, C, D & E are at the same magnification.

B: Terminals #1 & 2, containing medium to large vesicles, make symmetrical axosomatic synapses on the projection neuron. A neighboring terminal #3, containing medium to small vesicles, makes asymmetrical synapse with a HRP-negative spine. CAL = 0.5 μm.

C: Terminal #1, containing mainly large vesicles, makes symmetrical axosomatic synapses on the projection cell. Terminal #2 contains mainly small vesicles and does not synapse on the soma of the projection cell.

D & E: Adjacent sections showing the distal dendrite (ds) of a projection neuron. Two dendritic spines (white #1 & 2) make asymmetrical synapses with two terminals (black #1 & 2) containing medium to small vesicles. Their spine-shafts are oulined by the arrowheads. In comparison, a terminal containing larger vesicles makes symmetrical axodendritic synapse with a neighboring dendrite.

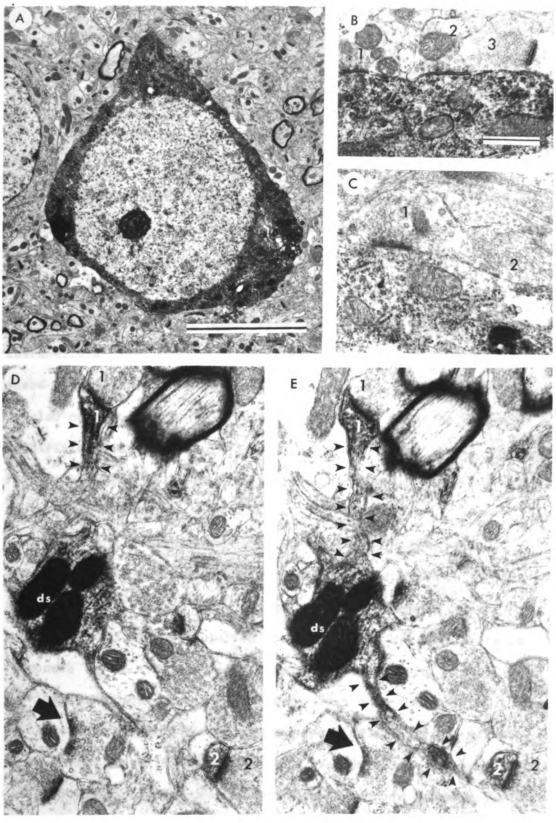

Figure 30

D. Rat Neostriatal Neurons Intracellularly Labeled with Horseradish Peroxidase

General Comments

In this study, we have analyzed the physiology and the morphology, at both light and electron microscopic levels, of the type I medium (medium spiny) neurons and two aspinous neurons corresponding to the type I large and type III medium neurons described in the preceding Golgi analysis. The sampling of two aspinous neurons out of more than one hundred intracellularly labeled neostriatal neurons in the present study is consistent with the results obtained in previous intracellular HRP labeling experiments in the cat and in the rat, where only type I medium neurons have been recovered. This sampling is also consistent with Golgi studies which show that the majority of neostriatal neurons are type I medium neurons (Kemp & Powell, 1971a, Fox et al., 1971/1972a, b; DiFiglia et al., 1976; also see preceding section A).

With the exception of the large aspinous neuron, whose response to CX stimulation was not tested, all of these intracellularly labeled cells had similar excitatory response properties following stimulation of CX and SN. This similarity in responses to two afferent sources is not surprising in light of previous anatomical studies which have shown that the various neostriatal neurons receive a similar range of afferent terminals (Kemp, 1968; Kemp & Powell, 1971b, c). Whether there are subtle differences in response properties of these neurons requires further investigation.

The morphology of the two lightly labeled aspinous neurons will be described first, subsequently the morphology of the medium type I neurons will be analyzed in detail since their sample size is much larger.

Aspinous Neurons: Light Microscopic Analysis

Two aspinous neurons have been lightly labeled in this study. One is a large aspinous neuron with smooth soma (SA = 435 square μm, or 34 X 20 μm in diameters) and five smooth primary processes which become varicose at various distances from the soma, some extend up to 450 μm in length (Fig. 31B). Because of the faint HRP labeling of this cell, it was difficult to distinguish the axon from dendritic process with light microscopic examination. However, electron microscopic analysis has revealed that the ventral process becomes myelinated (Fig. 32A, inset) at a distance of 50 μm from the soma. We have been unable to follow this axon to its termination, and no collateral fibers have been observed in the short length (200 μm) over which it could be followed. The somatic and dendritic morphological characteristics of this neuron indicate that it is one of the type I large neurons as defined in the preceding Golgi study. This cell responded with EPSPs to stimulation of SN (Fig. 31B1).

The other aspiny neuron found in this study (Fig. 31A and 34A) has a fusiform soma (SA = 115 square μm, or 15 X 8 μm in diameters) and three faintly labeled aspinous processes which divide into secondary and tertiary branches. It was not possible to identify the axon of this cell with either light or electron microscopic examination. This cell responded with EPSPs to stimulation of SN

Figure 31. Microscopic tracings of aspinous neurons labeled intracellularly with horseradish peroxidase. CAL = 50 μm.

A: Medium aspiny neuron, note its sparsely branched dedrites and their smooth dendritic surface.

B: Large aspiny neuron, note its long and aspinous varicose dendrites and the smooth soma.

A1 & A2: Excitatory responses of the medium aspiny neuron to substantia nigra and cerebral cortex stimulation respectively.

A3: Low gain record of A2, showing the action potential elicited by cerebral cortex stimulation.

B1: Excitatory response of the large aspiny neuron to substantia nigra stimulation. Dashed lines are extracellular controls. Scale in A1 also applies to A2. Arrowheads point to the onset of the stimulus.

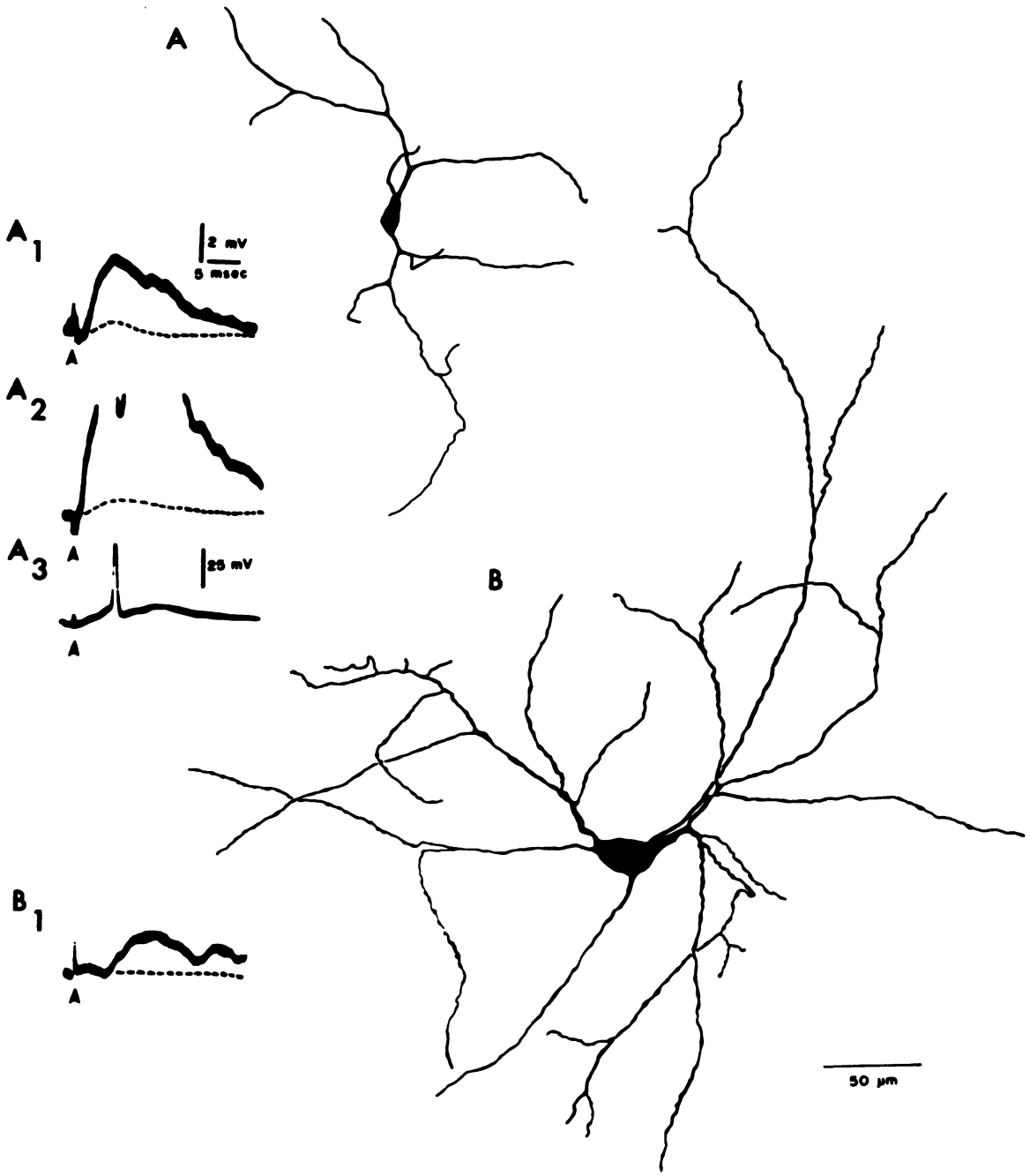

Figure 31

(Fig. 31A1) and CX (Fig. 31A2 and 31A3). Since its somatic cross-sectional area is larger than 100 square μm, it is tentatively identified as a member of the type III medium neurons as defined in the preceding Golgi study based on the appearance of its aspinous soma and poorly-branched dendrites.

Electron Microscopic Analysis:

Large Aspinous Neuron (Type I Large Neuron)

The large aspinous neuron (Fig. 32B) has an eccentrically located nucleus. Since the nucleus appears to be densely filled with HRP reaction products, the distribution of the heterochromatin remains unclear. It is nevertheless possible to see many deep nuclear membrane invaginations (Fig. 32B arrows), a characteristic of large neostriatal neurons well noted by the present and previous authors (Mori, 1966; Adinolfi & Pappas, 1968; Kemp & Powell, 1971a; Fox et al., 1971/1972b, Pasik et al., 1976; Dimova et al., 1980; see also preceding electron microscopic anlaysis of Golgi material). The surrounding perikarial cytoplasm has not been obscured by the HRP reaction product, thus revealing abundant rough ER and free ribosomes distributed throughout the somatic and proximal dendritic cytoplasm. A very well developed Golgi complex occupies a perinuclear position, and is continuous with the widely distributed smooth ER within the soma. Numerous multivesicular bodies and dense bodies resembling lipofuscin granules are found in both soma and dendrites. Elongated mitochondria, some of which are branched, have loosely packed cristae.

The proximal portion of the large primary dendrites of the large aspiny neurons contains all the organelles found in the

Figure 32. Photomicrograph and electronmicrographs of a type I large neuron labeled intracellularly with horseradish peroxidase.

A: Photomicrograph of the large aspiny neuron, the arrow points to the axon. Inset shows an eletron micrograph of this axon taken at about 60 µm away from the soma, note that it is thinly myelinated. CAL = 40 µm for photomicrograph and 1 um for inset.

B: Electron micrograph of the soma of the cell shown in A. Note that its nucleus (N) is deeply invaginated (arrows) and that most of the dark HRP reaction products are restricted within the nuclear envelope. The large cytoplasmic space is full of organelles, including the perinuclear Golgi complex (G), numerous free ribosomes (ri) and stacks of rough endoplasmic reticulum (RER). CAL = 2 µm.

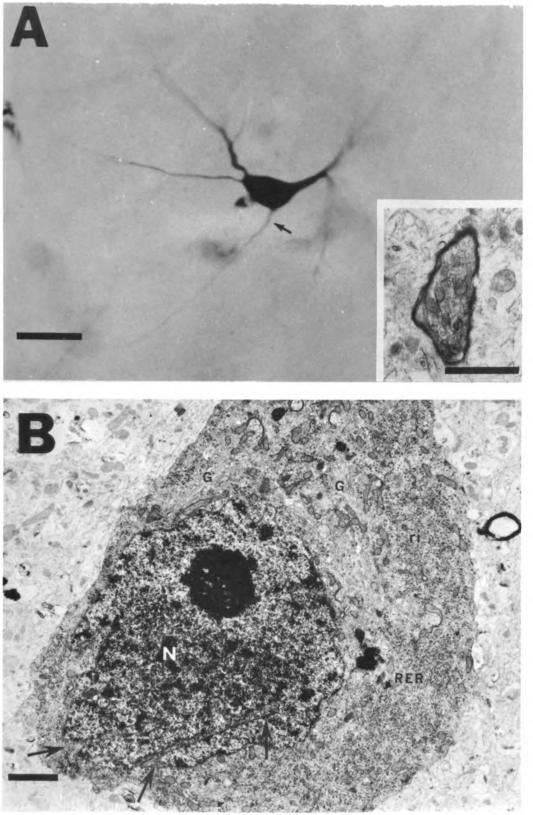

Figure 32

Figure 33. Electronmicrographs of dendrites of the intracellularly labeled type I large neuron.

A: A distal dendrite with a vacuolated swelling (*) makes slightly asymmetrical synapse with a terminal containing medium to small vesicles (arrows). CAL = 0.5 μm.

B: Varicose distal dendrite makes slightly asymmetrical synapses (arrows) with terminals containing medium to small vesicles. Note that the multivesiclular body (*) is present in the varicosity of the dendrite along with other organelles. CAL = 0.5 μm.

C: The large primary denrite of the large aspiny neuron. Note the abundance of organelles in this dendritic process, and especially the series of Golgi cisterns (arrows) extending along its long axis. CAL = 1 μm.

D: Lightly asymmetrical synapse (arrows) on a relatively straight portion of a distal dendrite. The preterminal contains medium to small vesicles. CAL = 0.5 μm.

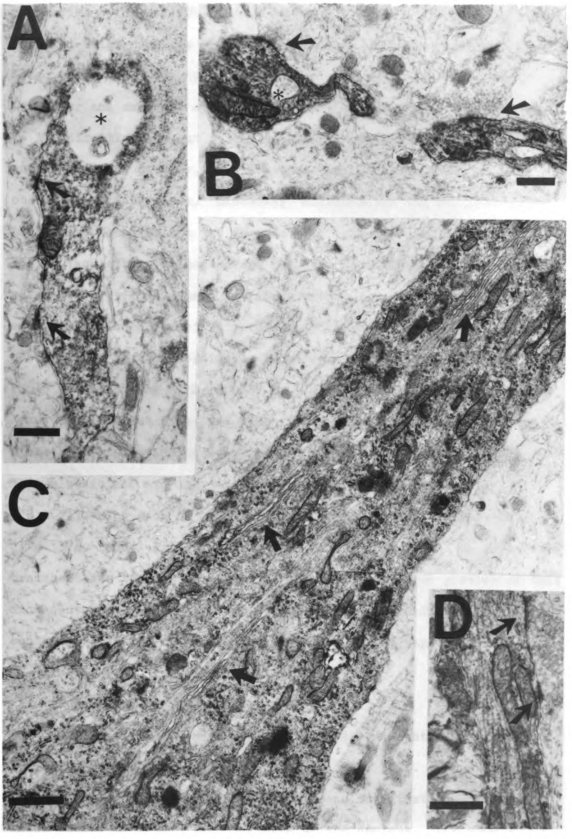

Figure 33

perikarial cytoplasm (Fig. 33C), including series of longitudinally oriented Golgi cisterns which are continuous with the perinuclear Golgi complex. Distally, the dendrites are characterized by their irregular and aspinous appearance. Occasionally, the dendrites appear to lie directly adjacent to the somatic or dendritic membrane of another neuron, however, no membrane specializations have been observed in these cases. Ribosomes, mitochondria and multivesicular bodies are found within the intermittent varicose swellings (Fig. 33B). Vaculated swellings (Fig. 33A) are frequently observed, whether these are artifacts caused by tissue preparation or from a possible exocytotic discharge activity remains unclear. Since the surrounding neuropil has not been optimally preserved, no distinct differences could be determined with respect to the morphology of synapses on the soma and dendrites (i.e. the size and shape of the vesicles, or the symmetry of the synaptic density). Most of the preterminals contain medium vesicles, and make slightly asymmetrical synapses (Fig. 33A, 33B & 33). The axon of this large aspiny neuron becomes myelinated (Fig. 32A, inset) at a distance of 50 µm from the soma. No collateral branches have been observed. The initial unmyelinated portion of this axon contains bundles of both microtubules and neurofilaments and is contacted by a few terminals making symmetrical synapses.

Medium Aspiny Neuron

The medium aspiny neuron has a large and centrally located nucleus within a narrow rim of cytoplasm (Fig. 34B). The nucleus is characterized by very deep nuclear membrane invaginations, some of which appear to bisect the long axis of the nucleus. The scanty

Figure 34. Photomicrograph and electronmicrographs a medium aspinous neuron intracellularly labeled with horseradish peroxidase.

A: Photomicrograph of the medium aspiny neuron. CAL = 10 μm.

B: Electronmicrograph of the same cell shown in A. Note that its nucleus has deeply invaginated nuclear membrane and the narrow rim of cytoplasm has many short mitochondria and a few distended rough endoplasmic reticulum. * marks the enlarged astroglial process. CAL = 2 μm.

C: A dendrite of the HRP labeled medium aspiny neuron synapses with a terminal containing medium to small vesicles (arrow). Note that the dendrite also contains multivesicular body (*). CAL = 0.5 μm.

D: An unlabeled neostriatal neuron which is similar to the HRP-labeled medium aspiny neuron in that it has a deeply invaginated nucleus and little of other organelles. Magnification is the same as in B.

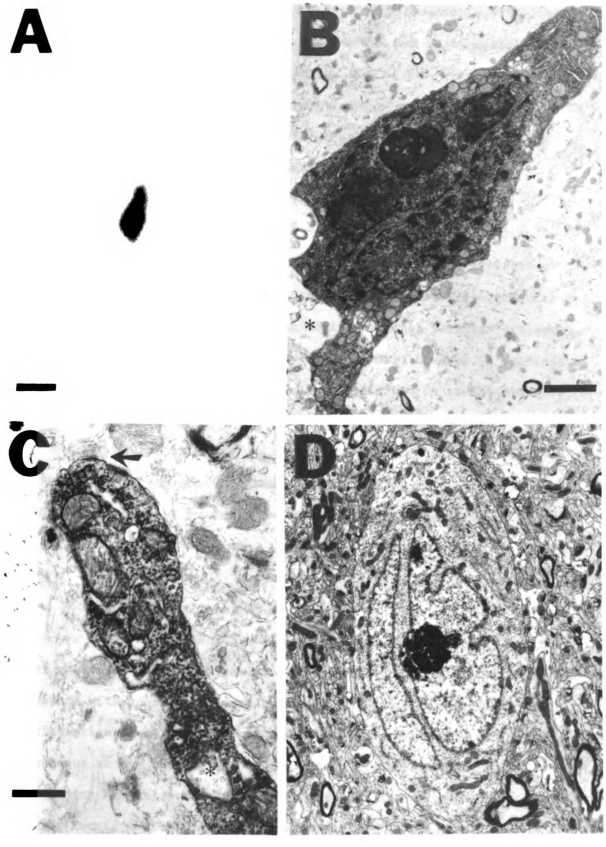

Figure 34

perikarial cytoplasm contains numerous short mitochondria with loosely arranged cristae. A small amount of rough ER has distended cisternae near the base of the dorsal pole of the soma. Free ribosomes, if any, are obscured by the granular HRP reaction product. A sparse smooth ER and a few small Golgi complexes are observed in the soma. Neurons with similar somatic morphology are rarely observed in normal neostriatal material. A comparable unlabeled neuron is shown in Figure 34D.

The dendrites of the medium aspiny neuron (Fig. 34C) are smooth, and contain mitochondria, ribosomes and some multivesicular bodies. Axon terminals containing medium to small vesicles are seen contacting soma and dendrites (Fig. 34C, arrow), however, a critical analysis of the synaptic relationship of this neuron is not possible because of the poor preservation of the surrounding neuropil.

No myelinated profiles containing HRP reaction product has been observed in the nearby neuropil and none of the labeled processes can be conclusively demonstrated as the axon of this cell.

Light Microscopic Analysis of Intracellularly Labeled Type I Medium Neurons

The most frequently encountered HRP labeled cell type in the rat neostriatum is the type I medium neuron (Fig. 35). These neurons respond with monosynaptic excitatory postsynaptic potentials to stimulation of cerebral cortex, substantia nigra and the intralaminar thalamus as has been described fully in previous intracellular recording and intracellular labeling studies in the cat (Kitai et al., 1976; Kocsis & Kitai, 1977; Kocsis et al., 1977; Kitai & Kocsis ,

1978; Kitai et al., 1978) and the rat (Park et al., 1979; VanderMaelen et al., 1979; Preston et al., 1980). These neurons have a spine-free soma (10-20 μm in diameter) and 4 to 6 spine-free primary dendrites (Fig. 35A, 35C). The dendrites become densely covered with spines at a distance of approximately 20 μm from the soma as they branch into secondary and tertiary segments. The majority of spines are pedunculated, with a round head on a stalk of variable length. Some sessile and some branched spines are also observed. The dendritic field of these medium spiny neurons covers a spherical domain with approximately 250 μm radius.

Although the somato-dendritic morphology of the type I medium neurons are more-or-less the same, the distribution pattern of their axons has been found to vary greatly. The axons of many of these neurons often give rise to 3 to 4 collateral branches which arborize entensively within the dendritic domain of the cells of origin (Fig. 35B). Less frequently, however, the intrinsic axon collaterals would ramify extensively outside the parent dendritic domain. An extreme example of this type of intrinsic axon distribution is shown in Figure 35C. This neuron has a main axon which courses ventrally and medially and gives rise to six main collateral branches which branch infrequently and extend beyond its own dendritic domain. These collaterals occupy a much larger area than those of the previously described medium spiny neuron (compare 35B to 35C). The parent axon of this spiny neuron has not been followed out of the neostriatum, as the HRP reaction products within the axon faded from view as it coursed within a fascicle toward GP.

Figure 35. Microscopic tracings of type I medium neurons labeled intracellularly with horseradish peroxidase.
A: Somatic and dendritic profile of a typical type I medium neuron, * mark the emergence points of the axon collaterals.
B: Axon collateral distribution pattern of the same cell shown in A. The dendrites are shown in dashed lines to demonstrate the relationship between the axon collaterals and the dendritic field of the same cell.
C: A less frequently encountered type I medium neuron with long intrastriatal collaterals. Note the similarities of the soma and dendritic appearances of the these two medium spiny neurons (A & C) and the differnce of their axon collateral distribution patterns.

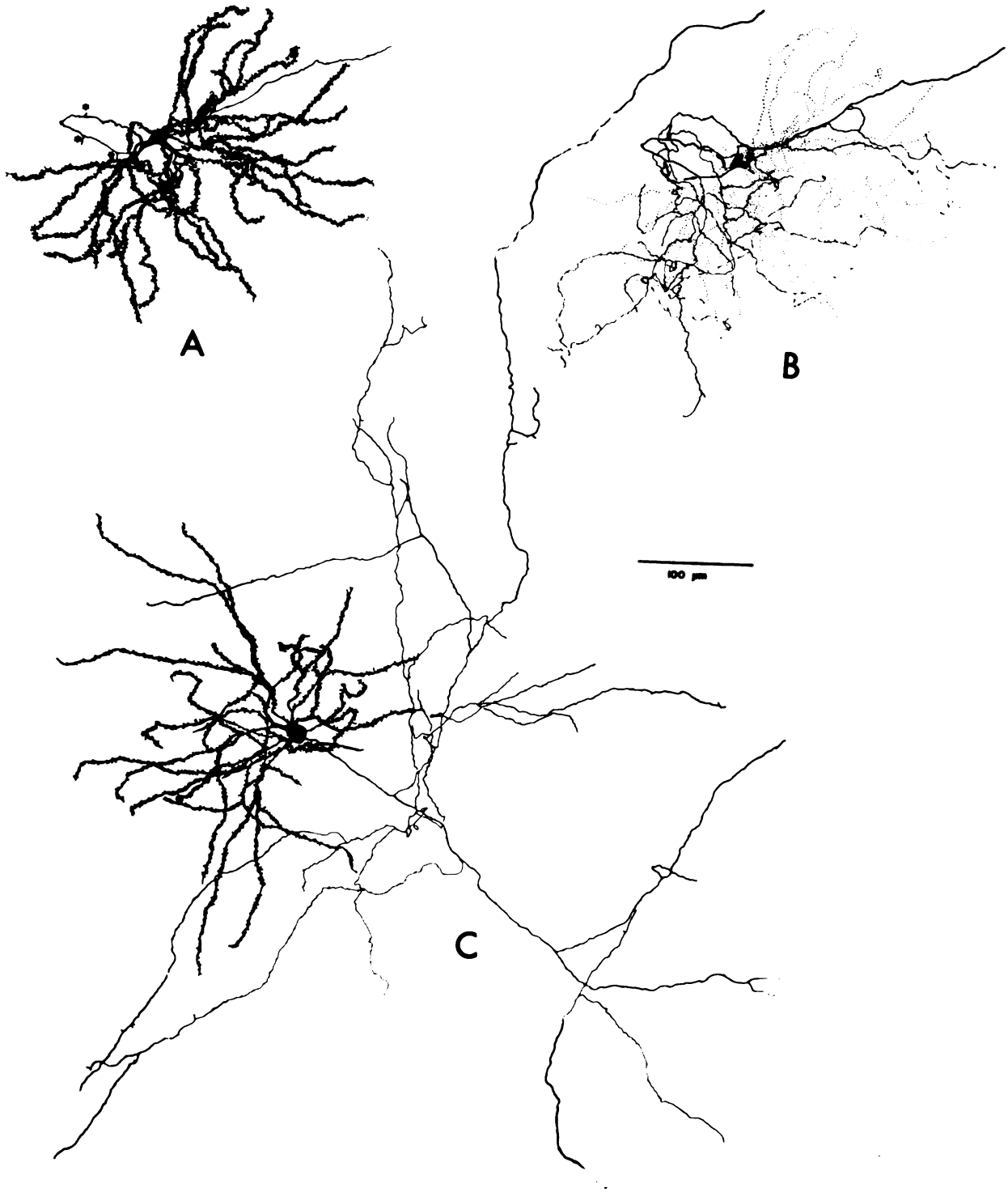

Figure 35

In addition to the differences observed with respect to the distribution of their intrinsic collaterals, it is also posssible to subdivide the medium type I neurons into two groups based on the differences in their efferent axonal morphology (e.g. pattern of projection to GP). In both groups of neurons, the main axon emitted several local collaterals in the striatum to form a complex intrinsic arborization near the dendritic domain of the neuron of origin.

In one group of cells (N = 7), the main efferent axons were unbranched throughout their course in the neostriatum, and gave rise to either none or only one or two thin collateral fibers along their course in GP. These observatios are in agreement with a previous light microscopic study of strio-pallidal axons (Preston et al., 1980). The main axons did not end in GP, but were observed to course caudally into the internal capsule. Some of the collateral fibers in GP became beaded in appearance, resembling "boutons en passant", but no elaborate axonal arborizations were seen. HRP reaction product within the thin collateral fibers often faded quickly beyond detection a short distance from the main axons.

In another group of neostriatal projection neurons (N = 6), the main axons divided within the neostriatum into two or three primary branches and these branches descend in a parallel course toward GP (Fig. 36A & 40A). Branching occured either close to the soma, or took place within the fiber bundles of the internal capsule. Upon entering GP, these branches immediately gave rise to a very localized but highly elaborate and overlapping axonal arborization in the rostral GP (Fig. 36B & 40B) Some primary axonal branches ended in this area while others continued caudally and then formed another

separate collateral terminal plexus in the caudal GP. These caudal axon arborizations also overlapped extensively. In the case shown in Figure 36A, one primary branch still continued caudally beyond GP into the internal capsule, but the HRP labeling within this axon became too faint to be traced any farther. The intrinsic axonal collateral arborization in the neostriatum and the terminal arborizations in both the rostral and the caudal GP appear to be similar in size, being approximately 400 μm in diameter.

Although all neurons of the second group showed similar basic pattern of axonal arborizations, the locations of the terminal fields in GP appeared to vary with location of the neuron of origin in the neostriatum. That is, more medially placed neostriatal cells gave rise to more medially placed fields in GP, and dorsally placed neurons arborized more dorsally in GP. This result agrees well with previous studies on the topographical organization of the neostriatal efferent system. However, no indication of a rostro-caudal organization was obtained, since all adequately labeled neurons of this group showed both rostral and caudal terminal fields in GP. No apparent differences were observed with respect to the spatial distribution of the cell bodies of these two groups (i.e. those with branched main axons vs. those with unbranched main axons) of medium type I neurons in the neostriatum.

Electron Microscopic Analysis of Intracellularly Labeled Type I Medium Neurons

Some of the type I medium neurons injected with HRP were not suitable for ultrastructural analysis because large amounts of HRP

Figure 36. Microscopic tracings of the axons of a type I medium neuron which projects into the globus pallidus.

A. The axon of a projection neuron is traced into the globus pallidus. The dendrites are omitted from the tracings to clarify the distribution of local intrinsic collaterals. Note the presence of two discrete terminal arborizations in the globus pallidus. CAL = 50 µm.

B. A higher magnification view of the rostral terminal field in the globus pallidus. The three main axonal branches are shown as solid, dashed and dotted lines to demonstrate the extent of their overlaps. CAL = 50 µm.

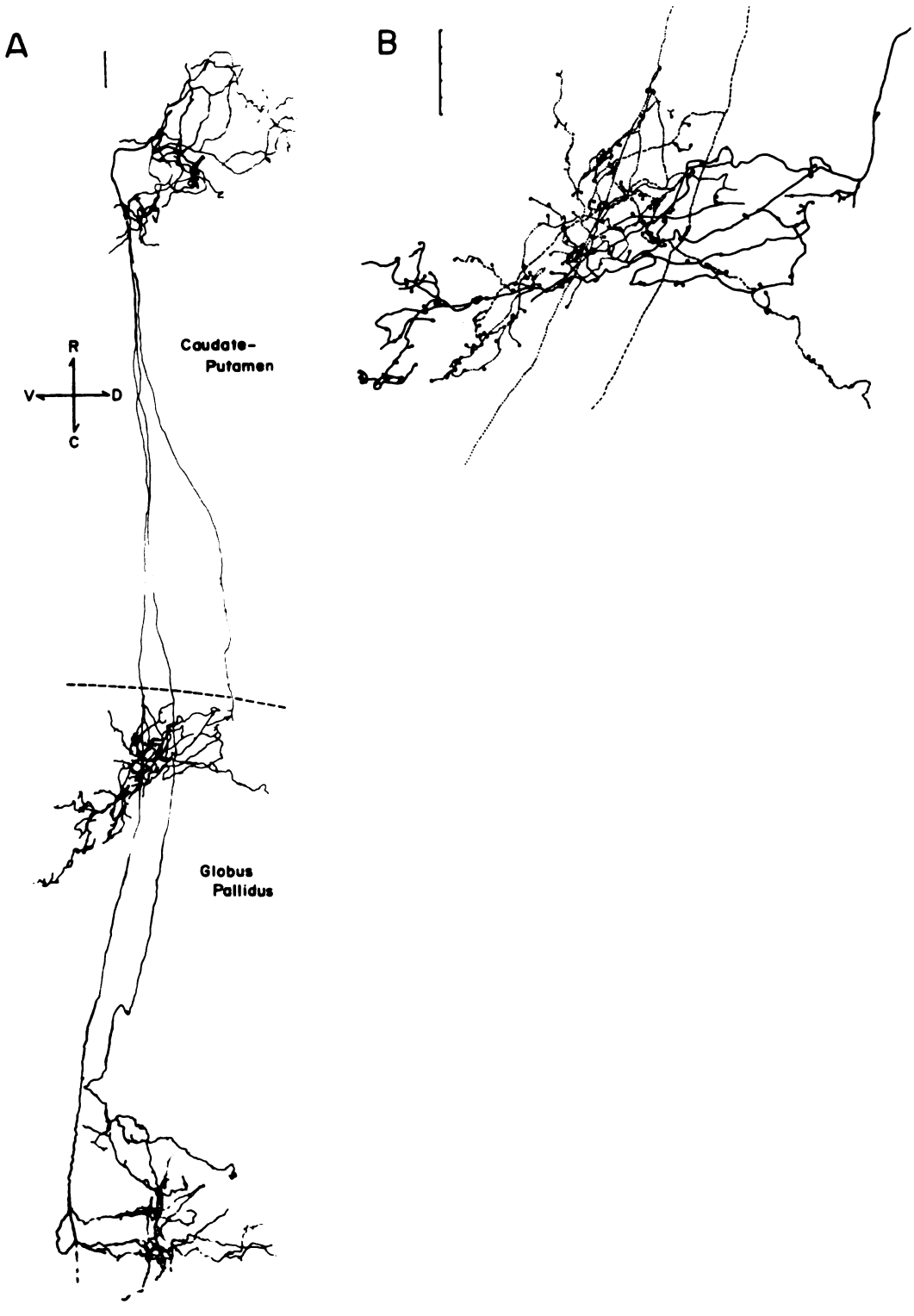

Figure 36

reaction product obscured the organelle morphology. When the nucleus was not penetrated by the intracellular electrode, the nucleus of the spiny I cell was found to be round and pale, without any noticeable nuclear membrane invaginations. On the other hand, in cases where the injected HRP was mostly restricted to the nucleus, the cytoplasmic detail could be analyzed (Fig. 37A & 37B). The relatively small perikarial cytoplasmic space formed a rim around the nucleus and contained abundant amount of free ribosomes, many in rosettes, and many mitochondria having densely packed cristae (Fig. 37D). The rough endoplasmic reticulum (ER) and smooth ER were not very numerous, and were rarely seen in stacked formations. Few Golgi complexes were present, but some appeared to be distended, possibly as a result of the HRP injection. A number of microtubules and some dense bodies resembling lipofuscin granules were distributed within the cytoplasm. Some cells also had a cilium (Fig. 37C) as had been observed by Rafols and Fox (1971/1972) in the monkey medium spiny neuron.

Axosomatic synapses were rarely seen, but when found were usually symmetrical synapses (Fig. 37D). At least two types of axosomatic terminals can be distinguished by the size of their vesicles. One type had large vesicles (Fig. 38A), the other had smaller vesicles (Fig. 38B). Subsurface cisterns next to the axosomatic synapses as described by Rafols & Fox (1971/1972) were rarely seen in our materials. Enlargement of astroglial processes around the soma of an injected cell was frequently observed (Fig. 37B) and this may reflect the physical damage caused by electrode penetration.

Figure 37. Electronmicrographs of the soma of a type I medium neuron intracellularly labeled with horseradish peroxidase.

A: Photomicrograph showing the soma and primary dendrites of the same cell shown in B. Magnification is approximately 1/4 of that in B.

B: Electronmicrograph of the soma of the same cell. The dark appearance of the nucleus (N) is probably due to accumulation of the HRP reaction products within the nuclear envelope. The slightly distended appearancce of the Golgi complex (G) and the perineuronal astrocytic processes (AS) may have been resulted from the recording microeletrode. The cytoplasm contain little rough or smooth endoplasmic reticulum, but has many free ribosomes (ri) and mitochondria. Block arrow points to a cilium of this neuron also shown in C. Arrow points to areas of axosomatic synapses and are also shown in D. CAL = 2 μm.

C: Higher magnification of the cilium (arrow). CAL = 1 μm.

D: Higher magnification of the axosomatic synapses (arrows) with symmetrical synaptic densities at all three synapses and the preterminals contain large pleomorphic vesicles. Also note that the rough endoplasmic reticulum (R) is not in stacked formation, but is surrounded with many free ribosomes (ri) and mitochondria (M) in the soma. CAL = 0.5 μm.

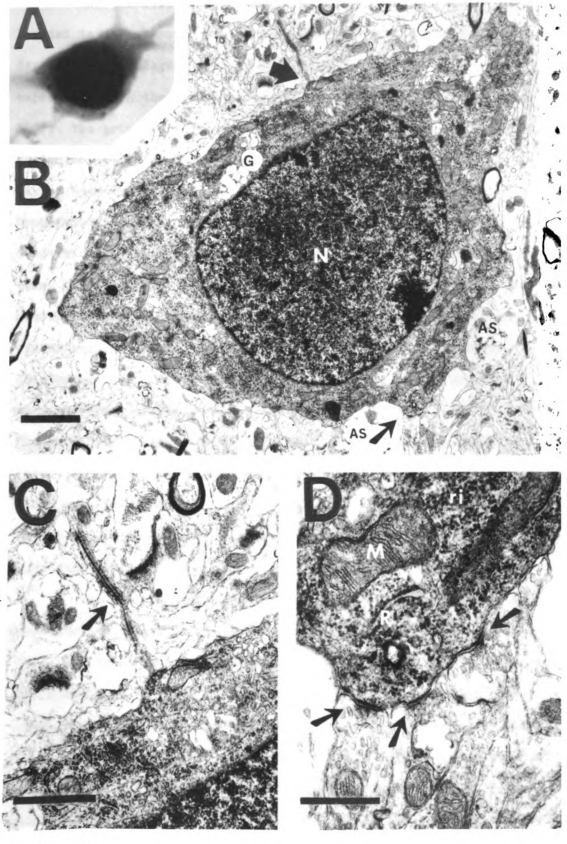

Figure 37

The HRP labeled spiny distal dendrites were usually easily located in the neuropil by the charateristic appearance of numerous dark spine appendages lined up along a dark dendritic shaft, especially when the dendrite was cut in longitudinal section (Fig. 38F). The primary dendrites, on the other hand, were smooth and were contacted mainly by preterminal containing large vesicles (Fig. 38D) making symmetrical synapses similar to those seen on the soma. Symmetrical axodendritic synapses were also occassionally seen on the dendritic shafts of the distal dendrites which bear spinous processes (Fig. 38E). However, the vast majority of the synapses on the distal dendrites were on the spines, which were contacted by preterminals containing medium round vesicles (Fig. 38G) and which probably originated from extrinsic sources as identified in the previous studies (Kemp & Powell, 1971b; Chung, Hassler & Wagner, 1978; Hassler, Chung, Rinne & Wagner, 1978; Hattori, Fibiger, McGeer & Maler, 1973). These terminals made asymmetrical synaptic contacts with the spines, which usually contained a spine apparatus. The dendritic shafts of the spiny I neurons were also characterized by their numerous microtubules and mitochondria (Fig. 38D, 38E, 38F & 38G).

The axon initial segments of the type I medium neurons were contacted by terminals containing large pleomorphic vesicles (Fig. 38C), and these ususally made symmetrical synapses. The intrinsic collaterals of the type I medium neurons in the neostriatum were unmyelinated, and made synaptic contacts of both "en passant" and "terminal" types. These boutons were usually filled with medium to large vesicles and appeared to make symmetrical contacts on dendritic shafts (Fig. 39C) and possibly the base of the spine stalk (Fig. 39D).

Occasionally, the synaptic vesicles could also be found within the narrow part of the collateral fibers, but no presynatic specializations were observed in these cases. The terminals of the intrastriatal collateral fibers of the type I medium neurons which ramify mainly outside the parent dendritic domain (Fig. 35C) also contained medium to large vesicles and appeared to make symmetrical contacts on the dendritic shafts of the neighboring neurons (Fig. 39E and 39F).

The main efferent axons of the type I medium neurons generally were thinly myelinated throughout their trajectory in the neostriatum and GP (Fig. 39A & 39B), and the collateral fibers within the GP were unmyelinated. Some of these neostriatal projection neurons responded antidromically following stimulation of the substantia nigra (SN), a known neostriatal target area distal to GP. These axons may thus represent the anatomical substrate for the inhibitory neostriatal axons projecting to both GP and SN. However, the branched efferent axons as shown in Figure 36A and 40A were thinly myelinated before branching in the neostriatum, but were unmyelinated subsequent to branching, both in neostriatum and in GP.

The neostriatal efferent plexus in GP consisted of thin unmyelinated fibers and boutons of both the "en passant" and "terminaux" types (Fig. 36B & 40B). These boutons, containing large and moderately pleomorphic vesicles (Fig. 40C), formed symmetrical synaptic junctions with pallidal dendrites of all sizes, including the occasional dendritic spines found on some dendrites (Fig. 41). Most of the synapses were formed onto the small to medium sized dendrites which predominate in the neuropil of GP. The ultrastructural

morphology of these HRP labeled boutons in GP appeared to be identical to the labeled intrinsic terminals of medium type I neurons in the neostriatum.

Analysis of serial thin sections through individual boutons demonstrated that every labeled bouton formed only one synapse with one postsynaptic element. On the other hand, two or more labeled boutons arising from the same axon collateral were often seen contacting the same pallidal dendrite, thus confirming a degree of the "longitudinal axo-dendritic" organization of the strio-pallidal connections as postulated from light microscopic analysis of Golgi impregnated materials (Fox & Rafols, 1975, 1976). However, even in areas of their highest density as shown in light microscopic reconstructed drawings, the labeled boutons represented only a minute fraction of the total number of boutons with similar strio-pallidal morphology, indicating a high degree of convergence of neostriatal axons upon pallidal neurons.

Figure 38. Electronmicrographs of synapses on soma, dendrites and axon initial segment of a type I medium neuron intracellularly labeled with horseradish peroxidase. A, B, C, D & E are at the same magnification. CAL in E = 0.2 μm.

A & B: Axosomatic synapses (arrows) on the cell shown in Figure 37 showing the difference in synaptic vesicle sizes of these two terminals.

C: Synapse (arrow) on the axon initial segment (is).

D: Synapse (arrow) on the primary dendrite (d).

E: Synapse (arrow) on the dendritic shaft of a spine-bearing distal dendrite(d). Emergence of the spine (s) is shown at the lower right hand corner.

F: Low magnification view of a longitudinally sectioned spiny distal dendrite darkly labeled with HRP. CAL = 1 μm.

G: Higher magnification of a lightly labeled spiny dendrite (d) showing the spine (s) containing stacks of spine apparatus. The axospinous synapses (arrows) are asymmetrical and the preterminal contains medium vesicles. For comparison, the blocked arrow points to another axospinous synapse on an unlabeled spine head. The arrow head points to an axodendritic contact which is out of the plane of this section. CAL = 0.25 μm.

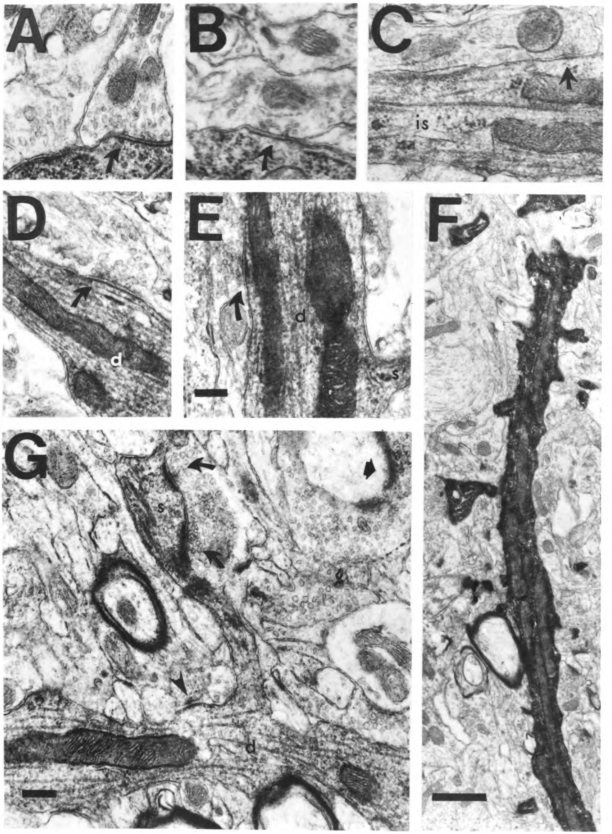

Figure 38

Figure 39. Electronmicrographs of the axons and intrinsic terminals of a type I medium neuron intracellularly labeled with horseradish peroxidase.
All at the same magnification, CAL = 0.5 μm.

A: The myelinated axon of a type I medium neuron, within the neuropil of the neostriatum.

B: The myelinated axon of a type I medium neuron in the fiber bundles at the lateral part of the globus pallidus.

C: Local axon collateral of a type I neuron makes an axodendritic synapse (arrow) on the dendritic shaft (d) of a neighboring neostriatal neuron. Note that the synaptic complex is symmetrical, and the preterminal contains large oval vesicles.

D: Another axodendritic synapse by the axon collateral of a type I medium neuron, the synapse appears to be at the base of a spine neck (arrow) of a dendrite (d). The block arrow points to the unmyelinated axon collateral fiber.

E & F: HRP labeled axon collateral terminals of the type I neuron shown in Figure 35C. Arrows point to the symmetrical contacts with the dendritic shafts (d) of neighboring neurons.

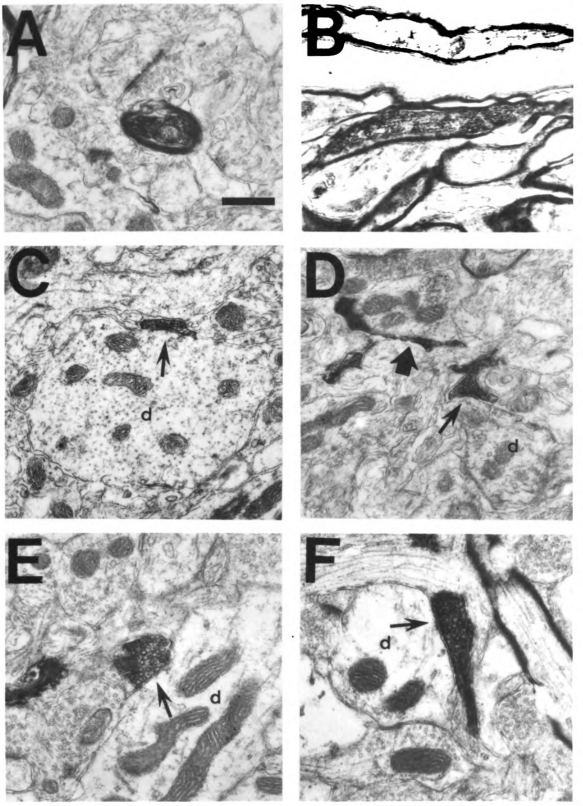

Figure 39

Figure 40. Photo- and electronmicrographs of the efferent axon of a type I medium neuron and its terminal arborization in the globus pallidus.

A: Photomicrograph of the first branching of the main axon of the neuron shown in Figure 36A. Although the branch forms at right angles to the parent axon, it immediately turns to take a parallel course toward the globus pallidus. The varicosity near the branch point is not presynaptic. CAL = 2 μm.

B: Photomicrograph of a portion of the rostral terminal arborization in the globus pallidus shown in Figure 36B. Arrows indicate the positions of electronmicroscopically identified presynaptic terminals such as shown in C. Same magnification as in A.

C: Electronmicrograph of a presynaptic bouton from an intracellularly labeled axon of a strio-pallidal neuron. The synaptic junctional complex appears to be symmetrical. The postsynaptic target is a small pallidal dendrite which also receives other unlabeled boutons, some of which (open arrows) exhibit morphology similar to that of the labeled terminal, while others (closed arrow) contain smaller vesicles and form asymmetrical synapses. CAL = 0.5 μm.

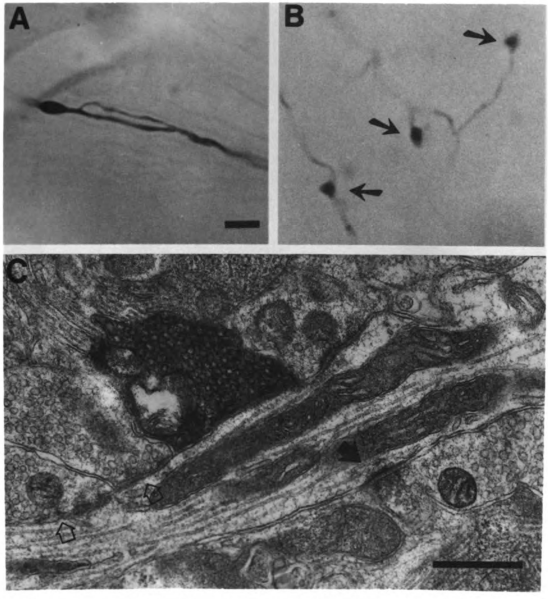

Figure 40

Figure 41. The distribution of the diameters of the pallidal dendrites which are postsynaptic to a neostriatal type I medium neuron.
Dendritic diameters were measured from single sections at the site of contacts with the labeled axon terminals, and for obliquely sectioned dendrites were taken perpendicular to the axis of elongation as indicated by the orientation of the microtubules. Most synapses are formed on small dendritic shafts less than 2 μm in diameters, although all areas of somato-dendritic surface of pallidal neurons can receive strio-pallidal synapses.

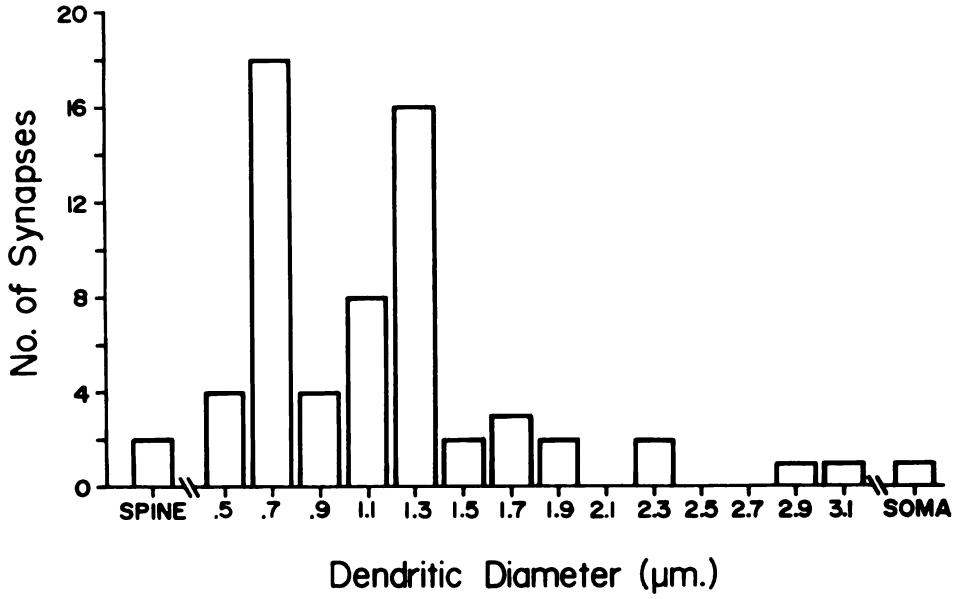
20
16
12
8
4
0
No. of Synapses
SPINE
.5
.7
.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
2.7
2.9
3.1
SOMA
Dendritic Diameter (µm.)

Figure 41

E. Somatic Size of Rat Neostriatal Neurons

Although somatic size has often been used as the main criteria for preliminary classification of neostriatal neurons in many clinical as well as experimental studies, analysis of the somatic size distribution of different types of neostriatal neurons as defined in this study reveals that somatic size alone is not a reliable parameter for predicting the dendritic or axonal morphology of any individual neostriatal neuron. Since the capriciousness of the Golgi impregnation prevents quantitative analysis of the population of different types of neurons within any nucleus, a distribution histogram showing the somatic size of all neostriatal neurons impregnated in our Golgi material would be skewed unavoidably to that of the type I medium neurons, the most frequently impregnated neurons in our preparation. Thus the somatic size of type I medium neurons (Fig. 42B) have been plotted separately from that of all other types of neostriatal neurons (Fig. 42C). The somatic size distribution of the neostriatal projection neurons labeled retrogradely with HRP extracellularly injected in the globus pallidus is also shown in Figure 42A. These histograms indicate that there is considerable overlap between the somatic size of the projection neurons with that of the type I medium neurons. However, the somata of many non-type I medium neurons also are within the size range of the projection neurons. Since the light microscope could not resolve adequately the distribution of dendritic spines of the HRP back-filled projection neurons, as mentioned earlier, it is premature to rule out the other medium neurons as projection neurons. Most large neurons, however, are probably not projection neuron (compare Fig. 42A and 42C).

Figure 42. The distribution of somatic sizes of rat neostriatal projections neurons labeled retrogradely with horseradish peroxidase extracellularly injected in the globus pallidus, and the somatic sizes of neostriatal neurons impregnated by the rapid Golgi method.

A. Distribution of somatic size of neostriatal projection neurons labeled retrogradely from HRP injections in the globus pallidus. The tissue sections have been dehydrated in alcohol and cleared in xylene. The ordinate scale is relative, total cells = 68.

B. Distribution of somatic size of type I medium neurons impregnated by the rapid Golgi method. The tissue sections have also been dehydrated in alcohol and cleared in xylene. The ordinate is relative, total cells measured = 100.

C. Distribution of somatic size of rapid Golgi impregnated neostriatal neurons which are not type I medium neurons. The tissue sections have been cleared in glycerin. Total number of cell analyzed = 69.

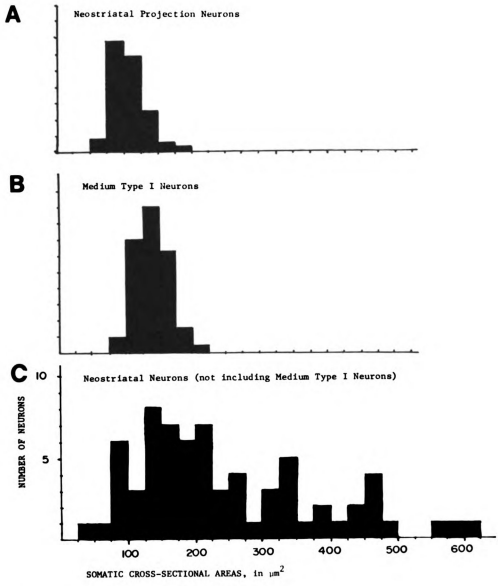
A
Neostriatal Projection Neurons
B
Medium Type I Neurons
C
Neostriatal Neurons (not including Medium Type I Neurons)
NUMBER OF NEURONS
10
5
100
200
300
400
500
600
SOMATIC CROSS-SECTIONAL AREAS, in μm²

Figure 42

Chapter 4. DISSCUSSION

A. Comparison of Classification Systems

Naming the rat neostriatal neuron types as type I, II and so on in this study has elimated the ambiguous and often misleading terms such as "stellate", "spidery", "aspiny", "less-spiny", "smooth" and so on in the designation of neuron types which existed in most of the previous Golgi studies (e.g. Mensah & Deadwyler, 1974; Lu & Brown, 1977; Danner & Pfister, 1979a, b). Since most of these reports provided only free-hand drawings (Mensah & Deadwyler, 1974; Lu & Brown, 1977) or partial photomicrographs (Danner & Pfister, 1979a, b), their published results were inevitably inadequate for comparison with each other and with those reports on other species which provided detailed camera lucida drawings (Kemp & Powell, 1971a, DiFiglia et al., 1976; Tanaka, 1980). A recent Golgi study of the rat neostriatal neurons has classified neurons into type I, II III and so on with accompanying camera lucida tracings (Dimova et al., 1980). However, no photomicrographs were provided in that particular study and the morphology of the axons was not described. The present study, therefore, extends the findings of Dimova et al. (1980) by providing both photomicrographic and camera lucida illustrations of different types of rat neostriatal neurons. Additionally, the ranges of quantitative values for several morphological parameters (e.g. somatic cross-sectional areas, number of dendritic stems vs. dendritic tips, etc.) have been described in the text. Since several new cell types as well as the axon morphology of various types of neurons have been found in this study, I have modified the classification system

provided by Dimova et al. (1980) in order to accomodate these new observations.

Idealy, anatomical classification of neurons should be based on the morphological characteristics of all parts of a neuron. However, since the axons are often incompletely impregnated in Golgi preparations, the somato-dendritic features have been the most important and reliable criteria used in analysis of neuronal morphology in the present and most of the other Golgi studies. Detailed somato-dendritic morphological descriptions are especially important for interpretating the results of the recently developed anatomical techniques, such as immunocytochemistry or retrograde labelings, which enable partial labeling of somata and dendrites. The axonal distribution patterns of indidvidual neurons have been analyzed whenever possible with the understanding that neurons with similar somato-dendritic morphology may have different axonal distribution patterns as illustrated by the type I medium neurons labeled intracellularly with horseradish peroxidase. A summary of the morphology of the different types of rat neostriatal neurons observed in this study is listed in Table 1.

As has been mentioned earlier in the INTRODUCTION, previous Golgi studies in other species have used rather descriptive terms for classifying the neostriatal neurons. Thus, found in the cat are medium spiny neurons with short axons, large and medium neurons with long axon, medium neurons with varicose or smooth dendrites and so on (Kemp & Powell, 1971a). However, since the presence of long axons has never been clearly demonstrated in that study, little distinction can be made between the medium long-axon cells and the medium smooth cells

Table 1. Summary of rat neostriatal neuronal types

	SOMA SIZE*	SOMA SURFACE	DENDRITES PROXIMAL**	DENDRITES DISTAL	AXONAL MORPHOLOGY***
LARGE					
I	L	SMOOTH	SPARSELY-SPINED or SMOOTH	SPARSELY-SPINED LONG DENDRITES, MAY BE VARICOSE DISTALLY.	HAS LOCAL COLLATERALS, HAS MYELINATED MAIN AXON.
II	L	w/SPINES	SPARSELY-SPINED	SPARSELY-SPINED DENDRITES, NO VARICOSE PROCESSES.	HAS MYELINATED MAIN AXON.
MEDIUM					
I	M	SMOOTH	SMOOTH	DENSELY COVERED WITH SPINES.	HAS MANY LOCAL COLLATERALS, AND MYELINATED MAIN AXON.
II	M	w/SPINES	SPARSELY-SPINED	SPARSELY-SPINED, SOME DENDRITES FORM POLYMORPHIC TERMINAL BRANCHES IN FASCICLES OF INTERNAL CAPSULE.	HAS LOCAL COLLATERALS.
III	M	SMOOTH	SMOOTH or SPARSELY-SPINED	SPARSELY-SPINED, POORLY-BRANCHED NON-VARICOSE DENDRITES.	HAS LOCAL COLLATERALS
IV	M	SMOOTH	SMOOTH or SPARSELY-SPINED	SPARSELY-SPINED, PROFUSELY-BRANCHED NON-VARICOSE DENDRITES.	HAS MYELINATED MAIN AXON, HAS LOCAL COLLATERALS.
V	M	SMOOTH	SMOOTH or SPARSELY-SPINED	SMOOTH or SPARSELY-SPINED, VARICOSE RADIATING DENDRITES.	HAS UNMYELINATED MAIN AXON, HAS MANY LOCAL COLLATERALS.
SMALL	S	SMOOTH or w/SPINES	SMOOTH or SPARSELY-SPINED	SPARSELY-SPINED, OFTEN HAS LONG DENDRITIC APPENDAGES.	HAS LOCAL COLLATERALS

* SOMA SIZE: L= larger than 300 μm^2; M= between 100 & 200 μm^2; S= less than 100 μm^2.
** PROXIMAL DENDRITES: include all those dendrites less than 20 μm away from the soma.
*** Data are based on both light and electron microscopic analysis of either Golgi impregnated or intracellularly labeled material.

based on their somato-dendritic morphology. Moreover, since the medium spiny neurons have been shown to be projection neurons, the "short-axon" title is no longer applicable.

Fox and his colleagues (1971/1972a, b, 1974) described the presence of spiny neurons and large aspiny neurons, spidery (medium) aspiny neurons and aspiny neurons with somatic spines in the monkey neostriatum. However, what exactly constitutes spidery remains unclear. A recent review by Rafols & Fox (1979) reclassified the monkey neostriatal neurons into six types: 1) medium neurons with many dendritic spines, 2) medium neurons with fewer dendritic spines, 3) large neurons with many dendrites, 4) large neurons with fewer dendrites, 5) small aspiny neurons, and 6) medium neurons with radiating beaded dendrites. Although this division of neurons based on their somato-dendritic morphology is similar to our present approach, naming each type with a descriptive title has the aforementioned disadvantage of ambiguity and can be misleading. For example, the "small aspiny neurons" included subgroups of neurons with medium somata, as well as neurons with either smooth or varicose dendrites, or dendrites with long-stalked beaded appendages and axons indicative of "local circuit neurons" (Rafols & Fox, 1979). However, since the medium neurons with radiating beaded dendrites also have varicose dendrites and locally arborizing axons, the distinction between these two types of cells becomes unclear.

On the other hand, the Pasiks and DiFiglia classified the monkey neostriatal neurons into Spiny I, Spiny II, Aspiny I, Aspiny II and Aspiny III cells, and they considered the Aspiny cells to be intrinsic cells and the Spiny I and II cells to be projcetion neurons

(DiFiglia et al., 1976; Pasik et al., 1979). However, since spines or dendritic appendages have been found on both Aspiny II and Aspiny III cells (Pasik et al., 1979), are these, therefore, Aspiny cells with spines? Since Fox's large aspiny cell is considered to be a large Spiny II cell (Pasik et al., 1979), then it must be a Spiny II cell without spines! By their classification criteria, then, if a sparsely-spined neuron had a long axon, it would be a Spiny II cell, otherwise it would be an Aspiny III cell. However, no definition has been provided for "long axon" other than the unwritten understanding that it is synonymous with efferent axon. Additionally, neostriatal efferent axons with accompanying somata and dendrites have never been clearly demonstrated in a Golgi study.

The above discussion demonstrates that most descriptive terms are not suitable for naming neuron types. On the other hand, since the somatic size is a measurable parameter and can be easily correlated with histological studies from different laboratories, the descriptive terms such as "large", "medium" and "small" have been used in the present study and their values have been clearly defined in order to better correlate with previous histological studies of the neostriatum. Since these definitions have artificial boundaries, several neurons with somatic sizes at the boundary regions have been found in this study. The classification of these neurons would then rely more on their other morphological characteristics.

The relationships of the present neuronal classification to those of previous Golgi studies is summarized in Table 2. In addition to establishing the morphological characteristics of different neuron types from the Golgi material, both extracellularly and

intracellularly labeled neurons have been included in this study in order to determine the morphology of the neostriatal projection neurons and to analyze the morphology of neurons which have been characterized physiologically by intracellular recordings. Further detailed comparisons of these observations are discussed below.

Table 2. Comparison of present classification with previous Golgi studies of the neostriatal neurons.

		LARGE		MEDIUM					SMALL
		I	II	I	II	III	IV	V	
	Dimova et al. 1980	Type 5	?	Type 1	?	Type 2 & 4?	Type 2?	Type 3	?
RAT	Danner & Pfister 1979a, b	Type 3	?	Type 1	?	Type 2 & 5?	Type 5?	Type 4?	?
RAT	Lu & Brown 1977	Large	?	Medium Spidery; Spindle; Long axon;	?	Medium w/ long dendrites	?	Medium w/varicose dendrites	Small?
RAT	Mensah & Deadwyler 1974	Large	?	Medium Stellate	?	Medium Smooth or long axon?	?	Medium w/varicose dendrites	Small?
CAT	Kemp & Powell 1971	Large	?	Medium Spiny	?	Medium w/ smooth dendrites; or w/long axon?	?	Medium w/varicose dendrites	?
DOG	Tanaka 1980	Aspiny II	Large Spiny II ?	Spiny I & Medium Spiny II	?	Aspiny III & Medium Spiny II?	Aspiny I?	Aspiny I?	?
MONKEY	Fox et al.* Rafols & Fox, 1979	Large Aspiny	?	Spiny	Aspiny w/somatic spines	Medium w/few dendritic spines	Spidery Aspiny	Medium w/ radiating varicose dendrites	?
MONKEY	DiFiglia et al. 1976	Aspiny II	Large Spiny II	Spiny I	Spiny II	Aspiny III	Aspiny I?	Aspiny I?	?

* Fox et al. references are from 1971/1972a, b and 1974.

B. Light Microscopic Morphology of Rat Neostriatal Neurons

Large Type I Neurons

Previous Golgi studies have described at least two varieties of large neostriatal neurons with aspinous soma, both of which are represented by the type I large cells in this study. One of these, represented by the neuron shown in Figure 1C in this study, is characterized by fusiform or triangular soma with long and often poorly branched dendrites, similar to the giant neurons described in the human (Ramón y Cajal, 1911), the cat (Kemp & Powell, 1971), the monkey (Fox et al., 1971/1972) and the rat (Danner & Pfister, 1979a & 1979b). The dendrites may be sparsely-spined, thus resembles the giant spiny II cells found in the monkey (DiFiglia, Pasik & Pasik, 1976; Pasik, Pasik & DiFiglia, 1976). These cells have been proposed to be projection cells by many authors based on the observations either that they have myelinated axon (Fox et al., 1971/1972) or that they have long axons (although none have been convincingly illustrated) which do not decrease in diameter (Kemp & Powell, 1971; DiFiglia et al., 1976; Danner & Pfister, 1979a, b). However, none of these cells have axons which have been actually traced out of the neostriatum.

The other group, represented by the neuron shown in Figure 1A and 2A in this study, is characterized by polygonal or fusiform soma with radiating dendrites which often become varicose in appearance and, in some instances, appear to swirl or revolve around the soma. These resemble the aspiny II cells in the monkey (DiFiglia et al., 1976) and the dog (Tanaka, 1980), or the giant neuron with many

dendrites in the monkey (Rafols & Fox, 1979), and the giant neurons in the rat (Mensah & Deadwyler, 1974; Lu & Brown, 1977; Dimova, Vuillet & Seite, 1980). The Pasiks and DiFiglia (1977) considered these to be interneurons based on the observation that their axons arborize locally near the parent somata. Additionally, large fusiform neostriatal neurons have been suggested to be cholinergic or cholinesterase-containing interneurons based on histochemical and immunocytochemical studies (Butcher & Bilezikjian, 1975; Kimura et al., 1980). However, since only partial somato-dendritic morphological features are revealed in these studies, it is unclear how these cholinergic cells are related to the large cell types described in this and previous Golgi studies.

Nevertheless, some large neostriatal cells have been demonstrated to be projection neurons in studies with retrograde axonal transport of HRP extracellularly injected in the midbrain (Grofova, 1978) or the auditory cortex in the cat (Jayaraman, 1980). Additionally, herpes simplex virus injected in the substantia nigra resulted in their uptake in large neostriatal neurons (Bak et al., 1978). Since these studies did not reveal enough somato-dendritic morphology, their identity remains unclear.

In the present study, we have observed that a large aspinous neuron with long and slightly varicose dendrites has locally arborizing axon collaterals (Fig. 2B), thus confirming the observation made in the baby monkey that some large neostriatal neurons have local axonal arborization (Pasik et al., 1977). Although this neuron obviously has intrinsic integrative functions of an interneuron, we cannot say that it does not have an unimpregnated projecting axon.

The only large neuron labeled intracellularly with HRP in this study has aspinous soma and dendrites, the dendrites being varicose distally. Its main axon becomes myelinated and no axon collaterals have been observed for the length over which the main axon could be traced (up to 200 μm from the soma). We cannot rule out, however, the possiblity that thin collaterals either very faintly labeled or not labeled with HRP may arise somewhere distally. Since no somatic spines have been observed at either light or electron microscopic levels, this intracellularly labeled neuron is considered as a type I large neuron as defined in this study.

Since the large neurons with smooth somata in our sample have more similarities than differences with respect to their somatic and dendritic morphology (e.g. they all have radiating, sparsely-spined dendrites which may or may not be varicose distally), they are all classified as type I large cells in this study. Further subdivisions into projection or intrinsic neurons will have to await future studies that can better demonstrate their complete axonal distribution patterns.

Type II Large Neurons

A distinguishing feature of type II large neurons is the presence of spines on their somata and proximal dendrites, indicating functional differences from the type I large cells with smooth somata.

Only one other laboratory has reported a similar type of large neostriatal neurons with somatic spines, the giant version of the Spiny II cells in the monkey (DiFiflia et al., 1976; Pasik et al.,

1976). These cells are reported to have long axons and are suggested to be projection neurons. However, since the somatic and dendritic morphological descriptions of Spiny II cells include virtually all sparsely-spined neurons (i.e. including features of both the large type II and the medium type II, III and IV neurons described in in this study), it would be difficult to distinguish the rat neostriatal neurons if we were to use this classification system originally proposed for the monkey. [See also review by Pasik et al.,(1979) in which the spiny II cells are correlated with many cells without somatic spines described in other species].

Although there are undeniably striking similarities between the type II large neurons and the type II medium neurons in this study, grouping these cells into two separate types according to their somatic size has the advantage of clarity and consistency in comparing these cells with other rat neostriatal neurons with similar somatic sizes.

In this study we are unable to demonstrate the existence of long axons of these large cells at the light microscopic level. However, electron microscopic analysis has shown that the large type II neuron shown in Figure 3A has a myelinated axon (Fig. 18B), suggesting that at least some of these cells have long axons. Whether they project out of the nucleus remains to be determined.

Type I Medium Neurons

The morphology of the type I medium (spiny) neurons, the most frequently encountered neurons in our material, has been well documented in previous Golgi studies as well as intracellular HRP

labeling experiments. In this study, one of the key features that characterize the morphology of this type of neuron, the density and the distribution of the spines, is found to vary noticeably. In addition, the axonal distribution patterns of these cells are demonstrated to be more complex than previously assumed.

Most of the medium spiny neurons have smooth soma and proximal dendrites, whereas the distal dendrites are densely studded with spines of all sizes and shapes, giving rise to a "bottle-brush" appearance. Several neurons (e.g. Fig. 5B), however, have relatively fewer spines than the majority of the medium spiny cells, yet the characteristic restriction of spines to the distal dendrites is still apparent. The morphology of these latter, spine-poor cells, appears to be similar to the medium spindle cells, a type of spiny cells with fewer dendritic spines described in the rat neostriatum by Lu & Brown (1977). They found significant differences in the density of the dendritic spine distribution between two populations of medium spiny cells and classified them as two different types of neurons. We are unable to confirm their observation at this point because of the overwhelming majority of medium spiny neurons in our material are the spine-rich variety, with "bottle-brush" distal dendrites. It is likely that the most of the medium spiny neurons have densely-spined distal dendrites, but some have fewer spines than others, and may represent members of a continuous spectrum of medium spiny neurons with different numbers of dendritic spines.

As has been mentioned earlier, we have observed in our intracellular labeling studies that several type I medium neurons have very different axonal distribution patterns. Many type I medium

neurons have local axon collaterals which arborize extensively mainly within the parent dendritic domain (e.g. Fig. 35B), indicating very localized intrinsic integrative activities. Less frequently, however, the intrinsic collaterals of these cells extend far away from the parent dendritic field (e.g. Fig. 35C), indicating a wider intrinsic influence of this neuron. It is difficult to state with any certainty the relative number of these two subclasses of type I medium neurons at this time. Indeed, it is probable that they, too, are just disparate examples of a continuum of axonal arborization patterns.

It is also possible to subdivide the type I medium neurons based on the distribution patterns of their efferent axons. Certain neurons appear to preferentially arborize extensively in the globus pallidus, while others appear to bypass the globus pallidus, indicating the presence of functionally different efferent neurons in the neostriatum with similar somato-dendritic morphology (i.e. type I medium neurons). These cells may represent the neurochemically-different neostriatal efferent neurons suggested in previous studies, such that striopallidal fibers convey enkephalin and GABA, whereas the strionigral fibers convey substance P and GABA (see INTRODUCTION).

Type II Medium Neurons

The type II medium neurons are characterized by the presence of somatic spines. These neurons are similar to the medium Spiny II cells (DiFiglia et al., 1976; Pasik et al., 1976) or the aspiny cells with somatic spines in the monkey (Fox et al., 1974). Since the spines are sparsely distributed over the entire length of the dendrites, they resemble the medium long axon cells described in the

cat (Kemp & Powell, 1971). Although the overall morphology of these neurons resembles the type II large neurons described in this study, and it may be argued that somtic size is not a critical factor in determining cell types, nonetheless, for the sake of ease in reference to previous studies of different species, they are placed in their own category.

Since the axons of these cells are not impregnated except for a portion of the initial segments, we are unable to confirm the existence of the long axons as reported by other authors in the cat (Kemp & Powell, 1971) and the monkey (DiFiglia et al., 1976). We have found a morphological feature previously unreported: some cells have polymorphic terminal dendritic processes (Fig. 4E) within the fiber bundles of the internal capsule which pass through the rodent neostriatum. These specialized dendritic endings could form synapses with fibers within the internal capsule. Whether they are pre- or postsynaptic to the capsular fibers should be the subject of further ultrastructural investigations.

Type III Medium Neurons

The type III medium neurons described in this study have the appearance of medium spiny neurons without the characteristic spines. They are aspiny or sparsely-spined cells, with only a few spines scattered on the radially directed dendrites. Similar neurons have been described in the rat (possibly the medium cell with long dendrites, 7e, of Lu & Brown, 1977; type II or the type IV medium neuron of Dimova et al., 1980), in the cat (medium smooth cell of Kemp & Powell, 1971), and in the monkey (Aspiny III of DiFiglia et al.,

1976). On some of these cells, we have found specialized dendritic endings in the internal capsule fiber bundle, similar to those observed on the type II medium cells.

Although the aspiny III cells of the monkey are said to be a type of intrinsic neurons with short axons (DiFiglia et al., 1976; Pasik et al., 1976; Pasik et al., 1979) and the medium smooth cell in the cat has locally arborizing axons (Kemp & Powell, 1971a), only one of the type III medium cells (Fig. 7B) has been found to have a locally arborizing axon in this study. Whether this is indeed the general pattern of axonal distribution of most type III cells remains unclear.

A few medium neostriatal neurons with very sparsely-spined dendrites have one or two visible somatic spines (Fig. 7D, 13G). However, since their overall appearance resembles that of other type III cells (Fig. 7A) rather than that of type II medium neurons (Fig. 4C, 4F), they are classified as medium type III cells in this study. The logic of this decision is similar to the conclusion reached by Fox et al. (1974) who have observed occassional somatic spines on the medium spiny neurons (i.e. type I medium neurons) which normally do not bear somatic spines in the monkey neostriatum. They, therefore, suggest that the presence of several or more somatic spines would be necessary to determine a neuron as an aspiny cell with many somatic spines in the monkey neostriatum (i.e. the equivalent of the type II medium neuron in the rat).

Type IV Medium Neurons

These neurons have smooth somata and many smooth or sparsely-spined dendrites. They are distinguished from the type III neurons by their profusedly-branching dendrites. This dendritic branching pattern resembles the spidery aspiny cell in the monkey (Fox et al., 1971/1972) or the medium cells with varicose dendrites in the cat (Kemp & Powell, 1971) and in the monkey (Aspiny I of DiFiglia et al., 1976). However, since the dendrites of the type IV medium neurons are rarely varicose in appearance as compared to those of the type V medium neurons in this study, only tentative correlation of these type IV cells with those neurons with varicose dendrites described in the cat or the monkey can be made at this time. Since electron microscopic analysis has revealed that the axon of the type IV neuron shown in Figure 8A and 9A is myelinated, suggesting that it has a long axon, it is likely that the counterparts of these neurons have not been described before. Only one medium type IV cell has an impregnated axon with beaded local collaterals, however, the complete picture of the axonal distribution patterns of this neuron type remains unclear, and it is premature to conclude whether it is a type of projection neuron or not.

Some cells have specialized terminal dendritic processes in the internal capsule bundles. Similar dendritic branches have not been described in previous studies. Whether this merely reflects species differences (i.e. internal capsule fibers do not pass through the cat or monkey neostriatum) or not remains unclear. Electron microscopic analysis has revealed that most of these processes are postsynaptic. Further investigations with either lesion-degneration

or orthograde-tracing techniques will be needed to establish the source of the afferents which terminate on these dendritic processes within the internal capsule bundles.

Type V Medium Neurons

The fifth type of medium neurons has smooth soma and aspinous varicose dendrites. Based on these varicosities alone, this cell type resembles the cell with varicose dendrites in the cat (Kemp & Powell, 1971) and the Aspiny I cells of the monkey (DiFiglia et al., 1976). However, the size and the branching pattern of its dendritic fields are much different from those of these two species. The type V medium neurons in the rat have radiating, stellate dendritic field extending up to 250 μm away from the soma, while in the cat and the monkey they have swirly dendritic branching patterns and shorter dendrites (120 μm in the cat, 150 μm for the aspiny I in the monkey) terminating closer to the soma. Recently, medium neurons with radiating beaded dendrites were reported in the monkey (Rafols & Fox, 1979) and the rat (Lu & Brown ,1977; Dimova et al. 1980) with similar soma and dendritic morphology to the type V cells in this study.

Regardless of what their counterparts are in other species, these cell in the rat neostriatum share similar axonal distribution patterns to most of the previously reported aspinous cells with varicose dendrites. The axons of these cells branch repeatly, forming a dense arborization of fine beaded axons within and near the parent dendritic fields. None of the branches can be traced away from the local arborization. However, since the projecting axons can easily be unimpregnated (as demonstrated by the debate on the identity of the

medium spiny cells as projection neurons in the recent past), it would be premature to consider these as only local circuit neurons at this point. Nevertheless, the distribution of the visible axons clearly indicates a very localized and focused influence, thus serving the function of a classical interneuron.

Small Neurons & Neurogliform Cells

Except that of Ramón y Cajal (1911), no other Golgi study has clearly demonstrated the presence of small neostriatal neurons with a defined axon. This has given rise to much controversy with respect to the identication of neurogliform or dwarf neurons in modern Golgi studies. Fox et al. (1974) considered their "aspiny neurons with somatic spines" to be good candidates for the dwarf or the neurogliform neurons described by Ramón y Cajal (1911). However, since the type II medium neurons with somatic spines in this study appear to be homologous to those described by Fox et al. (1974) in the monkey, it is unlikely that these are the dwarf cells in the rat. Instead, the small neuron with locally arborizing axon shown in Figure 7C is probably more closely related to the dwarf, short axon cell described by Ramón y Cajal (his Fig. 325E, 1911). This cell, clearly not glia-like, has a small round soma with a few short dendrites. It is interesting to note the similarity of this cell to that shown in Figure 7B, a type III medium neuron. Other than somatic size (91 vs. 160 square μm), they appear to be identical. This, again, points to the difficulties involved in classifying neurons based mainly on their somatic sizes.

We have also found examples (Fig. 16) similar to the neurogliform cells described in monkey (DiFiglia et al., 1976) or the small cell described in the cat (Kemp & Powell, 1971a, their Figure 12) and in the rat (Danner & Pfister, 1979; Dimova et al., 1980). Electron microscopic analysis revealed that this cell type in the rat has the morphological characteristics of glial cells (Dimova et al., 1980). Since no axon-like processes have been observed to arise from these cells, we agree with the conclusion reached by Dimova et al. (1980) that it is a type of perineuronal glia.

Other small neurons in the rat neostriatum in this study have sparsely-spined dendrites and may have a few somatic spines or appendages. These neurons have not been described previously. Their dendrites tend to align themselves along the course of the internal capsule fibers which pass through virtually all parts of the rodent neostriatum. However, since their axons are not impregnated to any appreciable extent, it is premature to speculate how these cells fit into the intrinsic organization of the neostriatum.

Rare Cell Types

Two neurons without apparent axons have been described in this study. One of which has a relative small soma and sparsely-spined dendrites (Fig. 12C, 13A, 13B) and is similar to the other sparsely-spined small neurons discussed earlier. In this case, it may simply have an unimpregnated axon. The other cell, with slightly larger soma, has aspinous dendrites with long dendritic appendages (Fig. 12F, 13C & 13D). None of the previous studies have described similar cells. If it were not a result of partial

impregnations, then it must be considered as a prime candidate as a type of neostriatal neuron engaged in dendro-dendritic or dendro-axonic connections with other neostriatal neurons reported in previous electron microscopic studies (Kemp & Powell, 1971b; Pasik et al., 1976; Hattori et al., 1979).

Several medium neurons rarely impregnated in our material have short and thin dendrites (Fig. 12D) while others have very long dendrites (Fig. 14A & 14B). Similarly named "medium cells with long dendrites" have been described previously in the rat, however, the dendrites of those illustrated neurons do not exceed 200 μm (Lu & Brown, 1977; Dimova et al., 1980) and thus are probably members of the type III medium neurons as defined in this study. On the other hand, medium cells with truly long dendrites have been described in the dog neostriatum by Tanaka (1980). Although he considered these cells comparable to the aspiny III cells in the monkey (DiFiglia et al., 1976), a similar conclusion is not justified in the present study since the type III medium cells of this study are closer related to the aspiny III of the monkey as has been mentioned earlier. Since the sample size is very small, these rarely-found cells have not been considered as members of a distinct neostriatal neuronal type in this study.

C. Electron Microscopic Morphology of Rat Neostriatal Neurons

Large Neurons

The two types of large neurons defined in this study share many of the somatic ultrastructural morphology described in many of previous electron microscopic studies of normal neostriatum. They both have deeply invaginated nuclei, abundant rough endoplasmic reticulum, a well developed perinuclear Golgi complex, many free ribosomes, mitochondria and dense bodies. They are, of course, distinguished from each other by the presence of somatic spines on the type II neurons. More often than not, however, the somatic spines are not observed in a random section through a type II neuron for the simple reason that there is more smooth somatic surface than areas occupied by somatic spines. Therefore, absence of somatic spines is not indicative of a type I large neuron in a random thin section. Another major difference between these two types of large neurons is that there are many more axosomatic synapses on the type II large neurons. Often, no more than three or four axosomatic synapses are visible in any one section through the soma of a type I large cell whereas many more synapses are observed on just a small part of the type II soma as shown in Figure 19A.

Axon terminals containing mainly medium to small vesicles have been observed to make synaptic contacts with the dendrites of both types of large neurons. Some of these terminals appear to be asymmetrical, resembling the afferent terminals described in previous studies (see INTRODUCTION), thus suggesting monosynpatic connections of the neostriatal afferents with these large cells. Further

investigations combining anterograde lesion-degeneration with Golgi gold-toned material or intracellularly labeled material should be able to resolve this issue.

At least some members of both the type I and type II large neurons have myelinated axons, as have been demonstrated from the results obtained from the intracellular labeleing study for the former (Fig. 32A, inset) and from gold-toned Golgi material for the latter (Fig. 18B). Since neurons with similar somato-dendritic morphological characteristics can have drastically different axonal distribution patterns as have been demonstrated for the type I medium neurons in this study (Fig. 35 & 36), it is premature to say that all large cells have myelinated axons, or that they have similar pattern of arborizations. However, we can conclude that some type I large neurons have local integrative functions through their local collaterals (as shown in Fig. 2B). Additionally, some type I as well as some type II large neurons have myelinated axons indicating they may have faster conduction velocity than other neostriatal neurons which have unmyelinated axons (e.g. type V medium neurons). According to Fox et al., (1971/1972) myelinated axons suggest a longer axon trajectory, and thus implies a possible efferent role of these neurons. On the other hand, it is also possible that these large cells are interneurons in the sense that they may serve some intrastriatal associative function, linking wide areas of the neostriatum.

Type I Medium Neurons

Ultrastructurally, the type I medium neurons appear to be the only type of neostriatal neurons with unindented nuclei. Although Mori (1966) has described a type of large neuron with unindented nucleus (Mori's Type I large neuron), it is likely that it is a longitudinally sectioned type I medium neuron. This conclusion is based on our observation that several medium type I neurons (previously identified via light microscopy) sectioned longitudinally (e.g. Fig. 21A) have remarkably similar dimensions and appearance as the diagrammatic drawing illustrated by Mori. The somata of longitudinally sectioned type I medium cells appear to be twice as large as the cross-sectioned neurons, the latter have the appearance of the small cell of Mori. Since no large neostriatal neurons with unindented nuclei have ever been found in this study or in any other previous study except Mori's, its existence remains doubtful.

The type I medium neurons also appear to be identical to the projection cells retrogradely filled with HRP extracellularly injected in the globus pallidus based on their similar somatic size and similar electron microscopic morphology. This finding agrees well with previous studies combining Golgi gold-toned and retrograde HRP transport techniques demonstrating that the medium spiny neurons of the rat neostriatum project to the substantia nigra (Somogyi & Smith, 1979). These cells also appear to be identical to the cholinesterase-negative projection neurons reported recently (Henderson, 1981). However, the existence of other types of neostriatal projection neurons cannot be ruled out as demonstrated by another study which showed that cholinesterase-positive medium neurons

also project to the substantia nigra (Kaiya et al., 1979).

At least two types of axosomatic terminals, containing either large or medium to small vesicles, synapse on the type I medium neurons, thus confirming the observations make previously in the monkey (DiFiglia et al., 1980), the cat (Frotscher et al., 1981) and the rat (Somogyi & Smith, 1979; Somogyi et al., 1981). The dendrites receive mainly asymmetrical axospinous synapses with terminals containing medium to small vesicles which are probably mostly extrinsic in origin (Kemp & Powell, 1971; Hattori et al., 1973; Chung et al., 1977; Hassler et al., 1978). Some of these have recently been demonstrated to be of cortical origin (Frotscher et al., 1981; Somogyi et al., 1981).

The intrinsic collaterals of the type I medium neurons labeled intracellularly with HRP make mainly symmetrical axodendritic synapses as has been reported in a recent study (Wilson & Groves, 1980). Additionally, we have observed that the main axons of the type I medium neurons are myelinated at least for part of their course toward the globus pallidus. The striopallidal terminal arborizations, labeled by intracellular injection of HRP, make mainly axodendritic synapses on small pallidal dendrites. Both the efferent terminals and the intrinsic terminals contain large vesicles, and the synaptic junctions appear to be symmetrical. These terminal boutons resemble the GAD-positive boutons identified in the immunocytochemical studies of the neostriatum the globus pallidus and the substantia nigra (Ribak et al., 1976, 1979). However, it is interesting to note that the soma of a GAD-positive neostriatal neuron has a slightly indented nucleus containing an intranuclear rod, which has never been found in any of

the type I medium neurons, but has been found in the type II medium neurons in this study and in another study (Dimova et al., 1980). On the other hand, the enkephalin-positive terminals in the neostriatum (Pickel et al., 1980) and globus pallidus (DiFiglia et al., 1980) have an appearance similar to the axon terminals of type I medium neurons. In addition, the enkephalin-positive somata in the rat neostriatum has a similar morphology to the type I medium neurons of this study (Pickel et al., 1980). The neurochemical properties of the type I medium neurons, therefore, remains unclear.

The results of this study together with findings from previous investigations indicate that type I medium neurons are responsible for most if not all of the neostriatal efferent projections to either the globus pallidus or the substantia nigra or both. However, despite their similar somato-dendritic morphological appearances, it is premature to conclude that these neurons have similar neurochemical properties. Whether they contain more than one putative neurotransmitters (i.e. GABA, substance P or enkephalin) awaits further investigations with both immunocytochemical and morphological techniques.

Type II Medium Neurons

Ultrastructurally, the type II medium neurons are distinguished by their deeply indented nuclei which often contain intranuclear rods which have been reported previously in some rat neostriatal neurons (Seite et al., 1977; Dimova et al., 1980). The intranuclear rod in the type II neurons consists of parallel bundles of filaments. These differ from other intranuclear inclusions

observed, which consisted of alternating sheets of filaments oriented at right angles to each other (Dimova et al., 1980). The functional significance of these intranuclear inclusions remains unclear, but it is noteworthy that they are found only in indented nuclei of rat neostriatal neurons.

In addition to nuclear morphology, the type II neurons are distinguished by the presence of many axosomatic synapses on both the smooth somatic surface and the somatic spines. In contrast, the axosomatic synapses on other medium neurons are not as numerous. Although synapses are found on dendritic spines, many axodendritic synapses occur on the dendritic shafts of type II medium neurons. In contrast, most of the axodendritic synapses of type I medium neurons are axospinous and few synapses are found on the dendritic shafts.

One partially impregnated axon of a type II medium neuron has been traced until the end of impregnation but did not show any sign of myelination. This demonstrates that myelination is not a necessary condition for partial impregnation of axons. The Golgi impregnation remains as enigmatic as ever.

Type III Medium Neuron

The somatic ultrastructural morphology of a type III medium neuron is distinguished by the presence of a nucleus with a few shallow indentations. Although no intranuclear inclusions have been observed, they have been reported to be present in all the neurons with indented nuclei in the rat neostriatum (Dimova et al., 1980). Similar to the type I medium cells, the cytoplasm contains only sparse rough endoplasmic reticulum or Golgi complex. In the

particular cell analyzed for this study, few microtubules or free ribosomes are observed, but it is not clear whether this appearance is an artifactual result of this particular preparation. Similar neurons have been described in the cat (Kemp & Powell, 1971a, their Figure 18). Their counterparts in the monkey remain unclear, but may correspond to the medium neurons with a few nuclear indentations in the monkey described by Fox et al., (1971/1972a) who considered these profiles as belonging also to medium spiny neurons.

Synapses are found on all parts of the neuron, including the axon initial segment. However, it is difficult to determine the presence of different types of synapses according to the criteria originally obtained from normal material (Bak et al., 1975; Chung et al., 1977; Hassler et al., 1978; Hassler, 1979). Nevertheless, we have found some differences with respect to the size of the synaptic vesicles within the presynaptic terminals. The terminals making axosomatic synapses with this neuron may contain either small, medium or large vesicles. These terminals may be analogous to the three types of axosomatic boutons observed in the monkey (Rafols & Fox, 1971/1972). However, tissue preparation artifacts preclude further detailed analysis. Boutons containing mainly medium to small round vesicles synapse on all parts of the dendritic processes, and may indicate monosynaptic inputs from the neostriatal afferents whose terminals have been shown to contain small or medium round vesicles (Kemp & Powell, 1971; Chung et al., 1977; Hassler et al., 1978; Hattori et al., 1973).

Although the axon has been traced through serial sections to the point where the impregnation stopped, no indication of myelination

was observed. Since the only well impregnated axon of the type III medium neuron in the present study (Figure 7B) appears to arborize locally, it would not be surprising if all type III medium neurons had similar short axons. Further investigations with intracellularly injected neurons should provide more conclusive data with respect to the nature of their axons.

It is very interesting to comment on the only medium aspinous neuron which has been labeled intracellularly with HRP to date (Fig. 31A, SA = 115 square μm). Although its light microscopic appearance indicates that it is a type III medium neuron as defined in this study, its deeply invaginated nucleus (Fig. 34B) revealed under electron microscopic analysis suggests that it may be a different type of neuron, perhaps more similar to the gold-toned small neuron (SA = 105 square μm) shown in Figure 28A which also has a very deeply invaginated nucleus.

Type IV Medium Neurons

The somatic morphology of the type IV neuron closely resembles the type III neuron described earlier. Its shallowly-indented nucleus is surrounded by small amount of rough endoplasmic reticulum and Golgi apparatus. Like other gold-toned neurons, the nucleus and the cytoplasm of the type IV neuron have a washed-out, or extracted appearance such that clumps of chromatin in the nucleus and other cytoplasmic organelles are separated by essentially empty spaces. However, unlike most of the other gold-toned medium neurons, the partially impregnated axon of this neuron was found to be myelinated at the point where Golgi

impregnation stopped, a finding similar to that observed for the large type II neuron described earlier. Since no collaterals have been observed to arise from the unmyelinated initial segment, it is likely that any, if at all, axon collaterals of this cell would arise relatively far from the parent soma.

The neuron analyzed in this study lies adjacent to another neuron with no intervening glia processes. Since this neighbor was not impregnated by the Golgi reaction products, its classification remains tentative. Based on its unindented nuclear morphology, this neighbbor is probably a type I medium neuron. Neostriatal neuron pairs with no intervening glial processes have been described in previous electron microscopic studies (Adinolfi & Pappas, 1968). The finding in this case therefore documents for the first time that different types of neostriatal neurons have somatic appositions with each other.

In addition to the axosomatic synapses similar to those on the type III medium neurons, the dendrites of the type IV neurons also make synaptic contacts with boutons containing mainly medium to small vesicles. The long dendritic appendages or spines observed in the light microscopic analysis have been demonstrated to be postsynaptic. One of the most important differences observed between the dendritic appendages of this cell and the dendritic spines of the type I medium neurons is that no spine apparatus has been observed within the dendritic appendages. Instead, a mitochondria is often observed to branch within the dendritic stem and pass through the thin neck of the appendage into its head. In very thin appendages, the amount of gold depositing precluded analysis of their cytological details.

The polymorphic terminal dendritic branches within the fiber bundles of the internal capsule have been found to be postsynaptic. Since the presynaptic terminals are embedded in the fiber bundles, their presence have been mostly overlooked in autoradiographic axonal tracing studies because radioactively labeled fiber bundles have been usually dismissed as fibers of passage. Our present finding indicates that this assumption, found in most light microscopic studies, is not valid. Electron microscopic analysis with axonal tracing methods such as lesion-degneration or autoradiographic methods are needed for re-evaluations of neostriatal synaptic connections embedded within the fibers of passage.

Type V Medium Neuron

The soma of the type V neuron is characterized by the presence of a large and indented nucleus surrounded by a very well developed Golgi complex and several stacks of rough endoplasmic reticulum. A few axosomatic as well as many axodendritic synpases have been observed. However, because of poor tissue preservation, the synaptic vesicles and junctional specializations in these cases have been very difficult to resolve and it is not possible to evaluate the similarity or difference of this neuron with the ultrastructure of the aspiny I neurons of the monkey (DiFiglia et al., 1980). Nevertheless, the compensatory yield of its well impregnated axons have provided much information with regard to its synaptic relationship to other neostriatal neurons.

The main axon, without forming any recognizable synaptic contacts with other presynaptic terminals, was traced for over 100 μm

in serial sections and did not become myelinated. Its collateral branches, with both bouton en passant and terminaux, formed symmetrical synapses with both dendritic shafts and somata of neighboring neurons. Most of the postsynaptic somata have round, unindented nuclei suggestive of type I medium neurons. Since more than one terminal from a type V medium neuron has been observed to make synaptic contacts on a single soma, the type V medium neurons are probably very effective in inhibiting the activities of the neighboring neostriatal projection neurons through these multiple axosomatic contacts. Exactly how this type of neuron interacts with other types of neurons awaits further investigation, preferably with combined intracellular recording and intracellular labeling to better correlate physiological and morphological informations.

Small Neurons

The small neuron analyzed for this study is characterized by its deeply invaginated nucleus surrounded by a narrow rim of cytoplasm. Although its soma resembles a glial cell body at first sight, the presence of a few axosomatic contacts clearly establishes its neuronal nature. Synapses have also been found on all parts of its dendritic tree. Unfortunately, no axon has been identified for this cell. Since similar small cells analyzed light microscopically have axons arising mostly from dendrites, it is perhaps not surprising that no axonal processes have been observed to arise from the soma of this cell under either light or electron microscope.

As has been mentioned earlier, the only medium aspinous neuron labeled intracellularly with HRP has a similar somatic size to

this gold-toned small neuron (SA = 115 vs. 105 square μm). In addition, they both have deeply invaginated nuclei surrounded by a very narrow rim of cytoplasm. This particular case again points to the difficulties involved in classifying neurons mainly on the basis of their somatic size. Previous studies have not described similar small neurons with sparsely-spined dendrites at either light or electron microscopic levels. Since the axons of these cells have not been impregnated to any appreciable extent, their roles remain unclear.

D. Intrinsic Organization of the Rat Neostriatum

a) Projection Neurons vs. Interneurons

In the present study we have demonstrated for the first time that the type I medium neurons (i.e. medium spiny neurons) have thinly myelinated axons, some of which terminate in the globus pallidus. Additionally, two patterns of efferent axonal distribution arising from type I medium neurons have been observed. These findings together with the observations that most of the neostriatal projection neurons are medium neurons (Grofová, 1975; Bunny & Aghajanian, 1976) and that virtually all neostriatal efferent axons are thinly myelinated (Fox et al., 1975) indicate that many neostriatal efferent neurons are type I medium neurons, and that the different axon distribution patterns may have arised from type I medium neurons which terminate selectively at different target nuclei (Szabo, 1981).

At least three other types of neurons are likely candidates for neostriatal projection neurons: Both types of large neurons and the type IV medium neuron have myelinated axons. Further investigations with either intracellular labeling or combined Golgi and retrograde labeling studies will be needed to demonstrate conclusively the destination of their aoxns.

The past description of some cells as Golgi type II (interneurons) in the neostriatum has been based on observations that some Golgi-impregnated cells have locally arborizing axons without apparent projecting axons. However, since the main projecting axons often fail to impregnate in Golgi preparations, mere absence of projecting axons cannot be taken as evidence that they do not exist.

Thus, designation of different types of neostriatal neurons into projection neurons versus interneurons based on Golgi data may be unwise and can be very misleading. The emphasis, instead, should be made primarily with respect to the distribution pattern and the extent of the observable axonal arborizations. In this study, except for the type II large cells, all types of neurons have been observed to give rise to some local collaterals with varying distribution, indicating that they all have some degree of intrinsic communicative function.

It is premature to ascribe any axonal distribution pattern strictly to any particular neuronal types because we have demonstrated that neurons with virtually identical somato-dendritic morphology can have very different patterns of axon distribution (e.g. Type I medium neurons have at least two different patters of local intrinsic axonal arborization as well as two different patterns of efferent axon distribution). Therefore, identification of neurons based on their somato-dendritic morphology should not imply a single pattern of axon distribution. Since intracellular labeling appears to be the best method available for demonstration of the distribution of very long neuronal processes (i.e. axons and their collateral branches), further analysis of intracellularly labeled material at both light and electron microscopic levels will be needed to provide detailed information with respect to the complete size of the local as well as the terminal axonal arborizations and their synaptic targets.

Neurotransmitters of the Rat Neostriatal Neurons

Cholinergic Neurons

Light microscopic immunocytochemical studies have shown that most choline acetyltransferase (CAT) containing neurons are either medium or large neostriatal neurons (see INTRODUCTION). However, the partial somato-dendritic morphology revealed in these studies precludes their comparison with either the type I or the type II large cells of this study.

The ultrastructural morphology of large AChE neurons which were not labeled retrogradely with HRP injected extracellularly in the midbrain (Henderson, 1981) appears similar to the large striatal neurons retrogradely labeled with herpes virus injected in the midbrain (Bak et al., 1978). Since neither of these studies mentioned the presence of axosomatic synapses or somatic spines, their identity as either type I or type II cells also remains unclear.

Medium CAT-positive neostriatal neurons have also been reported by the same laboratory which reported the presence of large CAT-positive neurons (Hattori et al., 1976). Their somatic ultrastructure, with specifically their unindented nuclei and sparse cytoplasmic organelles, resembles the type I medium neurons observed in this study. CAT-positive dendrites with spines have also been observed to make asymmetrical axospinous synapses similar to those seen on the dendritic spines of either gold-toned or intracellular HRP labeled type I medium neurons in this study. On the other hand, either AChE-positive (Kaiya et al., 1979) or AChE-negative medium neurons (Henderson, 1981) have been described to project to the substantia nigra and both appeared to have similar ultrastructural

appearance as the type I medium neurons of this study. Since AChE is not a reliable marker for cholinergic neurons, and the CAT immunocytochemical studies have produced conflicting results, it is premature to link any of the neostriatal neuron types deliniated in this study to cholinergic transmission.

GABAergic Neostriatal Neurons

The presence of glutamate decarboxylase (GAD), the synthesizing enzyme for gamma-aminobutyric acid (GABA), has been used as an indicator for neurons which use GABA as their neurotransmitters. Immunocytochemical studies have shown that GAD-positive rat neostriatal neurons are medium sized and have dendritic spines (Ribak et al., 1979). The morphology of the GAD-positive axon terminals within either the neostriatum or the globus pallidus resemble those of the intracellularly labeled type I medium neurons. In addition to biochemical studies which have shown that GABA is a characteristic chemical constituent of the neostriatal efferent system (see INTRODUCTION), recent electrophysiological studies have shown that GABAergic mechanism is involved in the possible autaptic recurrent inhibition of type I medium neurons (Park et al., 1980). These observations indicate that at least some type I medium neurons are GABAergic neurons.

On the other hand, some GAD-positive medium neostriatal neurons have intranuclear rods (Ribak et al., 1979), which have never been observed in the identified type I medium neurons either in the present or in previous studies (Dimova et al., 1980; Somogyi et al., 1981). Thus, at least some other type medium neostriatal neurons may

also be GABAergic. Presently, only type II medium neurons have been shown to have intranuclear rods resembling that illustrated by Ribak et al. (1979).

Other Neurotransmitters

Immunocytochemical studies at both light and electron microscopic levels have shown that enkephalin-containing neurons in the neostriatum of the rat (Pickel et al., 1980) and in the monkey (DiFiglia et al., 1980) both have features similar to those of the type I medium neurons. Furthermore, enkephalin-positive axon terminals in the globus pallidus (DiFiglia et al., 1980) also resemble the terminals of intracellularly labeled type I medium neurons. Whether the same neuron could contain both enkephalin and GABA remains unclear.

Biochemical studies have shown that there is a substance P projection from the neostriatum to the substantia nigra (see INTRODUCTION), however, immunocytochemical studies have not revealed the somato-dendritic morphology of substance P-containing neurons. Recently, antibodies to somatostatin have labeled a number of medium neurons in the cat neostriatum (Graybiel et al., 1981) and a few cells in the rat neostriatum (Bennete-Clark et al., 1980). Since detailed surface morphology of these cells were not revealed in these studies, their relationships to the Golgi-impregnated neurons also remain unclear.

Chapter 5. SUMMARY OF NEW FINDINGS

1. Based on the morphology of intracellularly labeled medium type I neurons, we have demonstrated that neurons with identical somato-dendritic morphology can have drastically different axonal distribution patterns, both intrinsically within the neostriatum and extrinsically in the target areas (i.e. GP): Some cells have dense local axon arborizations restricted mainly within the parent dendritic domain, others have widely distributed local collateral. Some efferent axon branch within the neostriatum and the daughter fibers arborize extensively within localized areas in GP, other efferent axons reamin unbranched in the neostriatum and give rise to few or no collaterals in GP. Thus, functionally different neurons may have identical somato-dendritic morphology.

2. This observation also demonstrates that emphasis of analysis of Golgi preparations should be placed on the distribution of the visible axonal arborizations rather than speculating on whether they are projection cells or not, since the axons in Golgi preparations are often only partially impregnated. We have proposed a system of classification of neostriatal neurons based on their somato-dendritic morphology in this study. We have extended the findings of previous Golgi studies of the rat neostriatum by providing both photomicrographic and camera lucida documentations of at least two types of large neurons and five types of medium neurons as well as a number of small neurons. Except for the large type II large neurons, every type of rat neostriatal neurons have some local collaterals with varying degrees of impregnation. None have been

ruled out as projection cells, and none have been determined to be strictly intrinsic to the neostriatum.

3. Electron microscopic analysis of the axons labeled intracellularly with HRP has confirmed and extended previous ultrastructural analysis of intracellularly labeled neurons (Wilson & Groves, 1980) and the gold-toned type I medium neurons (DiFiglia et al., 1980; Somogyi et al., 1981): The main axons of the medium type I cells are myelinated. Those efferent axons which branch within the neostriatum become unmyelinated after the branch point. On the other hand, the unbranched efferent axons remain myelinated throughout their trajectory within the neostriatum and the globus pallidus. The intrinsic collaterals of medium type I cells make symmetrical axodendritic synapses with neighboring neurons. Similarly, the efferent terminals of medium type I neurons contain large vesicles and make symmetrical synapses mainly with dendrites of GP neurons.

4. We have demonstrated the presence of large and medium rat neostriatal neurons with somatic spines - the large and medium type II neurons - thus confirming the existence of such neostriatal neurons in non-primates. Similar cells have been described in the monkey (DiFiglia et al., 1976) as the Spiny II cells, or the aspiny neuron with somatic spines (Fox et al., 1974). Although previous studies have suggested that these are projection neurons, we cannot confirm such suggestion due to insufficent data. Nevertheless. we have demonstrated that a large type II cell has a myelinated axon, suggesting that it may have a widely arborizing axon.

5. Ultrastructural differences have been found between the two types of large neurons: presence of more somatic synapses on the

large type II cells than on the large type I cells. Since previous histochemical or immunocytochemical studies have not describe the synaptic morphology of large AChE-positive or CAT-positive neostriatal neurons, it is premature to speculate either type of large neurons as cholinergic cells.

6. One medium type IV neuron has an myleinated axon, suggesting that it may have a long axon similar to the type I medium neurons, and may correspond to one of the several types of neostriatal efferent neurons containing substance P. enkephalin or GABA. We have observed the presence of terminal dendritic branches within the internal capsule fibers bundles, electron microscopic analysis of gold-toned materials shows that these terminal branches are postsynaptic. No presynaptic dendrites have been observed.

7. Based on the distribution of their local axon collaterals which arborize densely near the soma and appear to be equal if not more dense than those of type I medium neurons, the type V medium neurons must have at least as much local influences as the medium type I neurons. Since the intrinsic collaterals of medium type V cells make multiple symmetrical axosomatic synapses with neurons with unindented nuclei, they may have strong inhibitory actions on their neighboring porjection neurons (i.e. type I medium neurons).

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LIST OF REFERENCES

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