The Effect of Aging on Pars Compacta of the Substantia Nigra in Rhesus Monkey

ZAEE M. SIDDQI, MD, PhD, AND ALAN PETERS, PhD

Abstract. This aim of this study was to re-evaluate the types of neurons present in pars compacta of the substantia nigra in the rhesus monkey, and then to determine the effects of aging on the morphology of both the neurons and neuroglial cells. The substantia nigra was therefore examined in Golgi impregnated material, in sections labeled with antibodies to tyrosine hydroxylase and to GABA, and in tissue embedded in plastic for light and electron microscopy. Three types of neurons were encountered: (1) large multipolar neurons with prominent Nissl bodies, (2) bipolar neurons that are medium sized and spindle-shaped, with Nissl bodies confined to the poles of the deeply indented nuclear envelopes, and (3) small multipolar cells with sparse Nissl substance. The large and medium neurons are believed to be dopaminergic and the small neurons GABA-ergic. With age, all of the neurons accumulate lipofuscin, especially the small multipolar neurons. In addition, Marjorec bodies appear within the nuclei of the large multipolar neurons. The dendrites are most severely affected by age: many of them lose organelles and their cytoplasm can become filled with vacuoles, membrosum whorls, and dense bodies. In old monkeys many of the astrocytes have inclusions and in the neuropil there is a striking increase in the number of astrocytic processes. Also large spheroids appear. These appear to be derived from astrocytes and they have a core of dense granular material surrounded by a paler peripheral zone of cytoplasm from which processes can extend. Most of the oligodendrocytes have dense inclusions in their cytoplasm and many of the myelin sheaths break down. Microglial cells can become enormously swollen by phagocytosed material. Although both neurons and neuroglial cells are affected by age, no entities that could be construed to be dying neurons were encountered.

Key Words: Astrocytosis; Dopamine; Electron microscopy; Neurons; Normal aging; Primate.

INTRODUCTION

With normal aging in primates there is a progressive decline in the cortical and striatal levels of dopamine (1–4), which may be due to a loss of neurons from the dopaminergic nuclei that project to the cortex and striatum. These nuclei are the substantia nigra pars compacta (SNpc) and the ventral tegmental complex. Indeed, a number of reports have maintained that with increasing age there is a significant loss of neurons from the SNpc in humans (5, 6), monkeys (7), and mice (8, 9), although other studies of both aging monkeys (10, 11) and normally aging humans (12) have argued against such a loss.

Our own studies (13) using recently developed stereological methods to determine the numbers of neurons in the dopaminergic brainstem nuclei of 14 rhesus monkeys, ranging in age from 5 to 32 years, support the view that there is a significant age-related loss of neurons from the SNpc, as well as from the cortically projecting parainigral nucleus of the ventral tegmental area (VTA). In the SNpc, this age-related neuronal loss ranges from 23% to 25% and appears to affect the various regions of SNpc in a uniform manner. No effect of age was observed on the total number of neurons in the cortically projecting parabrachial pigmented nucleus of the VTA.

In contrast to the interest in age-related changes in neuronal number, little attention has been paid to effects of normal aging on the morphology and cytoarchitecture of the SNpc, although in Golgi impregnated preparations of the substantia nigra Cruz-Sánchez et al (14) have observed significant alterations in the morphology of neurons in older humans. These alterations include swelling and distortion of neuronal cell bodies, swelling and beading of dendrites, and a severe loss of dendrites and their spines.

The aim of the present study is to examine the effects of aging on the morphology of the neurons and neuropil of the SNpc in rhesus monkeys (Macaca mulatta) with particular attention to any evidence of neuronal degeneration that may be related to the cell loss we have observed. It may be pointed out that in the same population of monkeys, no evidence has been found for a significant age-related neuronal loss from either prefrontal (15), visual (16, 17), or motor (18) cortices (also see 19), and ultrastructural studies on these same cortical areas have shown no significant degenerative changes in the neuronal perikarya (15–17).

Depending on the morphological technique employed, previous studies on the primate substantia nigra have reported from 1 to 4 types of neurons. For example, Hiroseawa (20) described only a single type of pigment-containing neuron in the SNpc of the Japanese monkey (Macaca fuscata yakui) whereas based on an analysis of Golgi and electron microscopic preparatons of rhesus and squirrel monkeys, Schwy et al (21) described 2 types of neurons–1 large and 1 small. The large neurons have a rich content of cytoplasmic organelles with prominent Nissl bodies and long radiating dendrites, while the...
achromatic, small neurons are described as having a few, thin, short dendrites. Similarly, in Golgi preparations from monkey and man, Yelnik et al (22) have reported that each subdivision of the substantia nigra contains numerous projection neurons that have large cell bodies and sparsely branched dendritic trees, as well as a few small neurons with thin dendritic stems that branch within a short distance into even thinner processes. In Nissl preparations of monkey and man, Poirier et al (23) have identified 4 types of neurons in the substantia nigra, and these they term compacta, reticulata, intermediaria, and globular types. The compacta and reticulata types of neurons have a large amount of Nissl substance that is distributed as irregular patches in the compacta neurons and is more evenly distributed in the reticulata neurons. The intermediate type of neuron contains a moderate amount of diffusely distributed Nissl substance, while the globular type is small with a high nuclear/cytoplasmic ratio and a sparse Nissl substance. In contrast, on the basis of a pigmentoarchitectonic study of the substantia nigra in humans, Braak and Braak (24) have described 3 types of neurons. They describe a type I, or the medium to largesized neuron with a large number of neuromelanin granules that is predominantly present in the pars compacta, and a type II neuron, which is medium-sized, lacks neuromelanin, and occurs mainly in the pars reticulata. Many of the type II neurons contain a considerable amount of lipofuscin. Their type III neurons have a much smaller cell body, lack neuromelanin, and contain lipofuscin granules that are smaller than those of the type II neurons.

Since there is such an evident lack of consensus about how many different types of neurons are present in the primate SNpc, it was decided to first re-examine the neurons of SNpc in the rhesus monkey (Macaca mulatta) using Golgi, light and electron microscopic and immunohistochemical preparations. This was necessary before an assessment could be made of the effects of aging on the morphology of the nigral neurons.

Nine young monkeys (Macaca mulatta) between 3 and 11 years of age and 6 old monkeys between 26 and 35 years of age were used in this study. These monkeys reach sexual maturity at about 5 years of age, and an analysis of the life span of this species (25) has shown that about 25% of monkeys attain an age of 25 years and only 6% live longer than 30 years of age. Consequently in determining the effects of aging, monkeys that are between 5 and 11 years of age can be considered to be young, and monkeys that are over 25 years of age can be regarded as being old. The monkeys used were from an aging colony maintained at Yerkes Regional Primate Research Center and at Boston University School of Medicine. Both sites are fully accredited, and all animals in the colony are cared for under professional veterinary supervision in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 86-23).

Golgi Preparations

Two young rhesus monkeys, 3 and 4 years old, were used for the Golgi impregnations. The preparations were made with the Antonova (26) modification of the Golgi method, which shortens the time of fixation and impregnation, while adequately revealing the morphology of neurons. Briefly, blocks of fresh tissue measuring 5 mm × 2 mm were fixed for 5–6 days in a fluid containing 12% neutral formalin, 0.8% sodium tungstate, and 3% potassium dichromate. The solution was changed daily. The blocks were rinsed in a solution of 4% potassium dichromate and then transferred for 1 to 2 days to a solution containing 0.2% osmic acid, 4% potassium dichromate, and 2% chloral hydrate. The blocks were then dried on a filter paper, washed repeatedly in a 1.5% solution of silver nitrate, and left in the silver nitrate solution for 2 days. In the final step, the blocks were dehydrated in increasing strengths of alcohol, embedded in celloidin and sectioned at a thickness of 80 to 100 μm. Twenty-five well-impregnated neurons of each of the 3 types described in this study were drawn using a camera lucida.

Immunohistochemistry

For tyrosine hydroxylase labeling, frozen sections containing the substantia nigra were taken from the brainstem of a 5-years-old monkey that had been perfused with 4% paraformaldehyde. After being treated with hydrogen peroxide to remove any intrinsic peroxidase, the sections were immersed for 1 hour in a blocking solution containing 10% normal horse serum and 0.125% Triton-X in 0.05 M phosphate buffer at room temperature. The sections were then incubated with a mouse anti-tyrosine hydroxylase antibody (Incstar) for 12–16 hours at 4°C. The anti-TH serum was diluted 1:2,000. The antibody binding sites were visualized using the avidin-biotin method with a Vectastain ABC kit (Vector Laboratories, CA), with 3,3′-diaminobenzidine tetrahydrochloride as the chromogen.

For GABA immunocytochemistry, frozen sections from a cryoprotected brainstem of a 6-years-old monkey, and vibratome sections from the brainstems of 2 other monkeys, 6 and 10 years old were used. These 3 monkeys had been perfused with a mixture of paraformaldehyde and glutaraldehyde. The sections were treated with 0.1% sodium metaperiodate and 0.1% sodium borohydride for an hour. After a brief wash in 0.1 M phosphate buffer, the sections were immersed in a blocking solution containing 0.2% Triton-X and 3% normal goat serum. The sections were incubated overnight in the same solution containing 1:2,000 anti-GABA (Incstar, goat anti-rabbit) at 40°C. The antibody was visualized using the ABC kit (Vector Laboratories, CA) with 3,3′-diaminobenzidene tetrahydrochloride as the chromogen.
Electron Microscopy

For electron microscopic examination of the substantia nigra pars compacta, the brains of 5 young monkeys (5 to 6 years of age) and of 6 old monkeys (26 to 35 years of age) were used. The details of the fixation procedure used are given in Peters et al (15). Briefly, the monkeys were anesthetized and their brains fixed by intravascular perfusion through the ascending aorta with a warm fixing solution containing 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M cacodylate buffer. The brains were removed from the skull immediately after perfusion and the brainstem separated by making an incision above the mesencephalic-diencephalic junction. To achieve optimum fixation, the brainstems were subsequently immersed in a fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 at 4°C for at least a week prior to further processing.

The brainstem was cut into thick transverse slices, and blocks containing the dark band of substantia nigra were removed. The blocks were then osmicated, dehydrated, and stained en bloc with uranyl acetate before embedding in Araldite. Prior to thin sectioning, 1-μm-thick plastic sections were cut on an ultramicrotome in an axis perpendicular to the longitudinal axis of the brainstem. These sections were stained with toluidine blue to determine the precise location of SNpc in the block. To examine novel profiles encountered in old monkeys, a series of 2-μm-semithick plastic sections were cut and stained with toluidine blue. Thin sections were taken and stained with uranyl acetate and lead citrate before being examined using a JEOL 100S electron microscope.

RESULTS

Golgi Impregnated Neurons in Young Monkeys

Three types of neurons were identified in Golgi impregnations of the SNpc, the differentiation between them being based on the size and shape of the cell body, and the disposition of their dendrites. Also, as will be shown when the fine structural features of these neurons are described, these neurons differ in their nuclear morphology, the form of the rough endoplasmic reticulum, and cell surface features such as somatic spines and the extent to which the neurons were covered by astrocytic processes.

Large multipolar neurons are the most common neurons in all parts of the SNpc. In Golgi preparations their cell bodies range from 24 μm to 55 μm in their longest diameter (Fig. 1 upper row). Three to 6 thick primary dendrites emerge from the perikaryon. These dendrites may arborize soon after emerging from the cell body, but usually they extend for several hundred microns before branching to produce only a few secondary dendrites. Some dendrites pass ventrally to enter the pars reticulata (SNpr), whereas others course into a dorsomedial direction and traverse the fiber bundles in the tegmentum, dorsal to the SNpc. The primary dendrites have few spines, but spines commonly extend from the secondary dendrites.

The bipolar neurons are medium-sized and in Golgi preparations their cell bodies are spindle or fusiform shaped. One or 2 thick dendrites emerge from each pole of the cell body and these branch in the vicinity of the cell body to produce a few secondary dendrites (Fig. 1 middle row). The secondary dendrites are sparsely studded with spines and most of them pass ventrally into the SNpr. The axon usually emerges from the cell body, although in some examples, it may arise from one of the primary dendrites.

The small multipolar neurons are the smallest neurons in SNpc. Their cell bodies are between 12-μm and 18-μm long, and they are the least frequently encountered types of neuron in Golgi preparations. The cell body is round to ovoid and has 2 to 3 dendrites emerging from it (Fig. 1 lower row). The primary dendrites, which are thin, usually arborize in the vicinity of the cell body. The dendrites have a few spines and seem not to extend in any preferred direction. Occasionally, a thin filiform process arises from the cell body or from a primary dendrite, and this probably represents the axon.

Immuno- and Histochemical Features of Neurons

Preparations of the SNpc immunostained with antibodies against tyrosine hydroxylase (TH), a rate limiting enzyme in the formation of dopamine, or against gamma-aminobutyric acid (GABA), provide different views of the neurons of the SNpc. The TH-positive neurons comprise the bulk of the neuronal population. Most cell bodies are typically large and multipolar with several long dendrites, while others are bipolar or fusiform in shape with dendrites emerging from the 2 poles (Fig. 2). These features suggest that the TH-positive neurons correspond to the large multipolar and the bipolar neurons. Most of the TH-positive dendrites aggregate into bundles that pass ventrally into the pars reticulata, where they can be followed for a considerable distance.

In sections of substantia nigra labeled with antibodies to GABA only a moderate number of labeled neurons are encountered (Fig. 3). The cell bodies of the GABA-ergic neurons are small in comparison with the other, much more numerous neurons that are not labeled by GABA antibodies. These unlabeled neurons are readily apparent, because they are outlined by labeled axons terminals, which become more numerous along their dendrites. Because of the infrequency and small sizes of the GABAergic neurons relative to the other, unlabeled neurons in the SNpc, it is suggested that the labeled neurons correspond to the small multipolar neurons.

Electron Microscopic Features of the Neurons in Young Monkeys

In thin sections it can be seen that the large multipolar neurons have a plump cell body that contains a large
Fig. 1. Drawings of the 3 types of neurons in SNpc as they appear in Golgi preparations. Bar equals 50 μm. The large multipolar neurons (upper row) have several thick dendrites arising from their cell bodies and these usually enter the SNpc. Their cell bodies and primary dendrites have few spines, but the secondary dendrites are richly studded with spines. The bipolar neurons (middle row) have a fusiform cell body with 1 or 2 dendrites emerging from each pole. These dendrites have a moderate number of spines. The small multipolar neurons (lower row) do not frequently impregnate. These neurons have a small oval to round cell body from which a few fine dendrites extend.

nucleus with a prominent nucleolus (Fig. 4). In contrast to the other types of neurons, the nuclear envelope displays only a few shallow indentations, and the most striking feature of the cytoplasm of the large multipolar neurons is the large, irregular Nissl bodies that almost completely fill the perikaryon and extend into the bases of the thick dendrites (Fig. 4). The closely packed cisterns forming the Nissl bodies generally have rather short profiles, with many polyribsosomal rosettes in the cytoplasm between them. Another distinctive feature of the perikaryon is the presence of lysosomes. These are especially common in the perinuclear region, but they occur throughout the cytoplasm in the spaces between the Nissl bodies, so that their distribution is similar to that of the many mitochondria that occur in the cytoplasm. Dense core vesicles are not associated with the Golgi complexes in this type of neuron.

The perikarya of large multipolar neurons usually display a few short, stubby spines and typically, the cell bodies are completely surrounded by a thin astrocytic
sheath that separates these neurons from the surrounding neuropil (Fig. 7). The sheaths are only interrupted by a few axon terminals that synapse with the perikaryal surface and with the somatic spines. The dendrites of the large multipolar neurons are also covered by a thin astrocytic sheath and their shafts receive few synapsing axon terminals.

In electron micrographs profiles of longitudinally oriented bipolar neurons have a characteristic appearance (Fig. 5). The nucleus often contains a single nucleolus, and the nuclear envelope may display a number of indentations, some of which are deep. The Nissl bodies, which are most prominent at the poles of the perikaryon, are distinctive because they are composed of long, narrow cisterns that lie parallel to the nuclear envelope (Fig. 5). Another notable feature of the bipolar neurons is the numerous axon terminals that synapse both with their somata and dendrites. In contrast to the large multipolar neurons, the cell bodies of the bipolar neurons have only a sparse covering of astrocytic processes, and somatic spines appear to be absent.

The small multipolar neurons have the smallest cell bodies in the SNpc, and so they are easily recognized in plastic sections. Also they are the least common neuronal type in the SNpc. A distinctive feature of these neurons is the nucleus, which occupies a large portion of the cell body and has a remarkably irregular profile due to the presence of deep infoldings of the nuclear envelope (Fig. 6). The Nissl substance consists of only a few individual, long, curved and branched cisterns that are scattered throughout the cytoplasm (Fig. 6). Although numerous polyribosomes are present on the membranes of rough endoplasmic reticulum, there are only a few free polyribosomes evident in the perikaryal cytoplasm and because of this the cytoplasm is pale. However, the perikaryon is richly endowed with relatively small mitochondria that often occur in small clusters, and even in young monkeys some of the small multipolar neurons contain a large amount of lipofuscin (Fig. 6).

The perikarya of the small multipolar neurons may have a few spines and although a large part of the soma is covered by astrocytic processes, a few axosomatic synapses are present. It is also of interest that the perikarya...
of the small multipolar neurons frequently have a satellite oligodendrocyte. Because the small multipolar neurons have few dendrites, profiles of their dendrites are rarely encountered in the neuropil. But when the dendrites are seen it is evident that they have a pale cytoplasm with few organelles, and a sparse covering of astrocytic processes with only a few axon terminals synapsing with their shafts.

Age-related Changes in the Neuronal Somata

The most consistent age-related change in the neurons of the SNpc is the accumulation of lipofuscin pigment within their cell bodies. This is particularly striking in the small multipolar neurons, in which the lipofuscin can almost completely fill the perikaryal cytoplasm. As pointed out, some small multipolar neurons with abundant lipofuscin are occasionally encountered even in younger monkeys (Fig. 6), suggesting that the process of pigment deposition may start early in these neurons.

Ultrastructurally, the appearance of pigment granules in the 3 types of neurons is essentially similar. Usually the granules are dispersed throughout the perikaryon (Figs. 6, 7), but sometimes they are clumped in 1 location. The shapes of the granules are variable; some are almost round, whereas others are oblong and still others have irregular shapes, but in all cases the individual granules have an electron dense matrix in which 1 to 2 pale and round lipid droplets are embedded (Figs. 6, 7).

Another age-related change observed in the SNpc is the formation of intranuclear inclusions known as...
Marinesco bodies (27), which have been described previously in the human substantia nigra and locus coeruleus (28). These intranuclear inclusions are an aging feature of a small percentage of the large multipolar neurons and they are usually found close to the nucleolus. Marinesco bodies can be distinguished from the nucleolus because the nucleolus contains tightly densely packed granules surrounding lighter zones, whereas the Marinesco bodies have evenly spaced and less tightly packed granules (Fig. 8). Typical Marinesco bodies range in size from 2 μm to 2.5 μm and consist of 3 components; a central core formed by a rod-like lattice of filaments, a middle zone of loosely packed dense granules, and an outer halo or shell of pale, amorphous material (Fig. 8M). Frequently, a narrow space separates the middle granular zone from the outer amorphous shell. But in addition to this typical form, 2 other varieties of Marinesco body have been encountered (Fig. 8). The profiles of the most common variety are usually larger than those of the typical Marinesco body, and their diameters range between 4 μm to 5 μm (Fig. 8M₂). They are composed of numerous granules that are identical to those in the granular component of the typical Marinesco body, but they lack the central filamentous core and the outer amorphous zone. The other variety of Marinesco body is also round, but smaller, ranging in size from 1 to 1.5 μm, and it consists of dense granules intermixed with other granules that are somewhat smaller and less dense (Fig. 8M₃). These 3 different types of Marinesco bodies can occur within the same nucleus (Fig. 8).

Age-related Changes in Dendrites

As compared with the few morphological alterations in the neuronal perikarya, dendrites in SNpc commonly show obvious age-related degenerative changes (Figs. 9, 10, 11). Affected dendrites generally have a pale and watery cytoplasm and a sparse content of organelles, but their cytoplasm contains many membrane-bound clear vacuoles, as well as membranous whorls, similar to those that have been observed in the dendrites of pyramidal cells of old rats (29). The vacuoles occasionally become large (Fig. 9) and can almost completely fill the dendrite, so that its profile appears swollen with the cytoplasm limited to a thin rim. Sometimes, the dendrites also contain electron dense inclusions (Fig. 10), which may have the form of aggregates of round, dark granules (Fig. 10),
or of large discrete heterogenous "blobs" (Fig. 11). These inclusions are generally more electron-dense than the lipofuscin granules present in the neuronal perikarya and do not possess the pale lipid component typically present in the lipofuscin granules.

Spheroids

Large dense bodies like those illustrated in Figure 12 have been encountered in the neuropil of the SNpc of all of the old monkeys we have examined. They are similar to the spheroids that have been previously described in the human and monkey substantia nigra and globus pallidus (30, 31). The profiles of such bodies are generally round to oval and range in diameter between 10 and 50 μm and they can have processes extending from them. The appearance of these bodies is somewhat variable, but they always contain a core of dark inclusions that can be either amorphous, granular, or membrane bound. In some spheroids there are islands of very dark amorphous granules, as well as pockets of larger and darker granules that appear to be embedded in a less electron dense matrix. In contrast, the profile of the body shown in Figure 12 has a more homogeneous matrix in which island of granules of various sizes and electron densities are embedded.

The contents of other spheroids are small membrane bound spherical structures that resemble lysosomes. But in almost every case the profiles of these spheroids have a peripheral zone of pale cytoplasm that is free of the granules that abound in the core, but which contains fine filaments. This pale filamentous cytoplasm also fills any processes that extend from the bodies (Fig. 12p, p2).

In order to determine if these large dense bodies, or spheroids, are sections through cells we examined a number of them in series of semithick plastic sections. No nuclei have ever been seen within them, but it is common for the bodies to have 1 or 2 processes; however the processes could not be traced for more than a few tens of microns before they disappeared into the neuropil. Another point of interest is that although some of these bodies are of similar size to the perikarya of neurons, they do not seem to be degenerating neuronal perikarya because none of them have ever been seen to have axon terminals synapsing with them. The only possible clue to the origins of these bodies is that their surrounding membrane sometimes form zonulae adhaerentia with adjacent astrocytic processes (Fig. 13) and dense particles that resemble glycogen occur in the cytoplasm. These features
suggest that the spheroids may be derived from astrocytes.

Age-related Changes in the Neurropil

Other entities that have been encountered in the neurropil of these old monkeys are large round profiles with a central core of neurofilaments and periphery that contains numerous lysosomes, suggesting that they are dystrophic and dilated axons (see 31). Very few degenerating axons have been encountered, however, but it is very common for the myelin sheaths of axons in the neuropil to have split or swollen lamellae between which there is dark cytoplasm (Fig. 12F). This suggests that the sheaths are breaking down, and concomitantly many of the oligodendrocytes contain dense inclusions, which are of the type we have described previously in the cerebral cortices of these same monkeys (33).

Compared with that of young monkeys, the most notable feature of the neuropil in the SNpc in aged monkeys is the striking increase in the number of astrocytic processes. These astrocytic processes can frequently be traced for considerable distances through the neuropil as long, thick and curved strands that contain thick and robust bundles of intermediate filaments (Figs. 7, 10, 14).

In addition to the filaments, the astrocytic processes contain a large number of glycogen granules that may be dispersed or clustered (Fig. 14g). These features are in sharp contrast to those of the astrocytic processes in young animals, in which they form thin sheets that conform to the shape of surrounding elements and usually have few filaments and glycogen granules. As shown in Figure 14, the marked hypertrophy of the astrocytic processes may be particularly evident around abnormal profiles. In this extensive field, swathes of astrocytic processes are coursing around an unidentified structure that contains dense granular inclusions of various shapes and sizes.

Almost all of microglial cells in the substantia nigra of the old monkeys that we have examined contain phagocytic inclusions. As shown in Figure 15, some of the inclusions are so large that the cytoplasm of the microglial cell is confined to a thin rim and the nucleus is pushed to one side. Some of the inclusions are composed of clumps of dark granules, although others are paler and have a frothy appearance. Sometimes, as with the microglial cell shown in Figure 15, part of the inclusion is a seemingly empty, irregular space. Presumably these
empty spaces are ones from which lipid has been extracted during the processing of the tissue. The sources of these inclusions, which are similar in appearance to those encountered in microglia in the cerebral cortices of these same monkeys (34), are not known.

DISCUSSION

The present study confirms the presence of a diverse population of neurons in the SNpc in the rhesus monkey. Based on the size and shape of neuronal soma, distribution of dendrites, nuclear configuration, and disposition of rough endoplasmic reticulum, 3 neuronal types have been identified. With increasing age, the SNpc undergoes significant age-related changes that involve neurons as well as neuroglial cells. Further, numerous profiles displaying atrophic changes have been observed in the neuropil of SNpc. Some of these appear to be neuronal in origin, whereas others may be derived from neuroglia.

Neuronal Types in the SNpc of Rhesus Monkey

Previous morphological studies of the substantia nigra have usually employed only 1 type of preparation as the basis for classifying neurons. This may be why there are differences in the various accounts of the neuronal types in SNpc, since the sizes, shapes, and the Nissl patterns among the various neuronal types in the SNpc overlap to some extent, making it impossible to unambiguously categorize the neurons on the basis of a single morphological feature. The present classification of the neurons in SNpc into 3 types is more broadly based since it takes into account several morphological characteristics observed in a variety of preparations. Hence, the present study bridges the gap among the different techniques and helps to clear the discrepancies in the earlier descriptions of the neuronal population of the SNpc.

The hallmark of the large multipolar neurons in the SNpc is the intense basophilia produced by the large Nissl bodies in their perikarya. This feature of the large multipolar SNpc neuron has been recognized in a number of species including rodents (35), cat (36), and monkey (21). Similar intensely basophilic, large neurons have also been reported in the SNpr in the monkey by Schwyn and Fox (21) and in the rat by Gulley and Wood (37). The large multipolar cells in the SNpc differ from those found in the SNpr in several regards. While the large multipolar neurons in the SNpc are dopaminergic, those in the SNpr are GABA-ergic. Poirier et al (23) also noted differences between these cell types in Nissl preparations.
Fig. 10. Transverse section of a degenerating dendrite (D) that contains dense inclusions, vacuoles, and membranous whorls. Note the astrocytic processes (As) with prominent bundles of filaments in the surrounding neuropil. A 33-year-old monkey. ×15,000.

Fig. 11. A multipolar cell dendrite with a large, dense inclusion. A 33-year-old monkey. ×18,000.

They referred to the large neurons with abundant Nissl substance in the SNpc of the monkey as the “compacta” type of neuron and those in the SNpr as the “reticulata” type of neurons. Neurons of the “reticulata” type are round to triangular and contain discrete, less intensely staining Nissl bodies. These 2 types of neurons also can be distinguished on the basis of their ultrastructural features. Gulley and Wood (37) have noted several features of the large multipolar neurons in the SNpr that are not present in those in the SNpc. The perikarya of the large neurons in SNpr frequently contain subsurface cisternae of endoplasmic reticulum, large dense core vesicles associated with the Golgi apparatus, and occasional cytoplasmic inclusions with a characteristic lamellated appearance. These features have not been observed in the large multipolar neurons in the SNpc in the present study. However, the disposition of the rough endoplasmic reticulum appears to be similar in the 2 types of neurons.

The bipolar neurons are relatively less common than the large multipolar neurons in the monkey SNpc. Many studies have not recognized them as a separate category.

Hence, this type of neuron was not described in the substantia nigra by Domesick et al (35) or Juraska et al (38), and Schwyn and Fox (21) may have included the bipolar neurons in their class of large neurons. This may be because it is sometimes difficult to unambiguously differentiate between the bipolar and large neurons in Nissl preparations, since their Nissl patterns, sizes, and shapes overlap. For example, the large multipolar neurons frequently have a fusiform shape and, depending upon the plane of section, they can appear to have only a moderate amount of rough endoplasmic reticulum. Conversely in equatorial sections, the bipolar neurons may appear pale and round and resemble small multipolar neurons. It may be for these reasons that the bipolar neurons have been labelled as an “intermediary” type of neuron by some investigators (23). However, neurons like the bipolar cell clearly exist in the rat, since Gulley and Wood (37) have given a detailed description of the ultrastructure of a medium sized neuron in the rat SNpc that obviously corresponds to our bipolar cells.

Numerous studies have reported the existence of the small multipolar type of neuron in the substantia nigra of the rodent (39), monkey (40), and human (24), and most of these investigators agree on the morphological features
of this type of neuron. It has a small, ovoid to round cell body, an irregular nuclear profile, and lacks definite Nissl bodies. The small multipolar neuron is rarely encountered in Golgi preparations, but, when it does impregnate, its processes are seen to be thin and to arborize locally with no distinct polarity. Most authors have regarded the small multipolar neuron as a local circuit cell that may have an inhibitory role (41), and although the neurotransmitter content of these neurons has not been defined, the small multipolar neurons in the monkey (40) as well as the rat (35) are known not to contain dopamine. Our results suggest that the small multipolar cells in the monkey SNpc are GABA-ergic. This is in agreement with the observations of Smith et al (42) who examined the neurons labeled with a GABA antibody in the basal ganglia of the squirrel monkey and found the labeled neurons in SNpc to be of moderate number and of small size. Ford et al (43), also have reported small (10 μm to 15 μm) and small to medium (up to 20 μm) sized, GAD-positive cells codistributed with other cells in the rat SNpc.

Age-related Changes in the SNpc

The results of the present study suggest that the 3 types of neurons in the SNpc differ in the age-related morphological changes that they display. For example, the small multipolar neurons accumulate more lipofuscin than the large multipolar and the bipolar neurons. Furthermore, some of the small multipolar neurons start to accumulate
Fig. 13. In the lower portion of this electron micrograph is the periphery of a spheroid, which has a filamentous peripheral cytoplasm (p), and a core of dense granules. The bounding membrane of the spheroid is forming a zonula adherens (za) with an astrocytic process (As) in the surrounding neuropil. A 32-year-old monkey. \( \times 20,000 \).

Fig. 14. An unidentified structure in the neuropil. The structure has dense granular material in its cytoplasm, and it is surrounded by many astrocytic processes (As) that contain glycogen particles (g) and bundles of filaments (f). A 32-year-old monkey. \( \times 10,000 \).
lipofuscin much earlier than the other neurons so that they can have large accumulations even in monkeys less than 10 years of age. Different patterns of neuronal accumulation of lipofuscin have also been observed in other regions of the brain. For example in the cerebral cortex, the Betz cells in the motor cortex display marked accumulations of lipofuscin and age-associated decrease in perikaryal size in older monkeys (18), while the Meynert cells of the visual cortex and pyramidal cells of the prefrontal cortex accumulate only little lipofuscin (15, 17).

Another neuron specific age change is the formation of the Marinesco bodies. They only occur in the large multipolar neurons. These bodies have been previously described in the neurons of human substantia nigra and locus coeruleus, where they are associated with increasing age and are thought to originate from the nucleolus (28). The reason why these inclusions have a predilection for the large multipolar neurons is not known, but Curcio et al (44) have also observed a similar situation in rat pyriform cortex, in which rod-like intranuclear inclusions are only common in neurons in the superficial portion of layer II.

In view of our morphometric studies showing age-related neuronal loss in SNpc (13), one objective of this study was to determine if there is any ultrastructural evidence of age-related neuronal degeneration and loss. However, no entities that could be characterized as the cell bodies of dying neurons have been encountered. In retrospect this may not be surprising, because according to our morphometric data, the mean neuronal population in SNpc is about 150,000, of which about 25%, or 35,000 neurons are lost with age (13). If this loss occurs progressively over a span of about 25 to 27 years, then only about 1,300, or less than 1% of the neurons would be lost each year. Consequently, only a few dying neurons would be expected to be present at any particular point in time, and since only a minute fraction of the SNpc can be sampled by electron microscopy, there is a slender chance that the perikaryon of a dying neuron will be encountered. The time scale and the mechanism of age-related neuronal death in SNpc has not been determined. It is possible that neurons are being lost by an apoptotic process, in which case the cell death would occur rapidly without inflammation and involve single cells or small...
group of cells dying in an asynchronous fashion (45). This would make it difficult to observe neurons undergoing apoptosis without use of specific techniques, such as in situ end labelling. This technique specifically labels fragmented DNA in apoptotic nuclei. It has been used to show apoptosis in the SNpc in various neurodegenerative disorders (46, 47) and after metabolic injuries (48), and using the technique Tompkins et al (46) have recently reported apoptotic-like changes in the neurons in the normally aging human SNpc.

The profuse hypertrophy of astrocytic processes in the neuropil may be an indicator of the age-related loss of neurons from the SNpc, since in the old monkeys large areas are occupied by astrocytic processes and it may be that these are scars that have replaced lost neurons or their processes. In this context, Lindsay (49) has reported that in response to experimental lesions in the brain, astrocytes proliferate and occupy sites that were previously occupied by the neurons and their processes. Similarly, reactive astrocytosis is a feature of age-related degenerative disorders that are characterized by a neuronal loss in SNpc, such as Alzheimer disease, Parkinson disease and Huntington disease (50). Interestingly, in the same population of old monkeys, Peters et al (15) and Vincent et al (16) have not reported a comparable astrocytosis in the cellular layers in the prefrontal and visual cortices, in which the neurons show only mild age-related changes. However, in these same cortices, layer I becomes thinner with age due to degeneration of the terminal tufts of pyramidal cell apical dendrites and a loss of some of the synapses, and this leads to astrocytic hypertrophy (51). This hypertrophy is evident as a thickening of glial limiting membrane and an increase in the number and thickness of astrocytic processes in the neuropil.

The age-related astrocytosis in SNpc is accompanied by the appearance of abnormal profiles that resemble the “foamy spheroid bodies” or ‘spheroids’ that have been previously reported in the globus pallidus and substantia nigra pars reticulata of humans and old world monkeys (30, 31). Although the origin of spheroids and the sequence of events leading to their formation are not known, a number of observations suggest the spheroids may be astrocytic in origin. First, these structures usually have irregular shapes that follow the contours of the surrounding neuropil, a feature generally displayed by the astrocytic cells and their processes (50). Second, bundles of thin filaments have been observed in the spheroids, and filaments occupy their pale peripheral cytoplasm. Third, the membranes of these structures sometimes form adhering junctions with the adjacent astrocytic processes, a feature typical of astrocytic processes (52). Finally, dense granules, similar to the glycogen particles present in a typical astrocytic process (52), have been observed in the periphery of some of the spheroids. No nuclei have been found within these spheroids, and so it is likely that they represent distended processes and not the perikarya of astrocytes.

The spheroids have a core of electron-dense granular debris, together with dense secondary lysosomes and residual bodies. Whether this material represents degenerating astrocytic organelles or phagocytosed material is not known, but as shown by Bronson and Schoene (31), the spheroids appear to be a feature of normal aging in monkeys. In any case, the spheroids appear to be clinically “silent,” since the medical histories of the animals included in the present study were unremarkable.

In contrast to the paucity of age-related changes in the neuronal perikarya in SNpc, dendrites in old monkeys display degenerative changes. The dendrites commonly appear to be swollen, which is consistent with the beaded and deformed appearance of dendrites reported by Cruz-Sánchez et al (14) in the Golgi preparations of substantia nigra in older humans. Furthermore, the cytoplasm of many dendrites appears pale and watery, and organelles such as rough endoplasmic reticulum, mitochondria, and microtubules are sparse. Membranous whorls and clear vacuoles are frequently observed and they are similar to those reported in the degenerating dendrites in the brainstem of older rats (53). However, the dark inclusions observed in some dendrites seem not to have been described previously. These inclusions differ from the lipofuscin pigment that has been reported to accumulate in dendrites with increasing age in monkeys (54) because they are more electron dense and do not have the pale component typically associated with the lipofuscin pigment. Also, these inclusions are probably not related to melanin, because we have not encountered melanin granules in the neurons in SNpc. This is consistent with the observations of Schwy and Fox (21) who previously reported a lack of melanin granules in the neurons of SNpc in Macaca mulatta.

Axons in the neuropil of the aging monkey SNpc also show age changes. Most commonly the changes affect the integrity of their myelin sheaths, which frequently show split lamellae bounding dark cytoplasm. Such alterations in the integrity of the myelin are also reflected by the fact that oligodendrocytes in the aging SNpc have inclusions in their cytoplasm and sometimes show enlarged processes, as they do in the cerebral cortex (33). Usually, however, the axons themselves appear to be unaffected by age, although as stated, we have encountered large profiles that contains a core containing numerous neurofilaments and a periphery in which lysosomes predominate. These profiles undoubtedly represent swollen axons similar to those encountered in giant axonal neuropathy (32), an inherited disorder, in which the axons develop focal, bulbous swellings.

In summary, the age-related morphological observations in SNpc strongly suggest that it is vulnerable to aging. Changes such as those displayed by the dendrites,
axons, and glial cells of the SNpc have been encountered, but much less extensively, in the cerebral cortex. Indeed, the profuse astrocytosis that occurs in the SNpc in old monkeys is remarkable. The presence of extensive astrocytic scarring could be taken to indicate that these cells and their processes are occupying spaces from which neuronal cell bodies and their processes have been lost. But despite the fact that our morphometric studies indicate that there is a loss of neurons from the aging SNpc, no identifiable dying neurons have been seen.

ACKNOWLEDGMENTS

We wish to thank Dr. Thomas Kemper for his advice in interpreting the images that we encountered in this study and also for his critical reading of the manuscript. Our thanks are also given to Ms. Claire Sethares for her skilled technical assistance.

REFERENCES

35. Domescic V B, Stinus L, Paskevich P A. The cytology of dopaminergic and non-dopaminergic neurons in the substantia nigra and ventral tegmental area of the rat: A light and electron microscopic study. Neurousci 1983;8:743-65
SIDDQUI AND PETERS


42. Smith Y, Parent A, Segula P, Descaries L. Distribution of GABA immunoreactive neurons in the basal ganglia of the squirrel monkey (Saimiri sciureus). J Comp Neurol 1987;259:50–64.


Received January 6, 1999
Revision received April 7, 1999
Accepted April 9, 1999