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Brain Aging in Normotensive and Hypertensive Strains of Rats*

II. Ultrastructural Changes in Neurons and Glia

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Summary. A variety of age-related changes occur in the structure of neurons in the cerebral cortex of Wistar-Kyoto and spontaneously hypertensive rats. The most marked alteration associated with increasing age was the deposition of lipofuscin pigment, primarily at the bases of apical dendrites of pyramidal neurons. While no strain-related differences in the amount of lipofuscin pigment were observed in the youngest (3 months) and in the aged (22-27 months) groups of rats, it appeared that hypertensive rats had larger pigment deposits at 12 months of age. At the ultrastructural level, neurons of the aged brains exhibited numerous nuclear invaginations and filamentous nuclear inclusions, increased amounts of Golgi complex and two types of cytoplasmic inclusions. The number of degenerative structures in the neuropil (membranous whorls, dystrophic axons and alterations in myelin sheaths) was also apparently increased in the aged brains. Neurofibrillary tangles were observed in dendritic processes of a 27-month-old Wistar-Kyoto rat. Glial cells accumulated distinctive pigment granules by which the three types of glia could be identified.

Key words: Aging – Hypertension – Neurofibrillary tangles – Lipofuscin – Neurons

The role of vascular factors in the development of the morphological alterations which occur in the aging brain remains poorly understood. Chronic hypertension appears to accelerate age-related changes which occur in blood vessels (Karsner 1938), but the secondary effects of this process on nervous tissue have not as yet been evaluated at the ultrastructural level.

During normal aging, neurons undergo a series of changes in their cell bodies and processes. Within the perikaryon, marked alterations in the amount and distribution of certain organelles and pigments are observed (see reviews by Brizzee et al. 1976, 1980). Dendritic processes of neurons in aged individuals undergo loss of dendritic spines and are shrunken and distorted from their normal profiles (Scheibel and Scheibel 1975). Axons exhibit a variety of ultrastructural changes in aged mice (Samorajski et al. 1971). The effect of chronic hypertension on brain aging is currently unknown. In an earlier study of the zona reticularis of the adrenal cortex, Nickerson et al. (1980) reported that 95-week-old normotensive rats had a greater accumulation of age pigments than age-matched hypertensive rats. The purpose of the present investigation was to examine the ultrastructural characteristics of neurons and glia in aging normotensive Wistar-Kyoto rats and in the spontaneously hypertensive rats.

Material and Methods

A minimum of four female normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) were used in young (3 months), middle-aged (12 months), and aged (22–27 months) groups. The rats were given an i.v. injection of horseradish peroxidase for studies of blood-brain barrier permeability. They were subsequently killed by intracardiac perfusion of a fixative containing 1% glutaraldehyde and 1% paraformaldehyde. Blocks of frontal cortex (Kreig's area 10) were removed, osmicated, dehydrated, and embedded in Epon 812, as described previously (Knox et al. 1980). One-micrometer-thick sections were stained with toluidine blue and examined with a Zeiss II photomicroscope. Ultrathin sections exhibiting gold or silver interference colors were stained with uranyl acetate and lead citrate and examined in a Siemens 101 electron microscope.

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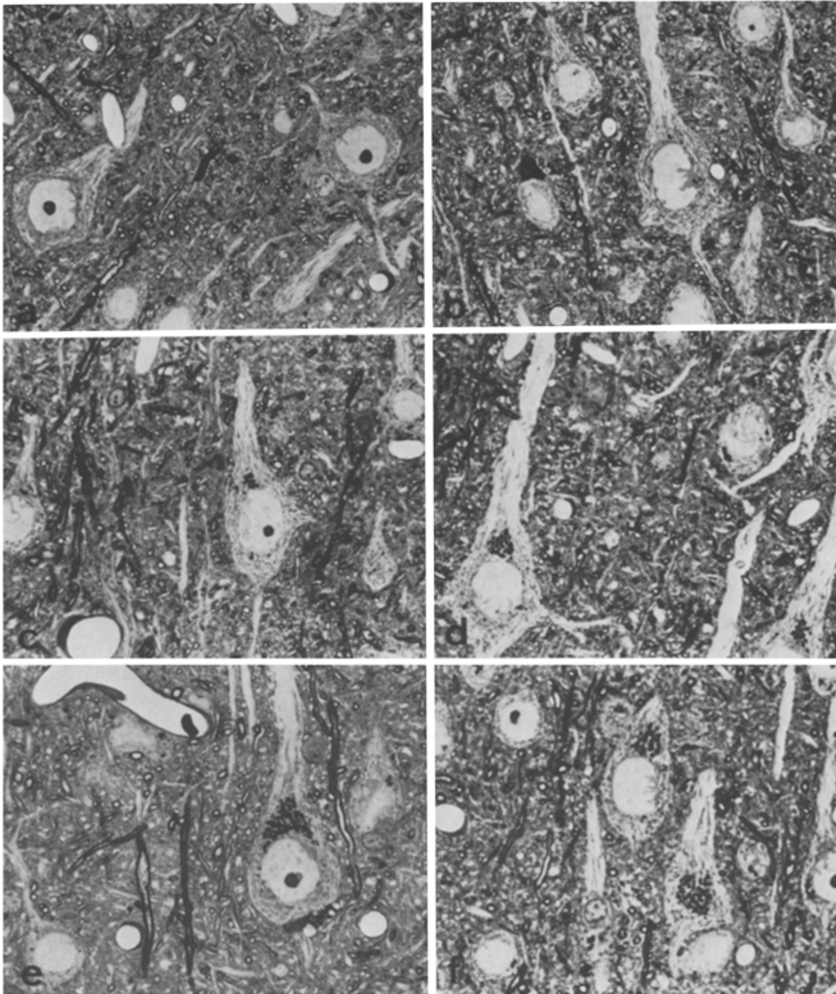


Fig. 1a–f. Layer V pyramidal neurons in young, mature, and aged rats of normotensive (left) and hypertensive (right) strains. **a** Two pyramidal neurons with distinct nucleoli are seen in this photomicrograph. Several small pigment granules are scattered throughout the cell bodies; 3-month WKY. Toluidine blue (TB). ×608. **b** The neuron in the center of the field contains well-developed Nissl substance with few pigment granules; 3 months SHR. TB. ×656. **c** In 12 months WKY, pigment granules are larger than in 3 months rats and are preferentially clustered at the bases of the appical dendrites. TB. ×656. **d** When compared to 12 months WKY, age-matched hypertensive rats sometimes exhibit greater accumulation of pigments within neuronal cell bodies. The neurons in this photomicrograph contain pigment deposits which approach the amount of pigment seen in aged rats; 12-month SHR. TB. ×709. **e** In aged WKY, pigment deposits may occupy large portions of the cell body. The smaller neurons of lamina V contain proportionately fewer pigment granules; 24-month WKY. TB. ×634. **f** Pigment deposits at the bases of the apical dendrites in neurons of 23 months SHR. TB. ×522

Results

In light-microscopic studies, it appeared that lipofuscin accumulation in neuronal cell bodies was proportional to the age of the animal. Few pigments were observed in neurons of 3-month-old WKY and SHR (Fig. 1a, b). At 12 months of age, moderate amounts of pigment were observed in neurons of both strains, but the hypertensive rats often appeared to exhibit a greater degree of pigment accumulation than age-matched normotensive animals (Fig. 1c, d). The aged rats of both strains exhibited extensive deposits of pigmented inclusions which appeared to occupy up to 50–60% of the perikaryon (Fig. 1e, f). The large pyramidal neurons in lamina V accumulated correspondingly larger pigment deposits than the smaller neurons of laminae II and III.

When examined by electron microscopy, neuronal lipofuscin granules were characteristically composed of an electron-dense core, a peripheral, electron-lucent vacuole, and a single limiting membrane (Fig. 2a). They were often scattered throughout the cytoplasm and apical dendrite, but were accumulated preferentially at the bases of the apical dendrites of large pyramidal neurons. Glial cells also accumulated distinctive pigment granules. Lipofuscin granules of astrocytes often had ultrastructural characteristics similar to those of neurons (Fig. 2b). In contrast, oligodendrocyte granules were more electron-dense than those of neurons and astrocytes. Oligodendrocyte granules usually contained distinctive electron-lucent clefts within the granules (Fig. 2c). Microglia accumulated a large, pleomorphic array of granules with similarities to the granules found in other glia (Fig. 2d). No other age-related changes were observed in the cell bodies of glial cells.

In addition to the accumulation of lipofuscin granules, several other age-related changes were observed in the amount and distribution of neuronal organelles.

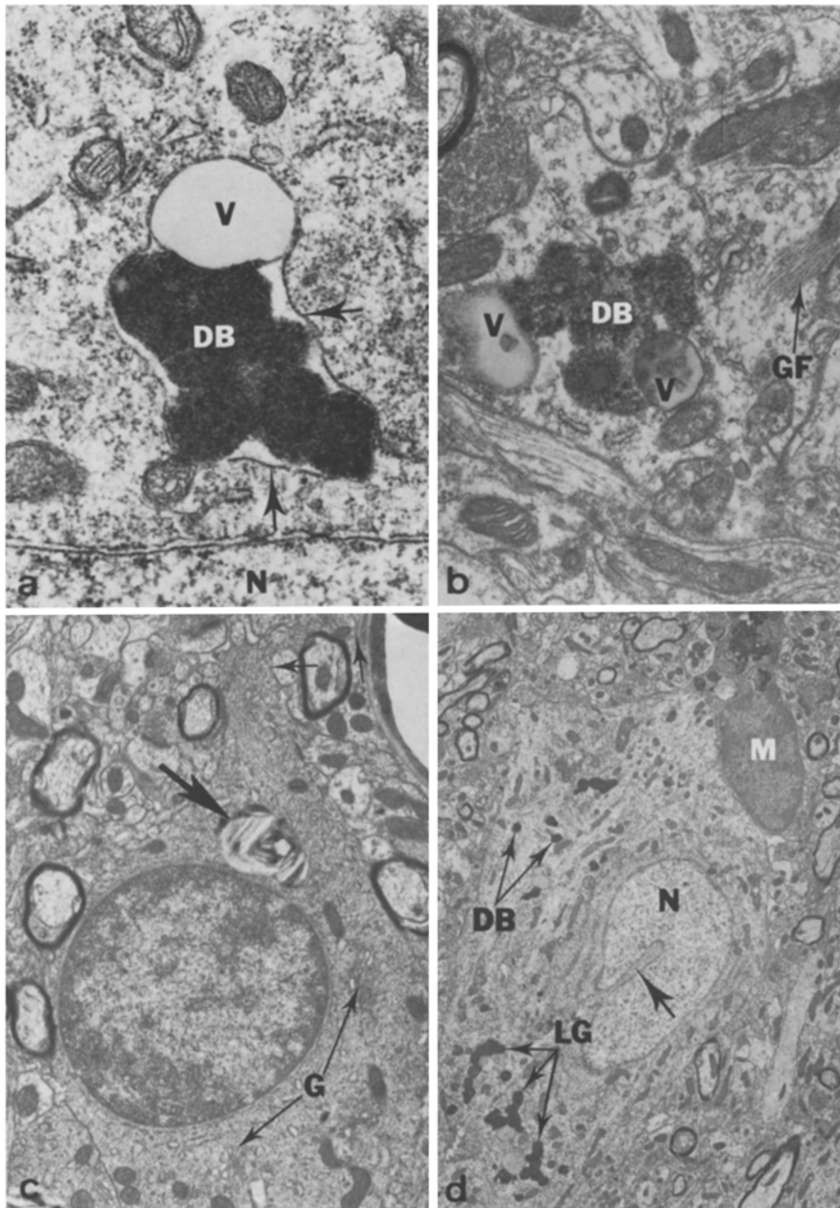
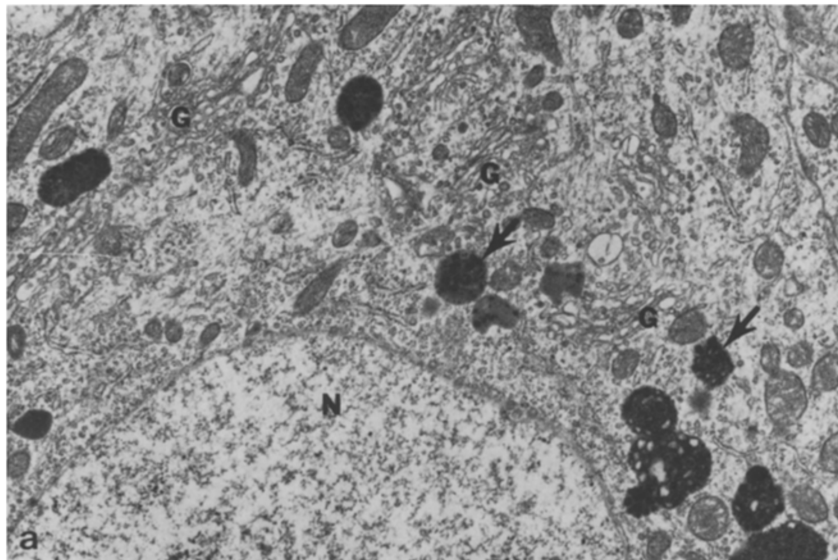


Fig. 2. **a** The characteristic neuronal lipofuscin granule contains three components: an electron-dense portion (dense body, *DB*), an electron-lucent vacuole (*V*), and a single limiting membrane *(arrows)*. Neuron nucleus, N; 12-month WKY. ×22,000. **b** The age pigment seen in this astrocytic process closely resembles the neuronal lipofuscin granule. The dense portion *(DB)* contains pleomorphic granules and is attached to two electron-lucent vacuoles *(V)*. The entire structure is invested by a single limiting membrane. Glial filaments, *GF*; 27-month WKY. ×12,700. **c** An oligodendrocyte with a perivascular process *(small arrows)* contains a characteristic pigment granule *(large arrow)*. The granule consists of electron-dense portions which are separated by large, electron-lucent clefts. Microtubules, rough endoplasmic reticulum, mitochondria, and two Golgi complexes *(G)* are seen in the dense cytoplasm of this cell. The nucleus is very electron-dense with a thick ring of clumped peripheral chromatin; 27-month WKY. ×6,200. **d** A neuron and a satellite microglial cell are seen in this photomicrograph. The neuron nucleus *(N)* contains a prominent nuclear invagination *(arrow)*. Numerous dense bodies *(DB)* and lipofuscin granules *(LG)* are seen in the cytoplasm. The microglial cell *(M)* contains granules with characteristics of both oligodendrocytic and astrocytic granules. Microglia contain little cytoplasm within the cell body; 27-month WKY. ×3,200

Many neurons exhibited an apparent increase in the amount of Golgi complexes in the perikaryon (Fig. 3a). Numerous stacks of cisternae of rough endoplasmic reticulum (RER) were observed in the neurons of the aged animals, but the accumulation of lipofuscin and Golgi complexes tended to displace the RER cisternae to peripheral portions of the perikaryon. While nuclear invaginations and filamentous nuclear inclusions were observed in neurons of young animals, these structures appeared to increase with the age of the rats (Fig. 3b, c). The nuclear inclusions often displayed a distinct lattice structure and measured up to 8 μm in length. Several types of cytoplasmic inclusions were observed in neurons of the aged rats. Nematosomes, originally described by Grillo (1970), were observed in approximately 1% of the perikarya examined by electron microscopy in the aged rats (Fig. 4a, b). Nematosomes consisted of numerous electron-dense strands measuring 40–50 nm in diameter which were embedded within an electron-lucent matrix. Filaments measuring 6–7 nm in diameter interconnected adjacent strands. Similar filaments bridged the intracellular space between nematosomal strands and a variety of adjacent organelles. A single nematosome was observed in a 12-month WKY (Fig. 4c); none were observed in the youngest age groups. Several examples of a second



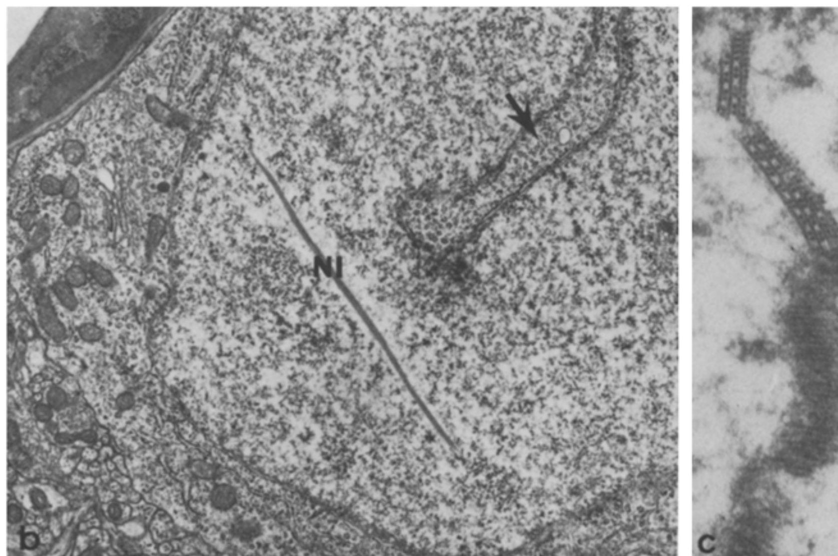


Fig. 3. **a** Neurons of the aged brain often exhibit an apparent increase in the number of Golgi complexes. In a neuron of a 24-month WKY, the perinuclear region is occupied by numerous Golgi complexes *(G)* and dense bodies *(arrows)*. Neuron nucleus, *N.* × 8,500. **b** The nucleus of a pyramidal neuron contains a filamentous nuclear inclusion *(NI)* and a large nuclear invagination *(arrow)*. An endothelial nucleus can be seen in the upper left; 24-month SHR. × 10,800. **c** Nuclear inclusions may exhibit a complex substructure. In this inclusion, a lattice-like arrangement of filaments measuring approximately 85 Å in diameter is seen. Note the striking difference in the appearance of the nuclear inclusion when sectioned in different planes at the top and bottom of the photomicrograph; 24-month SHR. × 79,700

cytoplasmic inclusion (Fig. 4d) were also observed in the aged rats. Both types of cytoplasmic inclusions measured approximately 1 μm in diameter.

A variety of degenerative changes were observed in the neuropil of the aged rats. While similar changes were occasionally observed in younger rats, the variety and prevalence of such alterations appeared to increase with the age of the experimental animals. Dendrites in aged rats often contained whorls of membranous material (Figs. 5a, b and 7c). Neurotubules in dendritic processes were occasionally distorted from their normal rectilinear profiles (Fig. 5c). Bundles of paired, helicallywound filaments were observed in the oldest animal used in this study (27-month WKY). These filaments measured 10 nm in diameter and exhibited a helical periodicity of approximately 34 nm (Fig. 6). Dystrophic axons and degenerative changes in the myelin sheaths (Fig. 7a–c) were also observed in the neuropil of the aged normotensive and hypertensive rats. The number of degenerative structures was not apparently different between the two strains.

Discussion

Our results confirm and extend earlier observations regarding the effects of aging on neurons of the rodent

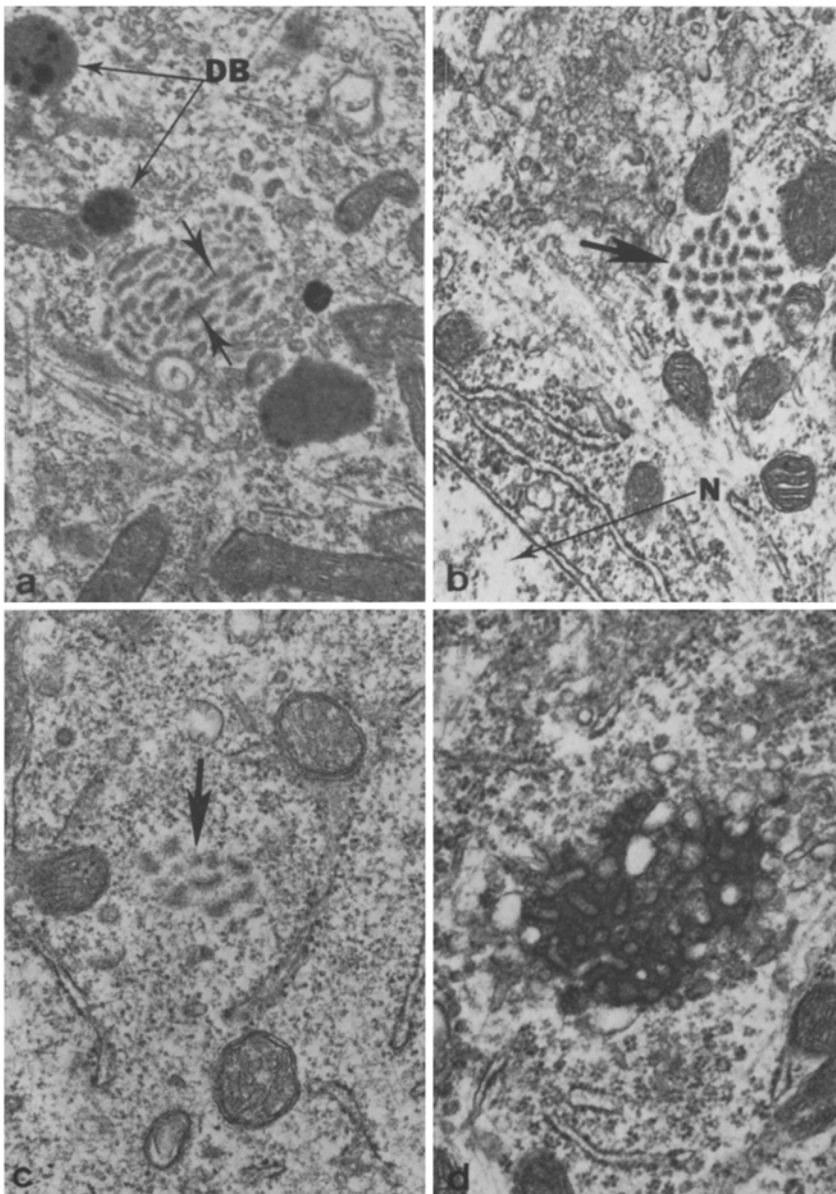


Fig. 4. **a** The nematosome consists of numerous electron-dense strands cut in cross-section and in longitudinal-section *(arrows)* embedded within a more electron-lucent matrix. No limiting membrane is present. Dense body, *DB;* 27-month WKY. ×27,100. **b** A perinuclear nematosome *(arrow)* is surrounded by numerous mitochondria. Neuron nucleus, *N;* 24-month WKY. ×23,230. **c** Nematosome in 12-month WKY is shown at arrow. ×21,110. **d** This unusual cytoplasmic inclusion consists of an electron-dense matrix which is pierced by numerous tubule-like structures; 27-month WKY. ×34,880

brain. In neurons of the lateral vestibular nucleus of aged rats, Johnson and Miquel (1974) also observed large numbers of nuclear invaginations and nuclear inclusions, disorganization of RER, and increased amounts of Golgi complex. Field and Peat (1971) reported a statistically significant increase in the number of nuclear inclusions in cortical neurons of aged mice. Anterior horn motor neurons of aged mice are characterized by the accumulation of lipofuscin pigments and a loss of RER, free ribosomes, neurofilaments, and mitochondria (Sekhon and Maxwell 1974). Hasan and Glees (1973) described increased numbers of nuclear invaginations and disorganization of RER in the perikarya of hippocampal neurons in aged rats. They also reported an increase in the number of neurofilaments and neurotubules in the dendritic processes and a decrease in the number of axosomatic synapses. .The present study of the cerebral cortex supports many of the findings described in these earlier qualitative studies. We observed an age-related dispersion of RER, an increase in the amount of Golgi complex, and greater numbers of nuclear invaginations and nuclear inclusions in pyramidal neurons. Recent quantitative studies of age-related changes in neuronal perikarya of the rat olfactory bulb (Hinds and McNelly 1979) and auditory cortex (Vaughan and Vincent 1979) have

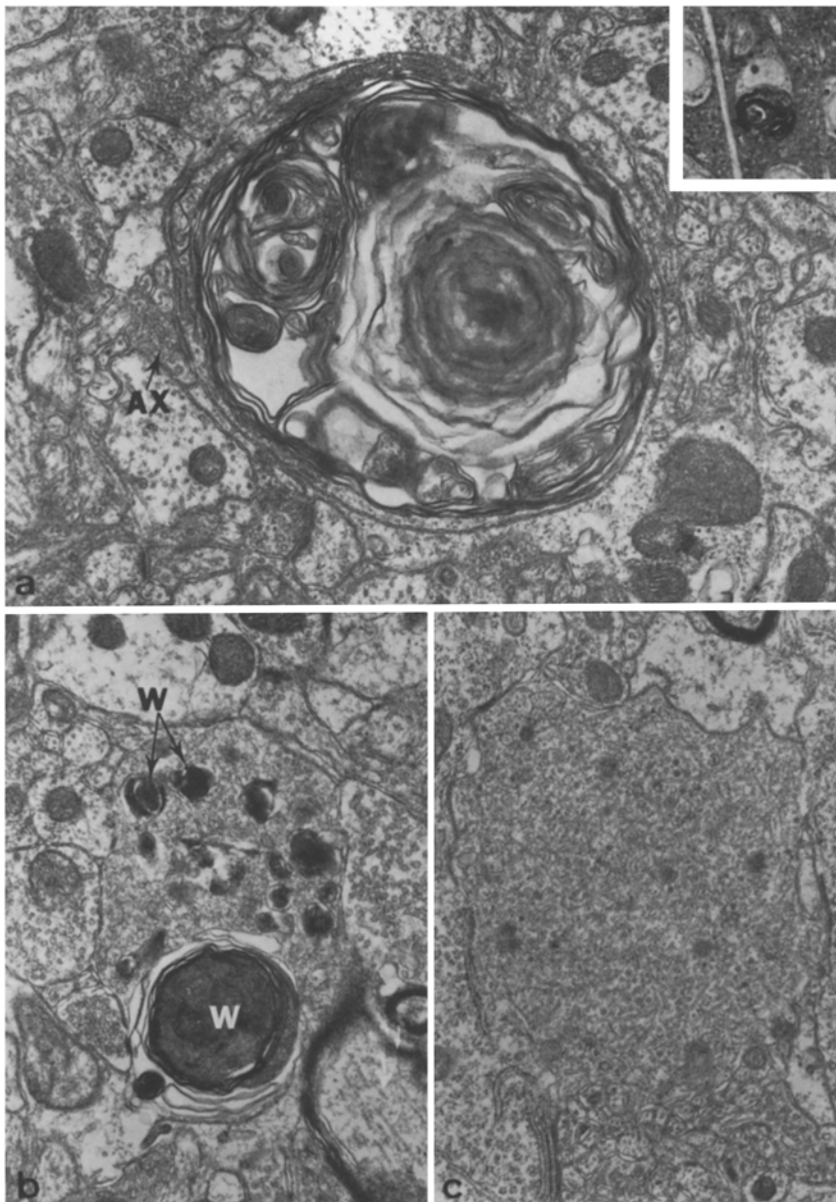


Fig. 5. **a** Multilamellar whorls of membranous material fill the process in the center of the field. An axon *(AX)* may be making synaptic contact with this process; 24-month SHR. ×9,830. *Inset:* Light-microscopic appearance of a similar whorl located adjacent to a neuronal cell body; 22-month SHR. Toluidine blue. ×700. **b** A degenerating process contains only electron-dense whorls *(W)* and a vesicular material; 27-month WKY. ×14,560. **c** A dendrite-like process is completely filled with intertwining and convoluted neurotubules; 27-month WKY. ×14,740

confirmed many of the earlier qualitative studies. Our observation of an increased accumulation of lipofuscin pigments in 12-month SHR (as compared to age-matched WKY) may indicate accelerated brain aging in this strain. Quantitative ultrastructural studies would provide significant data with regard to this hypothesis.

The presence of nematosomes in neurons of the cerebral cortex has not been previously reported. While only one nematosome was observed in neurons of the middle-aged rats, they were observed in approximately 1% of neuronal cell bodies in the aged rats. Multiple nematosomes within a single cell body were not observed. The function of this organelle remains unknown.

The present study demonstrates that paired helical filaments (PHF) may occur in the brain of aged rodents. While PHF have been observed in the hippocampus of aged rhesus monkeys (Wisniewski et al. 1973) and appear to be one of the most consistent findings in the brain of aged humans (Matsuyama et al. 1965), these structures have not been previously described in the brains of aged rodents. PHF are not specific to the aging process, however, since they have also been observed in a variety of neuropathological conditions, including lead encephalopathy (Nicklowitz and

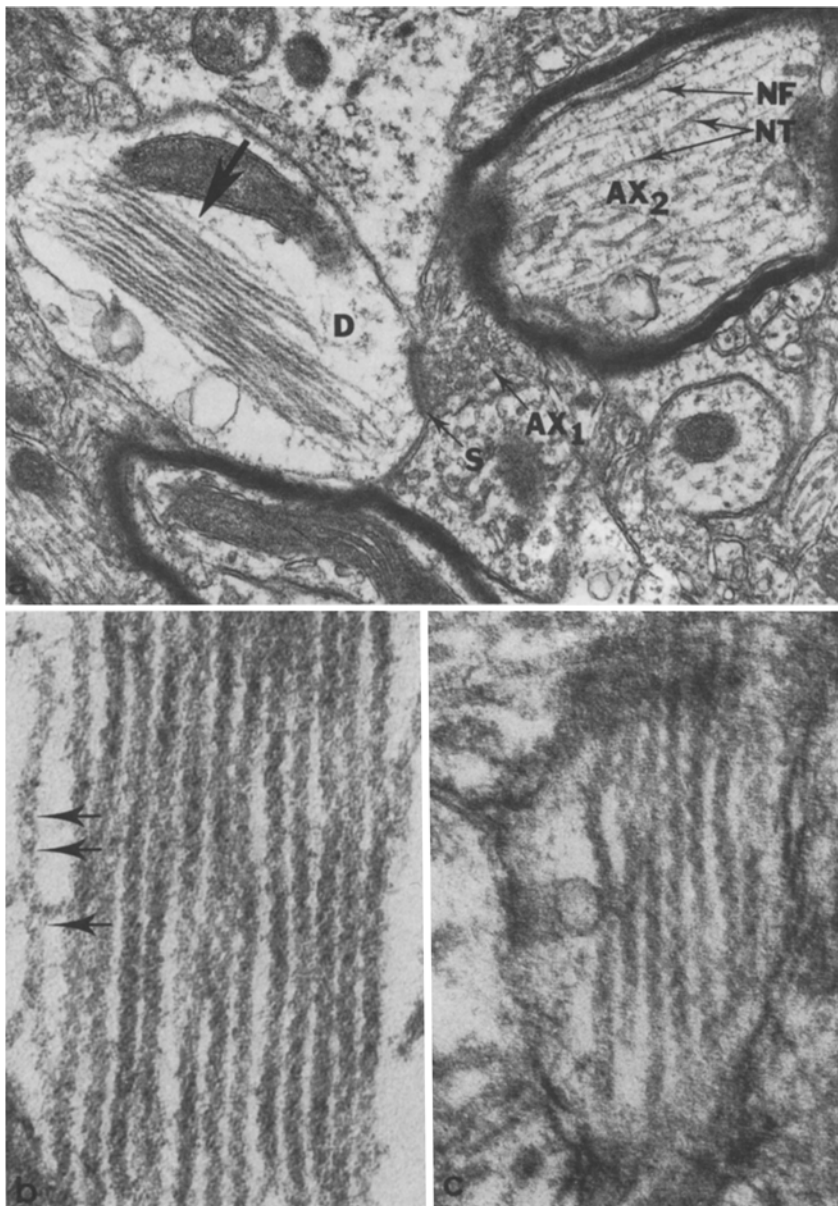


Fig. 6. a An axon terminal *(AX_1)* makes synaptic contact *(S)* with a dendritic process *(D)* containing a large bundle of paired helical filaments *(arrow)*. The normal structure of neurofilaments *(NF)* and neurotubules *(NT)* can be seen in the myelinated axon *(AX_2)* on the right; 27-month WKY. ×38,900. **b** Higher magnification from the region of the arrow in **a**. These structures appear to be pairs of helicallywound 10 nm neurofilaments rather than periodically constricted or twisted neurotubules *(arrows)*; 27-month WKY. ×142,700. **c** A bundle of paired helical filaments fills another dendritic process in 27-month WKY. ×106,000

Mandybur 1975), sclerosing angioma (Liss et al. 1979), neurovisceral lipidosis (Horonpian and Yang 1978), Down's syndrome, Guam-Amyotrophic Lateral Sclerosis-Parkinsonism-Dementia complex (Wisniewski et al. 1976), post-encephalitic Parkinsonism, Alzheimer's pre-senile dementia, and senile dementia (Wisniewski et al. 1970). The helical periodicity of these filaments in neurons of the human brain (65–80 nm), however, differs from the periodicity of PHF in neurons of aged monkeys (50 nm) and rats (34 nm). Our study supports the hypothesis that formation of PHF represents a non-specific neuronal response to a number of experimental and clinical conditions (Mortimer 1980).

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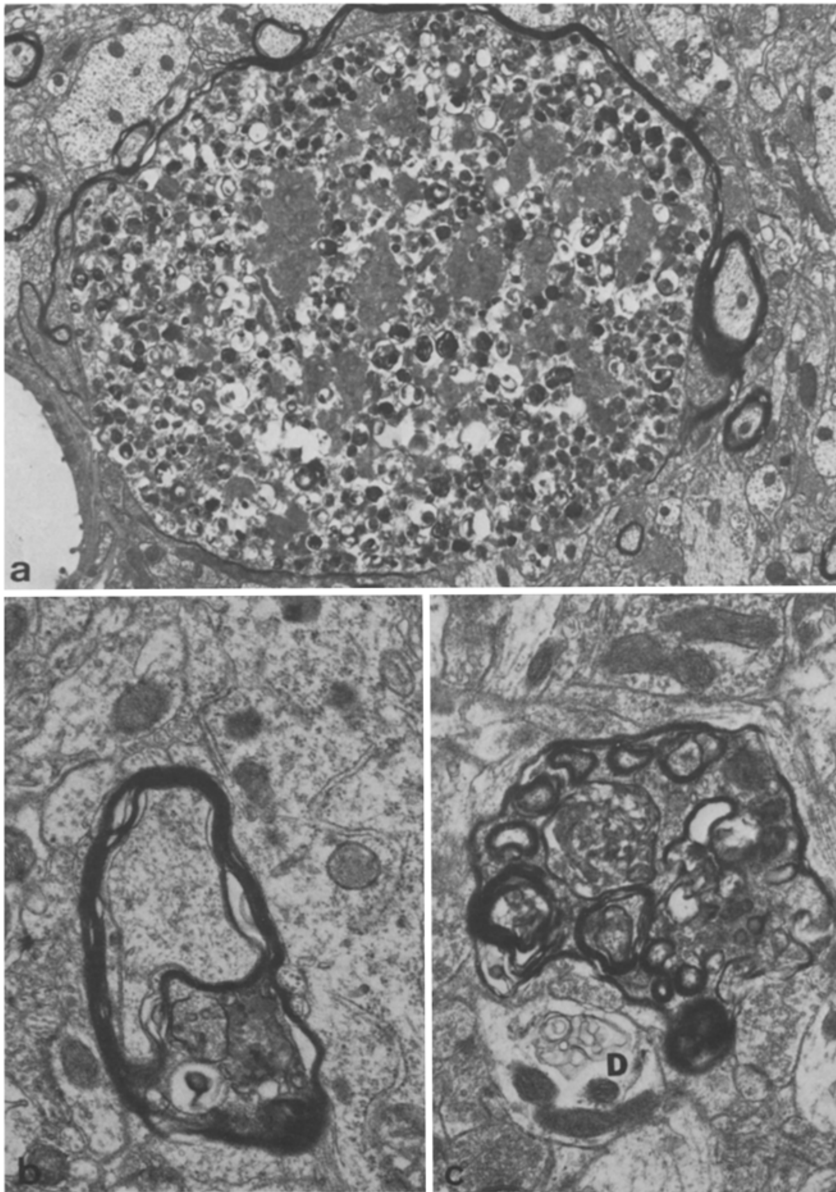

Fig. 7. a A perivascular myelinated axon displays the ultrastructural characteristics of neuroaxonal dystrophy. Membranous whorls, vacuoles and other degenerative structures can be seen within the axoplasm of this distended process; 24-month SHR. ×4,900. **b** Degenerating myelin sheath in 27-month WKY. ×9,600. **c** Myelin-like whorls fill the interior of a myelinated axon. An adjacent dendrite *(D)* contains a membranous structure. 27-month WKY. ×10,900

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