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Original Article

Histological structure of midbrain red nucleus in albino rats at different ages

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ABSTRACT

This study was conducted on twelve healthy male albino rats to investigate the histological structure of the midbrain red nucleus at different ages and to correlate these changes using light and electron microscopic preparations.

The animals were classified into three groups: neonates (early and late), adult and senile. Upon sacrifaction, midbrain samples were prepared for light microscope (L/M) examination using haematoxylene, eosin and silver stains. Other minute parts were processed for transmission electron microscopic examination.In this study, L/M examination revealed that the nerve cells of red nucleus were relatively small at the age of 7th and 21st days and became relatively larger at the age of 4 months of life, followed by reduction again occurring at senility. In the 7th days of life there was a morphological maturation affecting the nerve cells of the red nucleus was noticed by electron microscopic examination in the form of appearance of synapses. Each neuron contained a nucleus, which varied from eccentric to central position. Nuclei were usually indented and the cytoplasm contained prominent mitochondria, Golgi apparatus and rough endoplasmic reticulum. The neuronal cell membrane was regular. Growth cone could be detected, which had been seen at 7th and 21st days of life. At senile group the small nerve cells were detected in section of red nucleus in the form of darkly stained cytoplasm, ill-defined dark nucleus and irregular shape of nerve cell and marked reduction in all the constituents of the cytoplasm. The same findings were also confirmed by studying the semithin section. In conclusion, the previously observed histological alterations could explain the age related changes affecting the red nucleus, therefore it wasn't surprising to find different locomotor's impactions occurred with aging. © **Neuroanatomy. 2009; 8: 7–14.**

Key words [histology] [midbrain] [red nucleus] [rat]

Introduction

The nervous system played its function on the skeletal movement properly, through continuous sensory feedback information concerning the effects of its action [1]. Upper motor neurons were neurons in the brain, which influenced the control of skeletal muscles by alpha and gamma motor neurons [2]. The pyramidal system, represented with the corticospinal tracts, was the most important output pathway from the motor cortex [3].

Concerning the extrapyramidal nuclei, the tegmentum contained the red nucleus which was present at the superior level of midbrain and the substantia nigra that occupied both the superior and the inferior levels of midbrain which lied posteromedial to the crus cerebri [4].

Regarding the histogenesis of tegmental extrapyramidal nuclei of midbrain, Hanaway et al. [5] decided that the neurons of red nucleus were produced in days 13th and 14th of gestational life. Red nucleus was formed of two parts, rostral (upper) two-thirds called parvocellular and caudal (lower) one–third called magnocellular. Development of these parts occurred with a caudal to rostral gradient; the cells of the magnocellular preceded slightly those of the parvocellular.

Neurons of the red nucleus contained many of neurotransmitters such as glutamate, aspartate, nitric oxide [6] and chondroitin-sulphate proteoglycan, which were mainly found in the extracellular matrix, neuropil [7].

Povlishock [8] described the electron microscopic picture of the neuron population in different ages of rat red nucleus. He mentioned that during the first week of life there was a rapid morphological maturation occurred concomitantly with the onset of synapse. Later on Povlishock et al [9] continued that somal morphogenesis took place in the second week of life in the form of increased both in size and cytoplasmic constituents, while in the third week of life, the changes were mainly in the nuclei which became central and showed a distinct regular membrane.

The objective of this study aims to investigate the red nucleus of albino rat midbrain on the base of its participation in the extrapyarmidal system as a part of its locomotor function. In the meanwhile, the changes occuring at various age groups will be correlated using light and electron microscopic preparations.

Material and Methods

In the present work, twelve male albino rats were used and the animals fed a standard balanced diet and had water ad-libitum. These animals were classified into three groups according to Buckland et al [10].

Group I included six male rats (15-35 gm) aging 1-3 weeks, which subdivided into:

- Subgroup IA (early postnatal group): included three rats at the 7th day of life.
- Subgroup IB (late postnatal group): included three rats at the 21st day of life.

Group II (adult group): included three rats (150-200 gm) at the 4th month of life.

Group III (senile group): included three rats (250-300 gm) at the 18th month of life.

In all examined age groups, zona compacta of substantia nigra was studied for its histological changes, whereas samples from the adult age were considered as if they were a control group. The animals were dissected after decapitation of the skull, the vault of the skull was exposed and the brain stem for substantia nigra was separated and prepared for light and electron microscopy.

Preparation for light microscopy

The rat brain was kept as a whole in Bouin's fixative, dehydrated in ascending grades of alcohol, cleared with xylol, embedded and impregnated in paraffin wax, sectioned at 5 µm thick sections and subjected to the following stains:

- Haematoxylin and eosin [11]: for studying the general structure of substantia nigra.
- Silver staining (Holmes' method) [12]: to demonstrate the nerve cells, axons and dendrites.

Preparation for electron microscopy [13]

Minute specimens of the substantia nigra were selected, doubly fixed in gluteraldehyde and osmium tetroxide, dehydrated in ascending grades of alcohol, cleared in propylene oxide, embedded in epoxy resin and semithin sections (0.5 µm) were taken and stained with toluidine blue [14] to detect the proper area for ultrathin sections that was also performed to detect Nissl granules of nerve

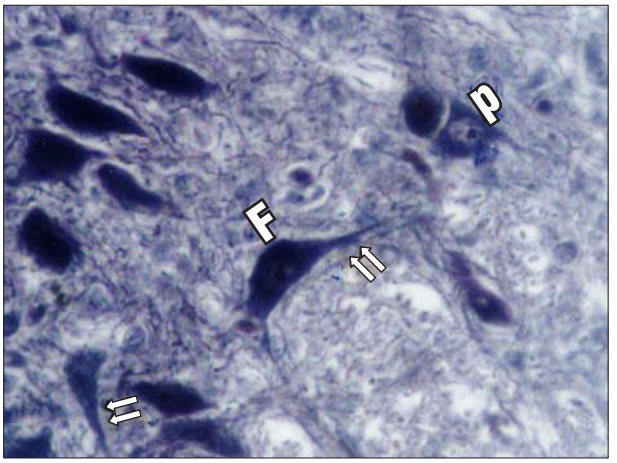

Figure 2. A photomicrograph of male albino rat midbrain on the 7th day of life showing axons (***double arrows***) of nerve cell of magnocellular red nucleus. Fusiform (***F***) and pyramidal (***P***) nerve cells are seen. (Holmes method for silver stain, X400)

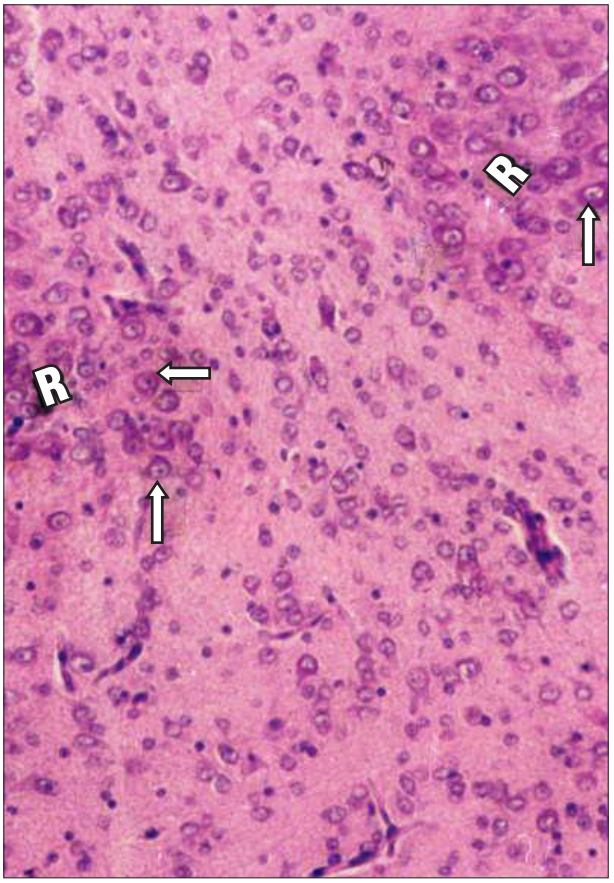

Figure 1. A photomicrograph of coronal section of male albino rat midbrain on 7th day of life showing the region of two magnocellular red nuclei (***R***). They are formed of a group of nerve cells (***arrows***). (HE, X100)

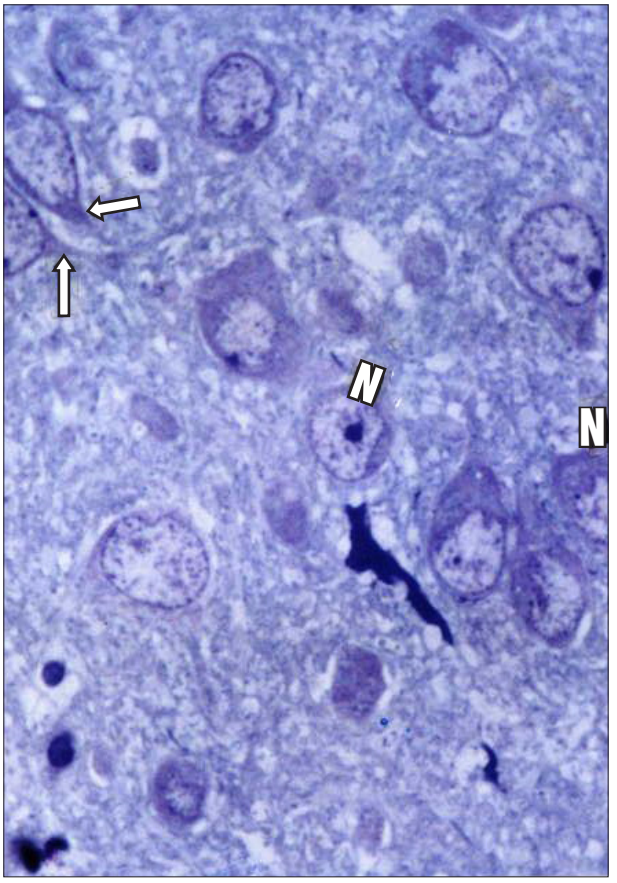

Figure 3. A photomicrograph of male albino rat midbrain on the7th day of life showing red nucleus nerve cells (***N***) with large prominent pale indented nuclei, prominent nucleoli. Cell processes (***arrows***) are seen. (Toluidine blue, X800)

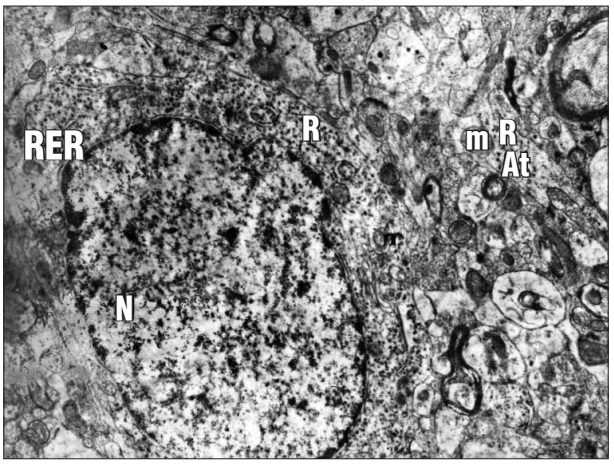

Figure 4. An electron micrograph of male albino rat on the 7th day of life showing nerve cell body with large central rounded euchromatic nucleus (***N***), numerous free ribosomes (***R***), mitochondria (***m***) and few cisternae of rough endoplasmic reticulum (***RER***). Axon terminal (***At***) containing mitochondria (***m***) and free ribosomes (***R***) are seen. (TEM, X9000)

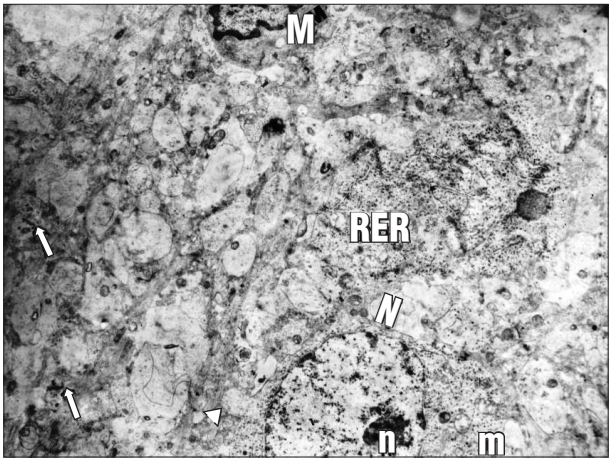

Figure 5. An electron micrograph of male albino rat on the 7th day of life showing red nucleus nerve cell with its large euchromatic nucleus (***N***), prominent nucleolus (***n***), rough endoplasmic reticulum (***RER***) and mitochondria (***m***). Growth cone (***arrow head***), synapses (***arrows***) and microglia (***M***) are seen. (TEM, X6500)

cells, glial cells, neuropil and myelin sheathes. Resin capsule previously prepared for the regions of substantia nigra were trimmed out, sectioned at 80-90 nm thick, stained with uranyl acetate and lead citrate, then examined at electron microscopy for the ultrastructure study of the nerve cells, myelin sheathes and neuropil.

Results

Subgroup I-A [early postnatal rats (7th day)]

Light microscopic examination. Sections of male albino rat of the midbrain at this age group showed two magnocellular red nuclei that are formed of group of nerve cells. Neither axons nor dendrites could be detected (Figure 1).

Silver stained sections at the same region of this group showed that the nerve cells of magnocellular red nucleus were of variable shapes; some of them were fusiform, while others were pyramidal. Axons of some cells could be seen (Figure 2).

The semithin sections stained with toluidine blue revealed that the red nucleus of this group had nerve cell with large prominent pale indented nuclei, prominent nucleoli and pale stained cytoplasm. The cells displayed processes, which appeared as direct extension of the cell membrane and contained cytoplasmic material (Figure 3).

Electron microscopic examination. Examination of ultrathin sections of this subgroup using an electron microscope showed that red nucleus nerve cells had large eccentric irregular euchromatic nucleus. The cytoplasm of these nerve cells contained numerous free ribosomes, mitochondria and few cisternae of rough endoplasmic reticulum. Neuropil (extraneuronal space) contained axon terminals with their large vesicles, mitochondria and synapses (Figure 4). Astrocytes could be seen having irregular large nuclei and scanty cytoplasmic organelles (Figure 5).

Subgroup I-B [late postnatal rats (21st day)]

Light microscopic examination. Sections of the superior level of midbrain of this group stained with H and E showed large nerve cells of the red nucleus than the

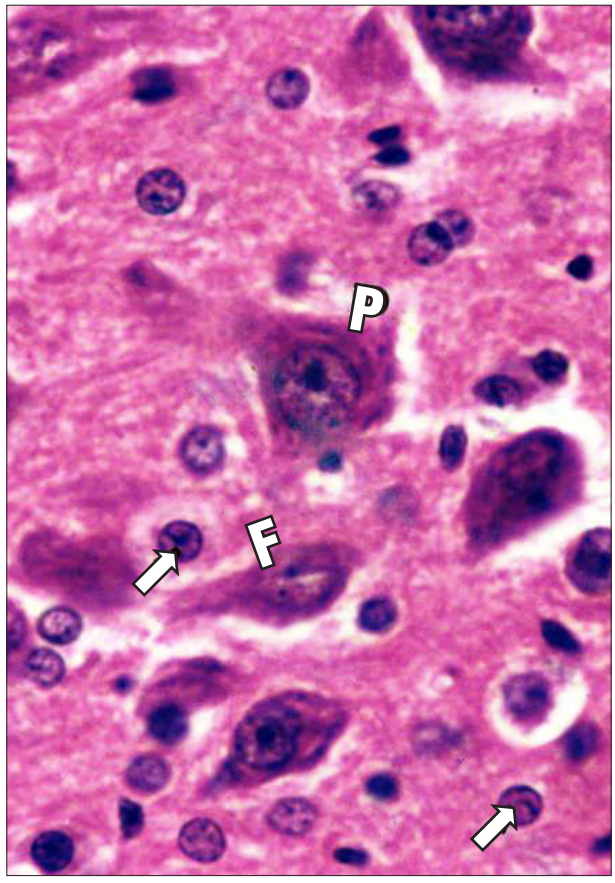

Figure 6. A photomicrograph of male albino rat midbrain on the 21st day of life showing large nerve cells of the red nucleus than the previous age. They are either pyramidal (***P***) or fusiform (***F***). Neuroglia cells are also seen (***arrows***). (HE, X400)

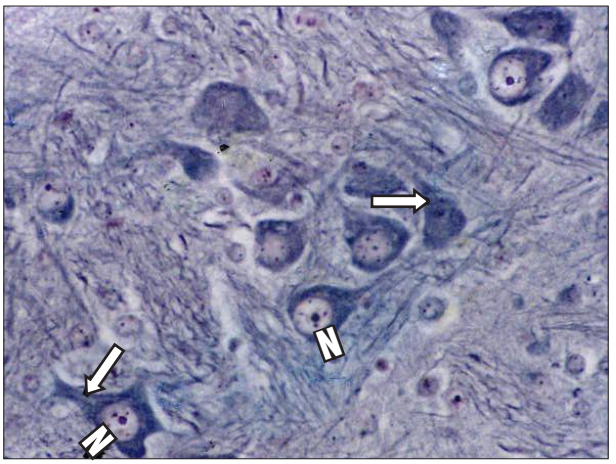

Figure 7. A photomicrograph of male albino rat midbrain on the 21st day of life showing nerve cells (***N***) of the magnocellular red nucleus with prominent nucleoli, unstained areas of nuclei and large area of cytoplasm. Angulations of the nerve cells and their axons (***arrows***) are seen. (Holmes method for silver stain, X400)

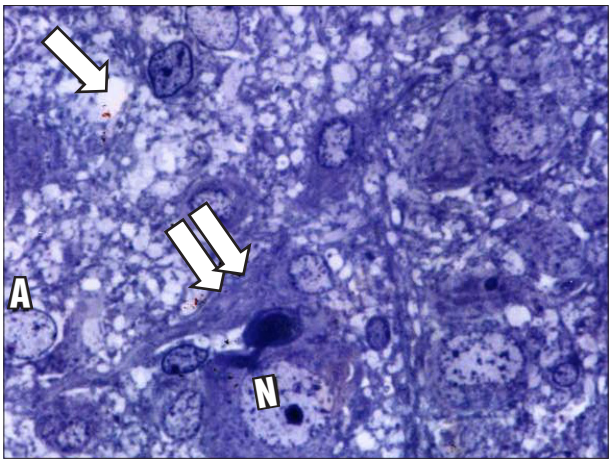

Figure 8. A photomicrograph of male albino rat midbrain on the 21st day of life showing nerve cells (***N***) of the red nucleus with larger amount of cytoplasm and regular contour of nuclei. Empty spaces of unmyelinated cell processes, axon hillock (***double arrows***) and sharply demarcated nucleus of astocyte (***A***) and blood capillary (***arrow head***) are seen. (Toluidine blue, X800)

previous age. They were of variable shapes which either pyramidal or fusiform. Neuroglia cells were also seen in between (Figure 6).

Silver stained sections at the same region of this group showed that the nerve cells forming the magnocellular red nucleus had prominent nucleoli, unstained areas of nuclei and large area of cytoplasm around the nucleus. Angulations of the nerve cells and their axons were clearly detected (Figure 7).

The semithin sections of the red nucleus at this group stained with toluidine blue revealed the nerve cells were of variable shapes. They had larger amount of cytoplasm and regular contour. Axon hillock, empty spaces of unmyelinated cell processes, sharply demarcated nucleus of astocyte and blood capillary were also seen (Figure 8).

Electron microscopic examination. Examination of ultrathin sections of this subgroup using electron microscope showed rounded nerve cell of red nucleus with nearly central rounded electron lucent nuclei. Their cytoplasm was seen to contain rough endoplasmic reticulum, numerous free ribosomes and mitochondria. Blood capillaries were also seen to be surrounded by empty spaces of perivascular feet processes (Figure 9).

Group II [adult rats (4th month)]

Light microscopic examination. Sections of the superior level of midbrain of this group at the region of magnocellular nuclei stained with H & E showed large nerve cells, which are mostly multipolar or stellate in addition to pyriform cells. Astrocytes with sharply demarcated nuclei were seen (Figure 10).

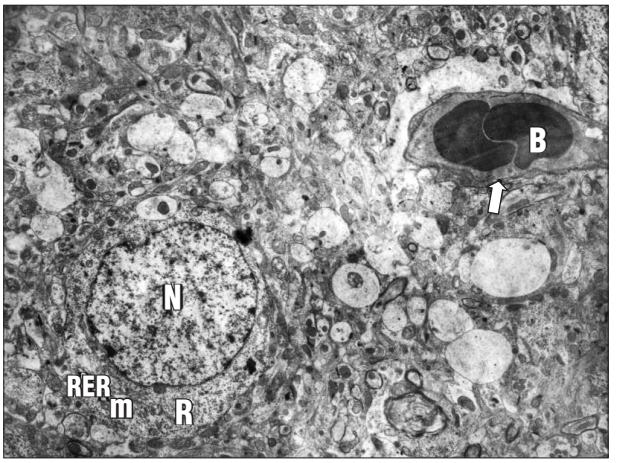

Figure 9. An electron micrograph of male albino rat on the 21st day of life showing rounded nerve cell of red nucleus with nearly central rounded euchromatic nucleus (***N***). Its cytoplasm contains rough endoplasmic reticulum (***RER***), free ribosomes (***R***) and mitochondria (***m***). Blood capillary (***B***) is surrounded by perivascular feet process (***arrows***). (TEM, X4500)

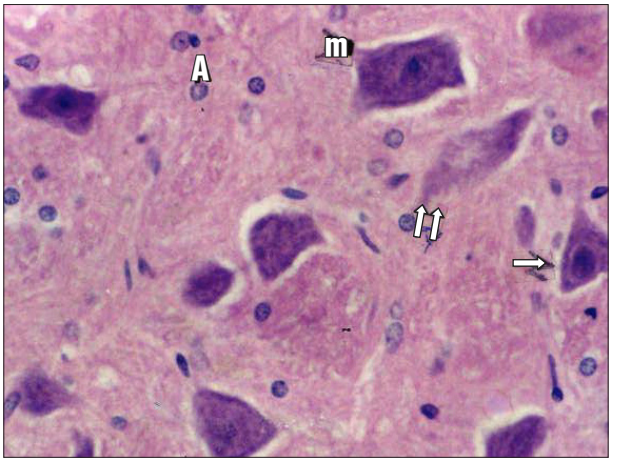

Figure 10. A photomicrograph of male albino rat midbrain on 4th month of life showing large nerve cells which are mostly multipolar (***m***), stellate or pyriform (***double arrows***). Astrocytes (***A***) with sharply demarcated nuclei are seen. (HE, X400)

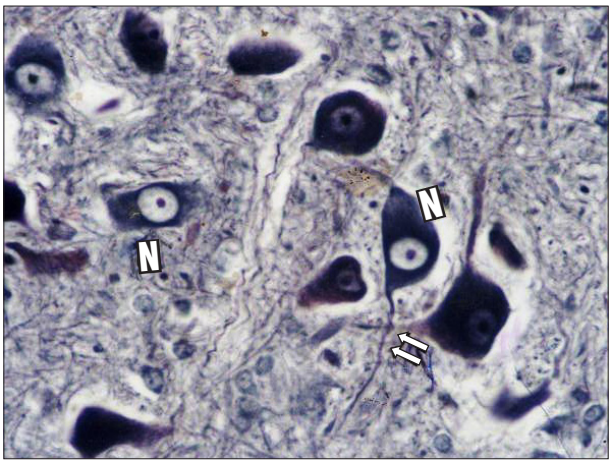

Figure 11. A photomicrograph of male albino rat midbrain on 4th month of life showing magnocellular nerve cells (***N***) having large area of cytoplasm. Long axons (***double arrows***) are seen. (Holmes method for silver stain, X400)

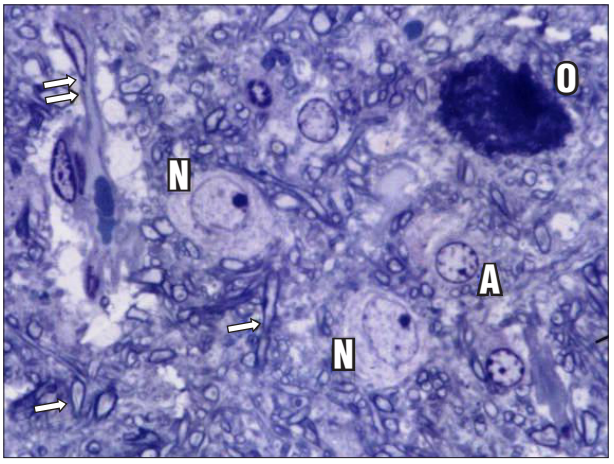

Figure 12. A photomicrograph of male albino rat midbrain on 4th month of life showing nerve cells (***N***) of red nucleus with small pale nuclei. Neuropil contains myelinated axons (***arrows***), astrocyte (***A***) and oligodendroglia (***O***) with irregular outline, dark nucleus and cytoplasm. Endothelium (***double arrows***) of blood capillary is seen. (Toluidine blue, X800)

Silver stained sections at the same region of this group at the region of magnocellular nuclei showed nerve cell angulations, long axons and dendrites. The area of nerve cells occupied by large area of cytoplasm (Figure 11).

The semithin sections of midbrain stained with toluidine blue at this age revealed nerve cells of red nucleus were small having pale nuclei and prominent nucleoli. Neuropil was packed with myelinated axons, astrocyte and oligodendroglia with irregular outline, dark nucleus and cytoplasm. Endothelium of blood capillary was seen (Figure 12).

Electron microscopic examination. Examination of ultrathin sections of this group showed that the nerve cell were large and rounded electron lucent with central euchromatic nucleus. Their cytoplasm contained numerous free ribosomes, mitochondria and rough endoplasmic reticulum (Figure 13).

Oligodendroglia had ovoid electron dense nucleus, prominent nucleolus and moderately electron dense cytoplasm. They surrounded multiple unmyelinated axons and partially myelinated axons were also seen (Figure 14).

Group III [senile rats (18th month)]

Light microscopic examination. Sections of male albino rat midbrain of this group stained with H and E showed that the nerve cells which had lightly stained cytoplasm

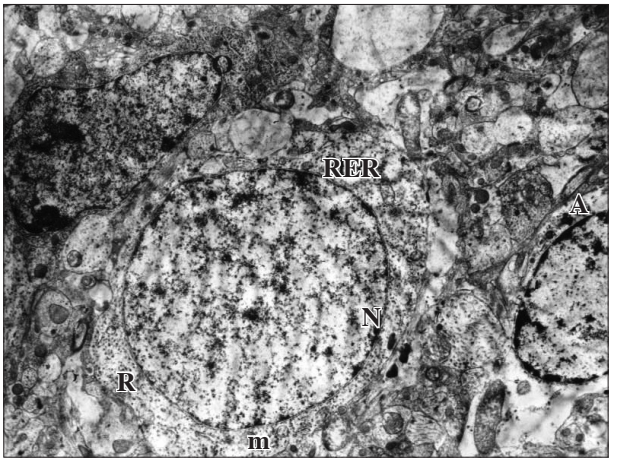

Figure 13. An electron micrograph of male albino rat red nucleus on 4th month of life showing large rounded electron lucent nerve cell with central euchromatic nucleus (***N***), numerous free ribosomes (***R***), mitochondria (***m***) and rough endoplasmic reticulum (***RER***). Oligodendroglia (***O***) and astrocyte (***A***) are seen. (TEM, X 6500)

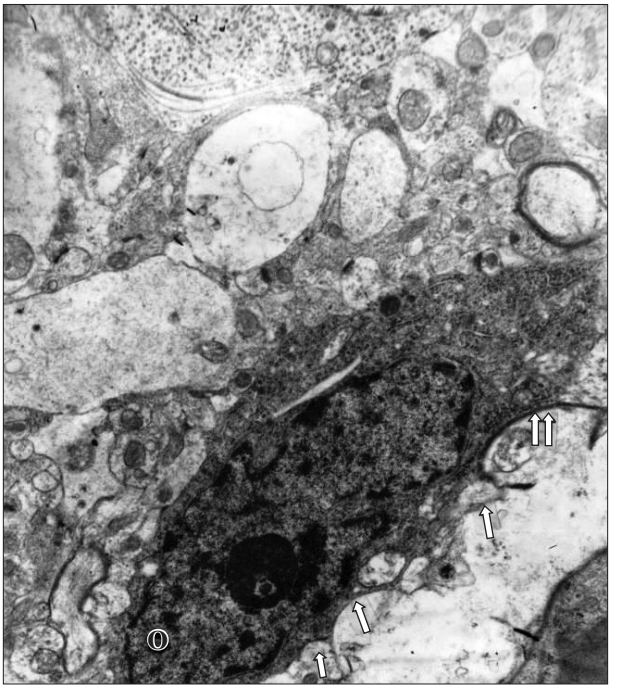

Figure 14. An electron micrograph of male albino rat red nucleus on 4th month of life showing oligodendroglia (O) with its ovoid electron dense nucleus, prominent nucleolus and moderately electron dense cytoplasm. It surrounds a partially myelinated (***double arrows***) and multiple unmyelinated (***arrows***) axons. (TEM, X11000)

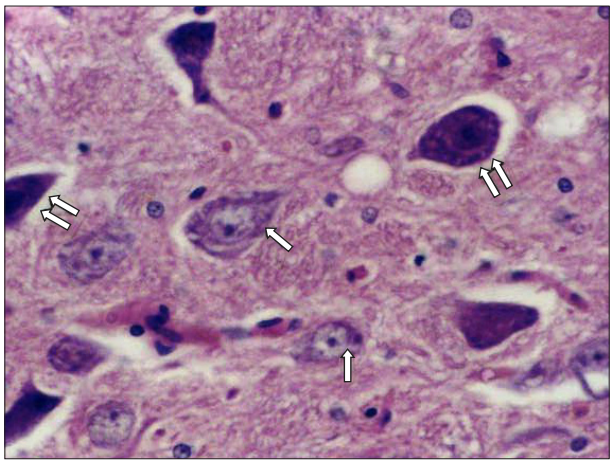

Figure 15. A photomicrograph of male albino rat midbrain on 18th month of life showing lightly stained cells (***arrows***) with pale nuclei and prominent nucleoli. Darkly stained cells (***double arrows***) have irregular shapes, condensed nuclei and empty spaces around them. (HE, X400)

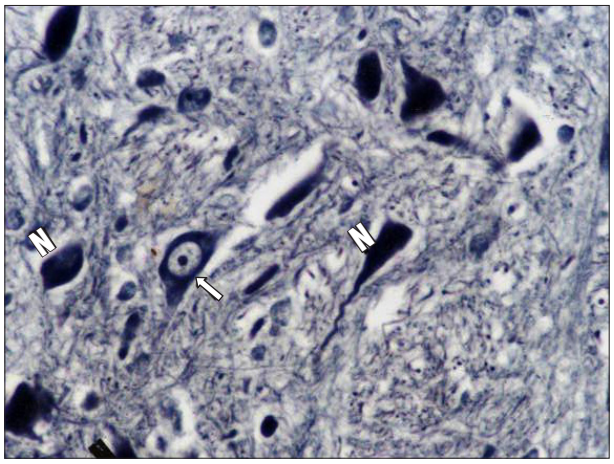

Figure 16. A photomicrograph of male albino rat midbrain on 18th month of life showing that most of the magnocellular nerve cells (***N***) of red nucleus are markedly shrinked with unapparent nucleoli. Normal shaped nerve cell (***arrow***) is also detected with its cytoplasm and prominent nucleolus. (Holmes method for silver stain, X400)

showed obvious nuclei and prominent nucleoli, on the other hand, cells with darkly stained cytoplasm had condensed nuclei as well as they were irregular in shapes and surrounded by empty spaces (Figure 15).

Silver stained sections of male albino rat midbrain at the same region showed that most of the magnocellular nerve cells of red nucleus were markedly shrinked with irregular shapes and nucleoli became unapparent. Normally shaped nerve cells were also detected with large amount of cytoplasm and prominent nucleoli (Figure 16).

The semithin sections stained with toluidine blue at this age group showed that there was either normal shaped nerve cell having regular contour with central rounded nucleus and prominent nucleolus or shrinked nerve cells with irregular eccentric nuclei with unapparent nucleoli. Nissl's granules were seen in the cytoplasm of the later one (Figure 17).

Electron microscopic examination. Examination of ultrathin sections of male albino rat of this group showed that nerve cells had irregular profile with moderate increase in its electron density. Nuclear envelope was of irregular contour. Cytoplasmic vacuoles and lipofuscin pigment could be seen. Neuropil showed myelin sheathes which were either separated or interrupted in addition to vacuolated axoplasm. (Figure 18).

Discussion

Functionally, the central nervous system has its role in integration, co-ordination of the neural signals and execution of higher mentality function such as thinking, learning and remembering [15]. Midbrain could be considered as a relay station for part of the extrapyramidal system. In a description of the extrapyramidal system [16]. Moreover, Carpenter [17] made isolated lesions of red nucleus of the monkey and got transient tremors, ataxia and ipsilateral occulomotor disturbances.

In this work, albino rats were chosen because they are easily obtained from the animal house and it is so easy

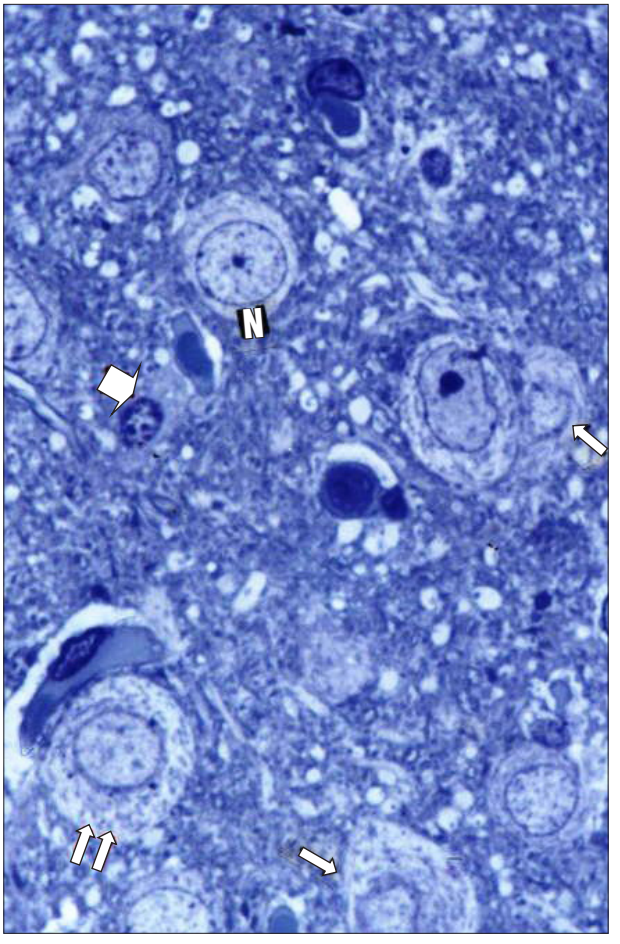

Figure 17. A photomicrograph of male albino rat midbrain on 18th month of life showing nerve cells of red nucleus which either have rounded nucleus (***N***) and prominent nucleolus or shrinked eccentric nuclei with unapparent nucleoli (***arrow***). (Toluidine blue, X800)

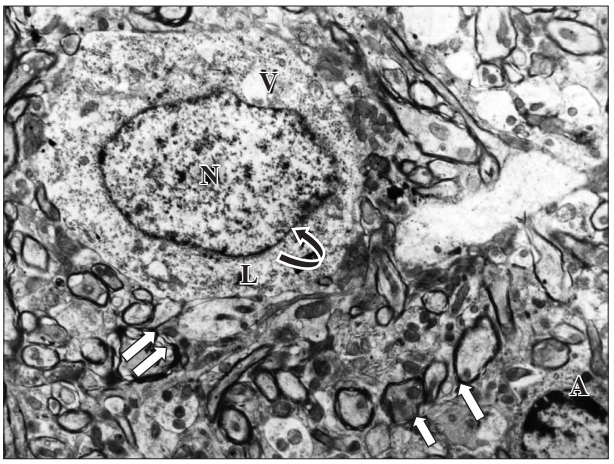

Figure 18. An electron micrograph of male albino rat red nucleus on 18th month of life showing nerve cell (***N***) with irregular nuclear envelope (***curved arrow***), lipofuscin pigment (***L***), large vacuole (***V***) and multiple myelinated nerve fibers, some of them have separated myelin sheath (***double arrows***) are seen. Astrocytes (***A***) are seen in one of the corners. (TEM, X11000)

to correlate the age of rat with the age in man [18]. Rats were classified into three main groups (newborn, adult and senile) and this classification of age groups was according to Buckland et al [10].

Throughout this work, the magnocellular red nucleus was found to be formed of groups of nerve cells, which took variable shapes. They could be multipolar stellate or pyriform and they were of relatively larger size. These observed data were in agreement with Cahill et al [19]. Silver- stained section magnocellular red nucleus showed that angulation of the nerve cells couldn't be clearly seen in early post-natal age group (7 days), while it appeared at the next subgroup (21 days). This condition may be explained by the proper development of axons and dendrites. The obtained results were in accordance with those of Gwyn and Flumerfelt [20].

In the current work, there were shrinked nerve cells in addition to the normal shaped nerve cells found in senile group, detected in H&E stained- section by empty spaces surrounding the shrinked nerve cells. Further examination of the semithin sections of the red nucleus, showed that these cells had irregular dark pyknotic or eccentric nuclei, nucleoli were mostly unapparent and there was dark cytoplasm due to aggregated Nissl's granules. Further examination of the sections using transmission electron microscope indicated that there was reduction in all the constituents of the cytoplasm in addition to the appearance of cytoplasmic vacuoles. Lipofuscin pigments were seen as an indication to the changes occurred at senility. The previously mentioned data was in accordance with those of Peinado et al [21], who mentioned that there was no neuronal loss occurred with age in several regions in the cerebral cortex of human and monkey, while there was little evidence of this point in rat. This opinion was also supported by Mukherjee et al [22] who described the changes that occur in nerve cells due to the effect of aging by apoptosis. Cotran et al [23] explained process of apoptosis by the presence of nerve growth factor, which is essential for the support of nerve cell during their life, whereas, in senility there is withdrawal of such support that can lead to apoptosis. Some scientist gave a controversed explanation to the presence of the extracellular space which appeared around apoptotic cell at the level of H and E. Marti et al [24] contributed this observation to the technical errors that causes shrinkage and retraction of cell during preparation that's why it only appeared in light microscopic preparation and not seen in semithin or ultrathin sections.

Myelination process is always a matter of interest as it differs every now and then, in the central nervous system, as it accompanies the process of maturation and ageing. It was seen in the first week of life and then took an ascending pattern of increase from one age group to the next. Myelin sheath couldn't be seen neither by H&E stain, as it dissolved during preparation, nor by Holmes' stain, which is selective for nerve cell body and axons. However, it could be seen by toluidine blue where it appeared as dark blue circle in cross section and by electron microscope where it appeared as electron dense circle around the axoplasm. These findings were in agreement with those given by Peters and Proskauer [25] and Vincent et al [26] who added that hematoxylin may demonstrate myelin sheath if potassium bichromate was used in addition to it.

Myelination in senile age group had different pattern than the previously described. There were separations of myelin sheath layers in addition to interruption of their continuity. This could be attributed by Lambeth and Blunt [27] to the ageing oligodendroglia, which could no more participate in their role of myelination process, in addition to the chemical alterations occurring with age in the myelin, sheathes.

In this work, HE- stained sections showed that at adult and senile age groups there were numerous cells other than the nerve cells, one of these cell types had very pale glassy nucleus, sharply outlined nuclear envelope, prominent nucleolus and clear cytoplasm which form very thin rim around its nucleus. This cell named astrocyte. The previous description was in accordance with those given by Al-Ali and Hussain [28]. The other cell type, which could be seen, was oligodendrocyte, which had irregular shapes. Holmes's method for staining gave little or even no details about supporting cells as it is selective for nerve cell as was given by Kiernan [12].

Toluidine blue–stained sections gave minor information about astrocytes. They appeared in sections of red nucleus at young age, (7 and 21 days) and increased markedly at adult and senile age group. Astrocytes were seen having pale nuclei and clear area of cytoplasm, while at senile age group, they showed dark nuclei. This condition, which could be also explained by apoptosis as, was mentioned by Ghatar [29].

From our study, examination of sections at the age of 7 days using toluidine blue could differentiate oligodendroglia into three different types according to the colour of cytoplasm and nucleus. They were dark,

medium or light oligodendroglia. These changes were recorded by Watabe et al [30].

In the same point of study, examination of oligodendroglia using electron microscope at senile age group showed elongated irregular nuclei with dark cytoplasm, numerous mitochondria and few tapering processes and multiple cytoplasmic vacuoles. Watabe et al [30] added that vacuolations of oligodendroglia were explained by their role in phagocytosis.

Moreover, electron microscope revealed more details about the three types of oligodendroglia. The dark one showed moderate amount of cytoplasmic organelles. The medium one had the least content of organelles, while the light one had abundant amount of organelles. The last one was known to be the most active cell in the process of myelination. Mayor et al [31] was in agreement with the previously given results. They added that light oligodendroglia myelinated large caliper axons with thick layer of myelin while the dark one myelinated the small caliper axons, whereas medium one myelinated medium to large caliper axons. Therefore cells had broad processes containing an organelle-rich cytoplasm and there was a continuity between their cell membranes and the outer myelin sheath lamellae which partially ensheathed the adjacent axons. Roza et al [32] referred the variations in shape of oligodendroglia to their role in maturation of the nervous tissue.

From the previously introduced work in this thesis, the effect of aging on the cytoarchitecture of both midbrain nuclei was studied aiming explain why locomotor affection considered a wide spread age- related disorder. Apoptosis could explain this finding properly.

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