ULTRASTRUCTURE OF THE AXON REACTION IN THE
IMMATURE RAT THALAMUS

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ABSTRACT

The axon reaction was studied using immature rats aged 4 or 14 days at the
time of unilateral removal of the cingulate cortex. After survival times of one
to 5 days they were sacrificed and the anterior thalamic nucleus was exam-
ined using ultrastructural methods. The degree of degenerative change was
much more intense in the 4-day group where the center of the reactive area
was characterized by pronounced lysis of the neuropil and the loss of most
neurons. Dark neurons were observed in both groups but mitochondrial
proliferation was restricted to the 14-day group. Evidence for microglial
formation from pericytes was observed in the 4-day group while reactive
astrocytes were observed in both groups. It was concluded that a rapid
maturation of the thalamic injury response occurs between the 4th and the
14th day in the rat so that by the 14th day the response is similar in many
respects to that of mature animals.

INTRODUCTION

The reaction of neuronal perikaryia after axonal damage (axon reaction,
retrograde degeneration) has been the subject of numerous ultrastructural
reports during the last decade and a half. More than 30 such reports are
mentioned in the 1971 review of Lieberman (19) and a substantial number (1,
12, 21–26, 34–39) have appeared since then. Most of these reports deal with
mature animals but there is a paucity of reports (13, 36) concerning ultrastruc-
tural aspects of retrograde degeneration in immature animals. As early as
1870 Gudden (14) stated that the axon reaction was more intense in immature
animals and for this reason Brodal (6) stressed the advantage of using
immature animals in anatomical studies utilizing the retrograde degenera-
tion method. Typically, injury to an axon results in a rapid degeneration of
immature neuronal cell bodies but with mature animals there is either a
much slower loss of neurons or the cells merely atrophy (36). It was the
purpose of the present study to examine the effect of a short time interval of
neural maturation upon the events which are observed during the first five
days of retrograde degeneration. Another purpose was to seek evidence to

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support the hypothesis that damage to axon terminals is a factor responsible for retrograde transsynaptic degeneration.*

MATERIALS AND METHODS

The study employed 24 rats aged 4 or 14 days at the time of lesion placement. The rats were divided into groups of three each and survival times were 1, 2, 3 and 5 days. The groups are herein referred to as 4 + 1, 4 + 2, etc. The unoperated side of the operated animals served as a source of control tissue. In addition 5 unoperated control animals aged 5–23 days were used. Lesions were made by aspirating the medial portion of the right cerebral cortex (cingulate region) through a 20 gauge needle. The skin over the area was closed with wound clips and the animals returned to their dams until sacrifice. Fixation was achieved by cardiac perfusion with 0.1 M phosphate buffer for one minute followed by 20 minutes with a 5% glutaraldehyde mixture in 0.1 M phosphate buffer. After 2–4 hours the brains were removed and stored in phosphate buffer until the following day when coronal slices were made. One or two blocks of tissue from the dorsal half of the most rostral part of the thalamus were used for ultrastructural investigation. Blocks from the left side of experimental animals and both sides of control animals were used to study the normal appearance of the anterior thalamic nucleus. After 2–4 hours post fixation in 1.0% osmium tetroxide the tissue was embedded in epon. Either the mesa method (17) or a resectioning technique (10) was used to locate the anterior thalamic nucleus.

RESULTS

Normal Tissue

The anterior thalamic nucleus was identified by locating an elevated mass on the dorsal surface of the thalamus lateral to the stria medullaris thalami (stria habenularis). The most dorsal group of cells, the anterior dorsal thalamic nucleus, was characterized by closely packed neurons. Using the electron microscope, the anterior dorsal nucleus was identified by extensive areas of contact between neuronal somas. Myelinated fibers were conspicuous after the second week of life in the stria medullaris thalami and after the third week in the anterior thalamic nucleus. Myelinated fibers formed a randomized network in the neuropil of the anterior dorsal nucleus but the anterior ventral nucleus was characterized by wide separation of neurons and aggregation of myelinated fibers into discrete bundles. Both of these subdivisions of the anterior thalamic nucleus were studied. Neurons were differentiated from glial cells by their larger size, smooth plasmalemma, prominent nucleoli, infoldings of the nuclear envelope, and evenly dispersed chromatin (Fig. 2). The endoplasmic reticulum consisted of a few widely separated cisternae and the Golgi apparatus was well developed and localized to one part of the cell. Glial cells were characterized by their small size, and irregular contour of the plasmalemma. Astrocytes had a low density cytoplasm ("watery") and a homogeneous chromatin pattern which was denser than that of the neurons but considerably less dense than that of all other cell types. Oligodendrocytes were associated with myelinated axons and were typified by dense cytoplasm and a dense nucleus. Nucleoli were indistinct or not observed in glial cells and nuclear infoldings were not observed. The

endoplasmic reticulum and Golgi apparatus were poorly developed. Pericytes were flattened and closely apposed to endothelial cells and a margin of heterochromatin was adjacent to the nuclear envelope.

**Experimental Tissue**

Aspiration of the medial part of the cerebral cortex resulted in loss of the cingulate cortex and retrograde degeneration of neurons of the anterior thalamic nucleus which projects to the cingulate cortex. The most striking difference was observed in the neuropil of the 4 day lesioned group. By the second day there was a great increase in the amount of extracellular space which gave the tissue a Swiss cheese-like appearance at the light microscopic level (Fig. 1). This extracellular area was clear and usually free of any debris at the ultrastructural level (Figs. 4, 5, 11, 13, 14, 15, 16) resulting in wide separation of tissue profiles. There was no increase in extracellular area in the 14-day lesioned animals. Most of the other features of the degenerated tissue were found in both the 4- and 14-day group but certain features were more prominent in one group or the other. Abnormal structures in the neuropil were the most frequent finding in the one-day survival animals of both age groups and degenerative profiles were common in the neuropil throughout the first five days after lesions were made.

Reactive cells (neurons and glia) contained five types of abnormal cytoplasmic bodies. These were multilaminate figures (Figs. 11, 12, 16), aggregations of dense granular material (Figs. 5, 12), dense homogeneous inclusions (Figs. 14, 15, 16), large lipid droplets (Fig. 17), and vacuoles (Figs. 15, 16), some of which were membrane bound. It was not always possible to differentiate between reactive cells of neuronal and glial origin since neuronal cytoplasmic and the nuclear compartments displayed an increase in density as degeneration proceeded. Cytoplasmic abnormalities were also found in a cell undergoing mitosis and in the cytoplasm of cells forming vascular sheaths (Fig. 11).

Two types of neuronal degeneration were observed. The first type of degeneration (dark neuron type) was characterized by an increased density of the nucleus and cytoplasm in both the 4- and 14-day groups. This change was seen as early as the first postoperative day in the 4-day lesion group. The chromatin of some of these cells appeared to coalesce into a nuclear body (Figs. 3, 4) which remained intact after dissolution of the nuclear envelope (Fig. 4). In the group operated on at 4 days, an apparent fragmentation of the plasmalemma and dispersal of cytoplasmic material into the extracellular space was observed after 2 days (Fig. 5). The nucleus of these cells (light neuron type) was remarkably stable and there was no increase in density of either the cytoplasmic or nuclear compartments. Early cytoplasmic changes in the 14-day group were disorganization of the endoplasmic reticulum and a proliferation of mitochondria. In the dark neurons a dense packing of mitochondria was observed (Figs. 7, 8). There was also a dilation of membranous cisternae in these cells (Fig. 7). Occasionally fingerlike invaginations of
Fig. 1. Semithin section of the anterior thalamic nucleus showing an area of extensive retrograde degeneration (D). 4 + 3 group, toluidine blue stain, 265 ×.

Fig. 2. Normal neuron from a section adjacent to the lower portion of Fig. 1. nucleus (N), 6,400 ×.

Fig. 3. Dark neuron with intact nuclear envelope (arrows), nuclear body (NB), extracellular space (E). 4 + 2 group, 8,380 ×.

Fig. 4. Nuclear body (NB) free in cytoplasm of a dark neuron, possible remnant of the nuclear envelope (arrow), extracellular space (E). 4 + 2 group, 12,100 ×.
"watery" cytoplasm into dark neurons of 14-day animals were observed (Fig. 8).

Mitochondrial aggregations in varying stages of degeneration were noted within astrocytic cytoplasm of the 14-day group (Figs. 9, 10). Other apparently phagocytic inclusions were chromatin-like masses and dilated processes (Fig. 9). The proliferation of astrocytic filaments and cytoplasmic sheaths was very evident by 5 days after surgery in the 14-day group (Fig. 10). Astrocytic filaments could not be demonstrated in the 4-day group but dense lamellar and granular bodies were often found in cells which were identified as astrocytes. This identification was based upon the characteristic cytoplasm, the wrapping of blood vessels with a cytoplasmic sheath (Fig. 11) and characteristic nuclei (Fig. 12).

Pericytes in the reactive area of the 4-day group were more rounded in appearance. The cytoplasm of some of these cells was increased in density and contained dense round masses (Fig. 13). Some of the nuclei had small infoldings and all had margination of nuclear chromatin (Fig. 13). Cells with identical characteristics were also found in the neuropil and not related to blood vessels (Figs. 14, 15, 16). These cells were designated as "M" cells due to their resemblance to principal reactive cells of other reports (23, 26). Some of the "M" cells were filled with electron lucent vacuoles, many of which were membrane bound (Fig. 16) and debris filled vesicles (Fig. 15). The Golgi apparatus was not involved in the vacuolization (Fig. 16). In one case two of these cells were found in close apposition as if they were incompletely separated post-mitotic daughter cells. Phagocytic activity was evident in some reactive "M" cells (Fig. 14). In the 14-day animals (Fig. 20) these cells were similar to those of the 4-day group but were not as abundant. Constricted mitochondria were occasionally found in these cells of both groups (Fig. 17).

Most of the changes observed were present by the second post-operative day but from that time through the fifth day the proportion of the cells involved increased.

**DISCUSSION**

Most of the degeneration seen in one day animals was observed in the neuropil. This may have been due to several factors. Probably the first degeneration which occurred was orthograde degeneration of corticothalamic fibers and terminals. Secondly, the neuronal processes which make up much of the neuropil may have been more susceptible to intracellular disturbances than the soma due to being further removed from vital organelles.

In many respects the changes reported in the present study were comparable with those previously described for newborn animals (13, 36) and with lesions in the adult rat thalamus (2) and rabbit thalamus (26). Basically, the present findings resembled an acceleration of the changes seen in the adult. The profound degeneration of the neuropil of 4 day old animals with lesions was very similar to that observed in the cerebral cortex of newborn rats due to mechanical damage (33). The increase in extracellular space was most likely
Fig. 5. Light neuron type of degeneration, apparent fragmentation of nuclear membrane, extracellular space (E), dense granular material (G), density of nucleus is increased slightly. 4 + 2 group, 6,800 x.

Fig. 6. Mitochondria filled process, 14 + 3 group, 12,100 x.

Fig. 7. Dark neuron with dilated cisternae and mitochondria filled cytoplasm, nuclear body (NB), 14 + 3 group, 9,000 x.

Fig. 8. Dark neuron with invaginations into soma (arrows) nuclear body (NB), 14 + 3 group, 8,500 x.
Fig. 9. Portion of astrocyte containing degenerating mitochondria (M), a dilated neuronal process (DP), and chromatin. The irregular outline of the cell is demarcated by arrows. 14 + 3 group, 7,500 X.

Fig. 10. Two astrocyte nuclei on the left, longitudinal (FL) and cross sectional (FX) views of astrocytic filaments, astrocytic sheets (S) and degenerating mitochondria (M). 14 + 3 group, 9,100 X.

Fig. 11. Astrocytic cytoplasmic sheath wrapping a blood vessel, multilaminate figures (arrows), extracellular space (E), 4 + 3 group, 5,530 X.

Fig. 12. Reactive astrocyte not associated with a blood vessel, large granular, multilaminate mass indents the characteristic nucleus, extracellular space (E), 4 + 3 group, 4,550 X.
Fig. 13. Juxtavascular pericyte in the process of transformation (left), note the margination of chromatin, infolded nuclear envelope and dense cytoplasm. On the right is a transformed pericyte/reactive "M" cell, extracellular space (E), 4 + 3 group, 4,000 ×.

Fig. 14. Reactive "M" cell in the process of phagocytosis (arrows), extracellular space (E), 4 + 3 group, 8,100 ×.

Fig. 15. Reactive "M" cell still associated with a blood vessel, debris filled vacuoles (V), extracellular space (E), 4 + 3 group, 5,400 ×.

Fig. 16. Reactive "M" cell with clear vacuoles (V) and a rather typical Golgi apparatus (GO), multilaminate figure (arrow), extracellular space (E); note nuclear infolding, 4 + 3 group, 6,000 ×.
Fig. 17. Reactive "M" cell of 14 + 3 group, lipid droplets (L), constricted mitochondrion (arrow), 8,100 x.

Fig. 18. Reactive "M" cell adjacent to a normal appearing neuron; the large residual body contains a lipid droplet (L), 14 + 3 group, 9,500 x.

Fig. 19. Mast cell between basal lamina (arrow) and an endothelial cell, 14 + 3 group, 2,100 x.

Fig. 20. Hypertrophy of endothelial cell with bulging of nuclear portion into the vascular lumen. Adjacent to the endothelial cell is a hypertrophied pericyte, 14 + 5 group, 9,000 x.
due to lysis of neuronal processes. The space around some masses in the neuropil was presumably due to dissolution of particulate material or uptake of particulate material by phagocytic cells.

The recognition of some reactive cells was difficult due to the occurrence of multilaminated bodies and other abnormal structures in cells which were clearly degenerating neurons or reactive glial cells. The finding of two types of neuronal degeneration has been mentioned for the adult rat thalamus (2) but not in the rabbit thalamus (25). The dense pattern of degeneration seen in the present study compares with “dark” neurons seen in thalamic degeneration in the adult rat (2) and in the lateral cervical nucleus of the kitten (13). Pale degenerating neurons were also seen in the adult rat thalamus (2) and empty swollen profiles were observed in the reactive neuropil of rabbits (25). The variable forms of degeneration may be indicative either of several functional types of thalamic neurons or are due to varying severity of axonal damage. Proliferation of mitochondria is a common finding in axon reaction (19). Mitochondrial increase was found in the soma, myelinated axons and large profiles in the neuropil, which were probably dendrites. These changes were found only in the 14-day group. If the neurons of the 4-day group were more vulnerable to axon damage, they may not have gone through a stage of attempted regeneration (19) characterized by mitochondrial proliferation. Disorganization of the endoplasmic reticulum is also a common finding in axon reaction of mature animals (19) and has been associated with a shift in the type of protein production.

The term nuclear body was used to describe the unusual structures found within the nucleus and later in the cytoplasm because it could not be determined whether they were composed of nucleolar substance, chromatin or both. It is possible that they were formed by a condensation of chromatin upon the nucleolus.

The astrocytic proliferation observed in the 14 day group is typical of that described in other studies (9); however, the appearance of filaments 5 days post-operatively is earlier than that found in older animals. The astrocytic sheet reaction appeared coincident with the appearance of filaments and was mentioned previously (15). Evidence for astrocytic phagocytosis was provided in the 14-day group by finding large masses of degenerating mitochondria and other material in astrocytic cytoplasm. Filaments were not found in the 4-day group but astrocytes were identified by their typical lightly staining cytoplasm and nucleus (30), irregular plasmalemma (30), and vascular ensheathment. The dense multilaminate and granular masses often found in these cells were indicative of some form of phagocytic activity but there was no evidence for phagocytosis of whole organelles. It must be assumed that cellular constituents were broken down before being taken into the astrocyte of the 4 day group.

The metamorphosis of pericytes has been a subject of much controversy. According to Maxwell & Kruger (27) pericytes can be transformed into cerebral macrophages (compound granular cells, gadget cells) while Hager (15) suggested their transformation into microglial cells. The apparent migration
of pericytes into the neuropil has been suggested by several authors (3, 28, 31) and phagocytosis by pericytes has been described (27) as well. There is a report of impregnation of pericytes with silver carbonate (29) and in another study (8) the uptake of exogenous protein by pericytes was described but no transformation of pericytes was observed. Microglia have also been said to be derived from juxtavascular mitotic cells (7, 32), juxtaneuronal cells (18) and leucocytes (1, 25, 26, 31), specifically monocytes (31). However, a compromise theory that both exogenous (hematogenous) and endogenous cells can give rise to microglia or "M" cells has been advanced (26, 31). The apparent transformation of pericytes seen in the present study indicates that they have the potential to migrate away from their perivascular locus and participate in the tissue injury reaction. While there was considerable similarity between the cells derived from pericytes described in the present study and the microglia and reactive "M" cells of Matthews (22), long endoplasmic reticulum cisterns were not demonstrated. Without serial sections it is difficult to know whether the apparent mitochondrial constrictions were not plane-of-section artifacts due to bending of mitochondria. Nevertheless, the term reactive "M" cell was used for these cells since it does not carry the definitive implication of the term microgliocyte (26). The reactive "M" cells of the present study had nuclear infoldings which were not seen in normal pericytes (11) but the dense bodies in the cytoplasm resembled the exogenous protein in pericytes described in a previous study (8). Nuclear infoldings may represent an attempt to increase the area for nuclear cytoplasmic exchange as a response to increased demand for synthesizing activity. The vacuolated reactive "M" cells were considered a terminal phase of differentiation. It was assumed that the vacuoles were produced by dissolution of debris within vesicles.

The differences between this study and others could easily be due to varying experimental conditions (age, type of lesion, species, and locus).

If cytolytic enzymes are released by immature neurons undergoing retrograde degeneration, neuropil processes of uninjured cells could be secondarily damaged. Evidence for extracellular release of lysosomal enzymes has been seen during experimental ischemia in adult animals (20). The more extensive destruction of the neuropil in the younger age group may be the basis for, or one of the factors responsible for a more marked retrograde transsynaptic degeneration in younger animals (4, 16). That is, the transsynaptically degenerating cells may be degenerating due to damage to their axon terminals rather than, or in addition to, loss of a retrograde transsynaptic trophic factor as was previously proposed (4).

REFERENCES


