Department of Histology (Head: Prof. G. I. Zabussov), Medical Institute, Kasan, USSR

SCHWANN GLIAL ELEMENTS IN THE CHANGES OF NEUROMUSCULAR JUNCTIONS

G. A. Polikarpova, L. G. Svatko and G. N. Shmeleva

(Received July 18, 1963)

The structure of the motor nerve endings in skeletal muscle has been known for long. In the last thirty years several observations have been published concerning the various structural alterations of the motor nerve endings due to different pathological processes as well as under experimental conditions. In 1934 and 1935, A. G. Filatova and B. I. Lavrentiev have established proliferative changes on numerous motor end-plates of the laryngeal muscles of men died, and proposed these characteristic structures to be termed "ending neuromas". These "ending neuromas" are characterized by the expansion of the growing terminals to the border of the end-plate or, in certain cases, to the border of the muscle fibre. Similar reactive changes of the motor nerve endings have recently been observed in various diseases by several authors, [9, 8, 2, 3, 6, 1, 10, 4, 5, etc.]. The changes in question were mostly involving the neurofibrillar component. In certain papers alterations relating to the nucleoplasmic component are also mentioned. The latter component, however, has not been separated into sarcoplasmic and glial parts, and the descriptions were restricted to the number and characteristics of the nuclei. All these results of course depend upon the methods applied some of which only allow detection of the neurofibrillar elements instead of the whole complex structure of motor nerve endings. According to the data yielded by electron microscopy and histochemistry, the motor end-plate is composed of three structural components: *a)* the muscular part containing the sarcoplasmic sole with nuclei and with a specific sarcolemmal structure, the folded postsynaptic membrane (the subneural apparatus); *b)* the neurofibrillar region, i.e. the terminal arborizations of the axon of the motor nerve fibre; and *c)* the glial part composed of the Schwann cells protoplasm with nuclei enveloping from outside the synaptic region [16, 18, 13, 15, 11, 17, 12, 14]. Obviously, each part of the neuromuscular junction has its specific function in the process of transmitting impulses from nerve to muscle and the various components of the motor nerve ending owing to their structural and functional differences probably react differently to pathological influences.

The aim of the present investigations was to study with certain new methods the glial part of the motor nerve endings under various pathological conditions and some observations as to the changes occurring in the sole plasm of the motor end-plate.

Material and methods

Silver impregnation according to Bielschowsky-Gross completed with phase contrast microscopy allowed a reliable observation of the structure and changes of each component in the neuromuscular junction. The first method made it possible to study the neurofibrillar component and phase contrast microscopy for studying nucleo-plasmic structures. As material, various striated muscles of men suffering from cancer, nephritis, leucosis, radiation injury, etc., and of the cat were used; the muscles were removed either post mortem or at operation.

Results

The changes could clearly be observed on the neurofibrillar component and the Schwann glial elements of the ending. The glial nucleo-plasmic part, characterized by the presence of small, dark nuclei of an oval shape, showed conspicuous changes, especially in the altered motor nerve endings. First a swelling of the glioplasm takes place and the number of the Schwann nuclei increases; at the same time the nuclei begin to situate themselves in a cone at the border of the motor end-plate (Fig. 1). In the nerve endings, showing progressive changes of the neurofibrillar apparatus, with an increase of the axon terminal, a further growth with structural changes of the glial part takes place. A compact structure is preserved in the centre of the increasing end-plate only. In the lateral increasing parts the protoplasm is composed of isolated dark regions of irregular shape; these are united by bundles across light, larger or smaller spaces (Fig. 2). In perpendicular sections one can observe the topography of the increasing glial mass, which, extending along the muscle fibre, the sarcolemma and the endomysium separates into layers (Fig. 3). Parallel with the growth of the neurofibrillar mass and its transformation into an ending neuroma a considerable increase of the glial component occurs which is transformed into a massive nucleo-plasmic body of syncytial structure. All parts of the neuroma seem to be embedded in glial protoplasm (Fig. 4). The latter is a necessary structural component of the growing terminals. Even on the neurofibrillar branches growing out quite far from the end-plate one can always observe the protoplasmic layer with typical Schwann nuclei closely enveloping the growing nerve branches (Fig. 5). There are no unmyelinated terminal branches at the border of the end-plate. The glial origin of the syncytial nucleo-plasmic structures observed in the region of the changed endings is shown by the direct connection between them and the elements of the Schwann sheath enveloping the preterminal nerve fibre (Fig. 6). In motor

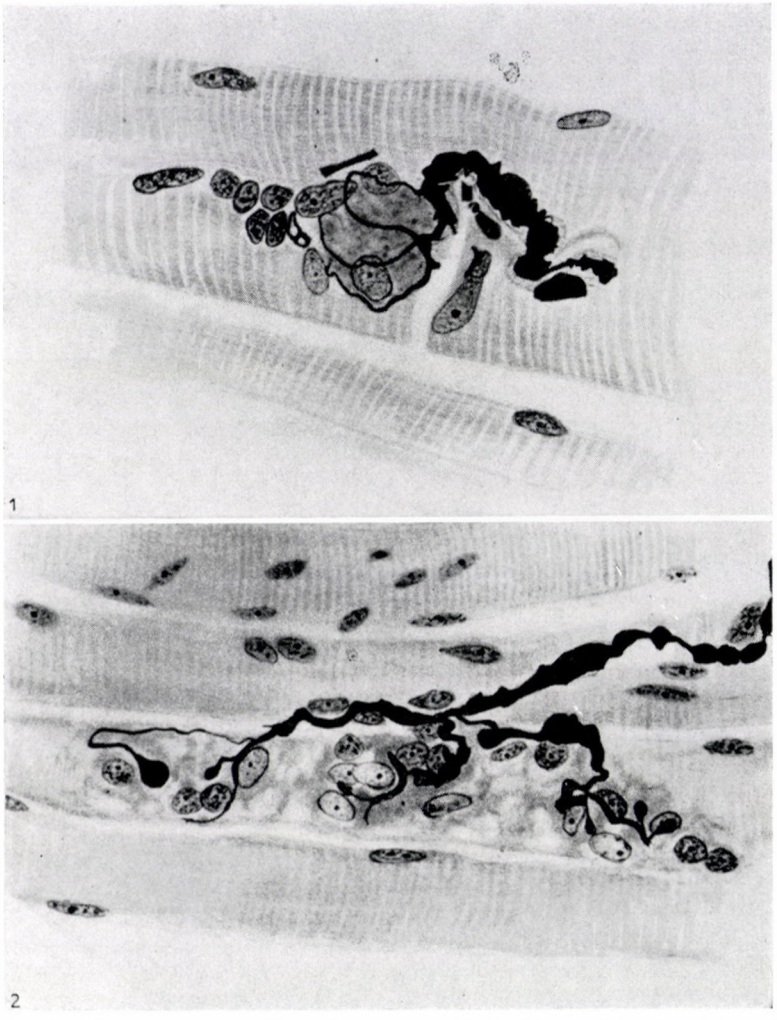

Fig. 1. Beginning changes of the glial nucleoplasmic component in the motor end-plate of the musculus rectus superior oculi of the cat. Acute radiation injury. — Latent period. Bielschowsky-Gross impregnation, phase-contrast. × 900

Fig. 2. Progressive proliferative reaction of the neurofibrillar and glial components of the motor end-plate, from the musculus thyreoarytaenoideus of the cat. The glioplasm has a syncytial structure in the peripheral regions of the ending. Acute radiation injury in the period of agony. Bielschowsky-Gross impregnation, phase-contrast. × 900

nerve endings with a neurofibrillar apparatus showing signs of advanced degeneration, the nucleo-plasmic component exhibits still more expressed changes. The site of the original normal end-plate cannot be defected. The whole plasmic mass consists of dark regions of various shape and size connected with each other by numerous bundles and light spaces. The number

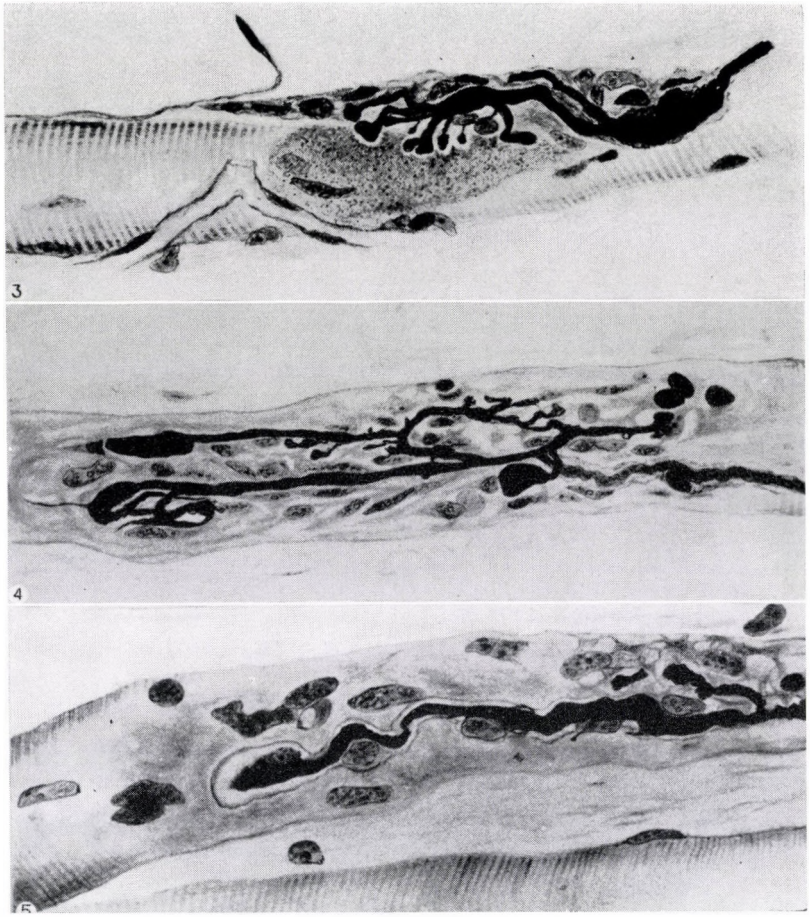

Fig. 3. Extension of the overgrowing glial component of the motor nerve ending between the endomysium and the sarcolemma of the muscle fibre, Man, musculus thyreoarytaenoideus internus. Cancer of larynx. Surgical material. Bielschowsky-Gross impregnation, phase-contrast. ×900

Fig. 4. Ending neuroma in the musculus thyreoarytaenoideus internus. Man. Overgrowing terminal branches enveloped by glial nucleo-plasmic mass of syncytial structure. Cancer of the larynx. Surgical material. Bielschowsky-Gross impregnation, phase-contrast. ×900

Fig. 5. Growing terminal of a changed motor end-plate covered in its whole length with a layer of glial protoplasm containing Schwann cell nuclei. Musculus thyreoarytaenoideus internus. Man, Cancer of larynx. Postmortem material. Bielschowsky-Gross impregnation, phase-contrast. ×900

of the nuclei in such a sole is many times that in a normal end-plate. Morphologically they are closely similar to the Schwann glial nuclei (Fig. 7).

The changes of the sarcoplasmic part of the nerve ending are less expressed. In the beginning stages of the reactive changes, the size of the nuclei

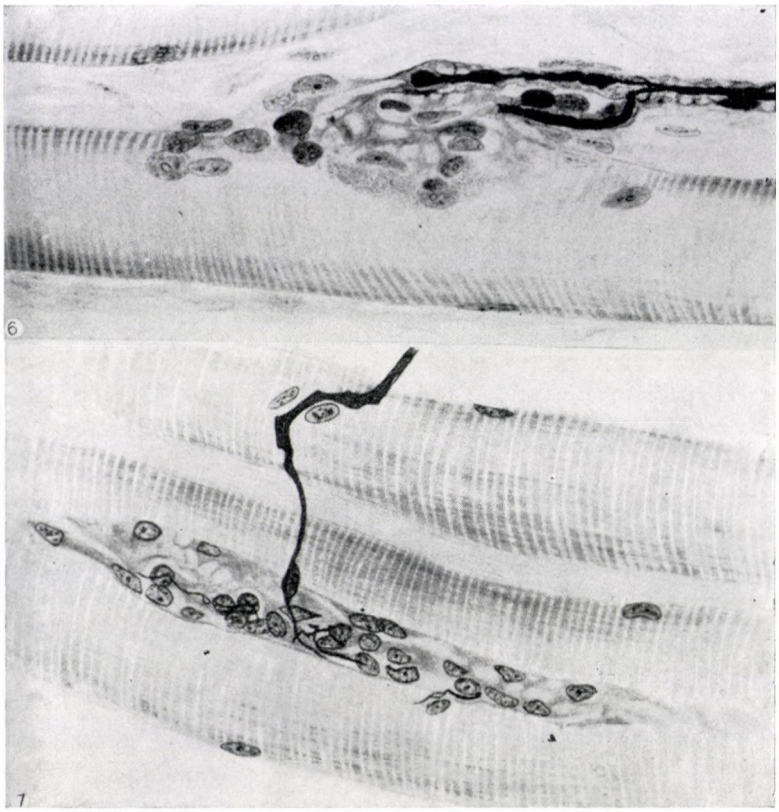

Fig. 6. Total destruction of the neurofibrillar apparatus of the motor end-plate. One can well see the connection between the syncytial glial component and the sheath of the preterminal motor nerve fiber. Musculus thyreoarytaenoideus internus of the man. Cancer of the stomach. Necrotomic material. Method: Bielschowsky-Gross: phase-contrast. ×900

Fig. 7. Considerably increased nucleo-plasmic mass with numerous Schwann cell nuclei on the site of a destructed motor nerve ending. Musculus thyreoarytaenoideus internus of the cat. Acute ray-disease. Acmic period. Method impregnation Bielschowsky-Gross impregnation, phase-contrast. ×900

in the sole plasm increases significantly. Subsequently they disappear and only Schwann cell nuclei can usually be observed in the substance of the neuroma. The sarcoplasm of the sole of intensively changed nerve endings obtains a granular character. No signs could be found of syncytial structures being former in the region in question.

Discussion

The described changes of the Schwann nucleo-plasmic component of the motor nerve ending have been observed by us in various diseases (cancer, nephritis, leucosis, acute experimental radiation injury). Similarly to the

changes of the neurofibrillar part, they cannot be regarded as specific for any disease. An analysis of our data, however, has allowed to recognize certain characteristics in the development of changes in various parts of the motor nerve endings. First of all, there exists a doubtless correspondence between the reactions of the neurofibrillar and the glial component. In all our cases the growth of the terminal was followed by the growth of the glia. The ending neuromas were hitherto regarded as unmyelinated overgrowths of the irregularly interlacing terminals bearing varicose widenings and bulbs, situated freely at the border of the end-plate sole and the muscle fibre. We, however, did find such neuromas without glial elements. However large the neuroma has grown, it always remained embedded in a nucleo-plasmic mass. Even single terminal branches overgrowing far the borders of the ending were enveloped by a protoplasmic layer with nuclei. Consequently, there exists a well-expressed parallelism between the character of reaction of the neurofibrillar apparatus and the glial part of the neuromuscular junction. Proliferative-reactive changes of the former structure are always followed by the same alterations of the latter. Each increase in the neurofibrillar apparatus of the motor nerve ending under changed conditions must evidently lead to a mass growth and a structural transformation of the glial component. This speaks for the existence of an interconnection and functional interdependence of these two structures of the neuromuscular synapse. The formation of a syncytial structure in the overgrowing glial part of the ending can also be regarded as a regular phenomenon. The reactive proliferation of the motor nerve endings induced by various diseases always contains nucleo-plasmic masses of syncytial structure, connected with the Schwann sheath of the motor nerve fibre. It is remarkable that the destruction of the neurofibrillar component of the motor nerve ending does not lead to the death and destruction of the glial part. After having transformed its structure into a syncytium, the glial part continues to live at the site of the former motor ending.

The sarcoplasmic part, in contrast to the glial and neurofibrillar parts, reacts in a different manner on the trophic changes caused by different diseases. After a short reacting period characterized by a significant increase of the nuclei, the elements show degenerative changes which later cannot be seen. This part is evidently less resistant and less reactive.

As to the functional importance of the described changes of the Schwann glial elements of the neuromuscular synapses and as to their causes, the problem remains open until there will be satisfactory data in this connection.

Summary

The changes of the Schwann glial cells of the motor nerve endings in striated muscles of the cat and of humans suffering from grave diseases (cancer, nephritis, leucosis, acute radiation injury) have been investigated by silver impregnation and phase-contrast microscopy.

The latter method allowed to reveal all components of the neuromuscular synapse, namely the terminal branches of the motor axons, the Schwann glial elements, as well as the sarcoplasmic sole with the nuclei. The occurrence of proliferative changes of the glial elements in some end-plates was observed. First, the number of the glial nuclei increased, later an increase of the protoplasmic mass and a change of its structure took place. The protoplasm looking like a symplast in normal neuromuscular synapses became syncytial in the changed ones. In such synapses it has a form of irregularly shaped plates containing dark oval nuclei connected by protoplasmic bands. The increasing mass of the syncytial Schwann protoplasm with its nuclei was situated on the surface of the muscle fibres lying between the sarcolemma and the endomysium. The increase of the Schwann elements was always simultaneous with the proliferative changes of the motor axon terminals. No naked terminals were found in the proliferating neuromuscular synapses, all the growing and newly formed terminal branches were covered with a layer of the Schwann protoplasm containing oval dark nuclei. While the neurofibrillar component of certain changed neuromuscular synapses was completely destroyed, the syncytial glial structure remained intact and did not lose its connection with the Schwann elements of the sheaths of the destroyed motor nerve fibres.

REFERENCES

1. *Анисимова-Александрова, В. В., Старшинова, В. В.:* (1958) Морфологические изменения нервного аппарата глазных мышц под влиянием ионизирующей радиации. Тр. Смоленск. гос. мед. ин-та, *8*, 151. — 2. *Жаботинский, Ю. М.*: (1950) Патологические изменения внутримышечных нервных стволов и их окончаний в поперечнополосатой мускулатуре при гнойных процессах с общими септическими или токсическими явлениями. Архив патологии *6*, 15. — 3. *Полянцев, А. А.:* (1946) Изменения в диафрагмальном нерве и диафрагме при воспалительных процессах в легких и плевре. Сборник научн. трудов клиники общей хирургии Башкирского мед. ин-та, *1*, 138. — 4. *Поликарпова, Г. А.:* (1961) Состояние иннервационного аппарата поперечнополосатых мышц после воздействия на организм рентгеновских лучей. Проблемы морфологии, патморфологии и реактивности периферических отделов нервной системы. Казань, 304. — 5. *Поликарпова, Г. А., Сватко Л. Г., Шмелева, Г. Н.:* (1961) Морфологические выражения дистрофических процессов в двигательных нервных аппаратах поперечнополосатых мышц при тяжелых заболеваниях организма. Проблемы морфологии, патоморфологии и реактивности периферических отделов нервной системы. Казань, 237. — 6. *Сватко, Л. Г.:* (1954) Изменения иннервационного аппарата мышц гортани человека при раковой болезни и некоторые особенности афферентной иннервации этих мышц. Диссертация. Казань. — 7. *Филатова, А. Г., Лаврентьев, Б. И.:* (1935) Гистопатология нижнегортанного нерва и его окончаний при туберкулезном ларингите. Тр. Тат. ин-та теор. и клинич. медицины, *2*, 183. — 8. *Фалин, Л. И.:* (1960) Об изменениях двигательных нервных окончаний скелетных мышц при хронической сердечной недостаточности. Архив патологии, *1*, 69. — 9. *Хазанов, А. Т.:* (1940) Об изменениях нервных волокон и моторных окончаний в мускулатуре диафрагмы при туберкулезе легких. Архив патол. анатомии и патол. физиологии, VI, *5*, 45. — 10. *Шмелева, Г. Н.:* (1958) К вопросу об изменении периферической нервной системы при хроническом нефрите. Тезисы докладов научн. конференции Казанского мед. ин-та, посвящен. Великой Октябрьской Соц. Революции, Казань, 16. — 11. Anderson-Cedegren, E.: (1959) Ultrastructure of Motor End-plate and Sarcoplasmic Components of Mouse Sceletae Muscle Fiber. Ultrastruct., Suppl. I, 1. — 12. Birks, R., Katz, B., Miledi, R.: (1960) Physiological and Structural Changes at the Amphibian Myoneural Junction, in the Course of Nerve Degeneration. J. Physiol. (kond.) **150**, 145. — 13. Couteaux, R.: (1958) Morphological and Cytochemical Observations on the Postsynaptic Membrane at Motor End-plates and Ganglionic Synapses. Exp. Cell. Res., Suppl. **5**, 294. — 14. Csillik, B.: (1960) Contributions to the Development of the Myoneural Synapses. Ontogenetic Aspects of the Subneural Apparatus. Z. mikr.-anat. Forsch., **52**, 150. — 15. Csillik, B., Sávay Gy.: (1958) Die Regeneration der subneuralen Apparate der motorischen Endplatten. Acta neuroveg. (Wien), **19**, 41. — 16. Reger, J. F.: (1957) The Ultrastructure of Normal and Denervated Neuromuscular Synapses in Mouse Gastrocnemius Muscle. Exp. Cell Res., **12**, 661. — 17. Robertson, J. D.: (1960) Electron Microscopy of the Motor End-plate and the Neuromuscular Spindle. Amer. J. phys. Med., **39**, 1. — 18. Zelena, J., Szentágothai, J.: (1957) Verlagerung der Lokalisation spezifischer Cholinesterase während der Entwicklung der Muskelinnervation. Acta histochem. (Jena) **3**, 284.

BEOBACHTUNGEN ÜBER DIE SCHWANNSCHEN NEUROGLIA-ELEMENTE BEI DER VERÄNDERUNG DER NEUROMUSKULÄREN SYNAPSEN

G. A. POLIKARPOVA, L. G. SVATKO und G. N. SHMELEVA

Es wurden im Laufe verschiedener schwerer Krankheiten (Krebs, Nephritis, Leukose, akute Strahlenschädigung) die Veränderungen der in den motorischen Nervenendigungen der quergestreiften Muskel befindlichen Schwannschen Zellen bei Menschen und Katzen untersucht. Die neuromuskulären Synapsen wurden nach Bielschowsky-Gross mit Silber imprägniert und phasenkontrastmikroskopisch untersucht. Diese Methode ermöglichte die Beobachtung aller Elemente der neuromuskulären Synapsen, d. h. der Endzweige der motorischen Nerven, der Schwannschen Glia-Elemente, sowie des Sarkoplasmas und dessen Kerne. In den Glia-Elementen einzelner Endplatten waren Zeichen von Proliferation sichtbar. Erst nahm die Zahl der Neuroglia-Kerne zu, dann wuchs die Masse des Protoplasmas und seine Struktur veränderte sich. In normalen neuromuskulären Synapsen ist das Protoplasma dem Symplast ähnlich, nach der Veränderung zeigte dieses eine syncytiale Struktur. In den veränderten Synapsen wies das Protoplasma unregelmäßige Lamellen auf, die dunkle ovale Nukleolen enthielten und untereinander mit protoplasmatischen Bändern verbunden waren. Die wachsende Masse des syncytialen Schwann-Protoplasmas und dessen Kerne befindet sich auf der Oberfläche der Muskelfasern zwischen Sarkolemm und Endomysium. Die Zunahme der Masse der Schwannschen Elemente ging mit proliferativen Veränderungen in den Axonenden der motorischen Nerven einher. In den veränderten neuromuskulären Synapsen waren keine bloße Endigungen zu beobachten. Alle wachsende und neugebildete Nervenendigungen waren von einer Schwannschen Protoplasmaschicht bedeckt, in der dunkle ovale Nukleolen sichtbar werden. Obwohl der neurofibrilläre Komponent einzelner neuromuskulärer Synapsen vollkommen zugrunde gegangen ist, blieb die syncytiale Gliastruktur unverändert und ihre Verbindung mit den Schwannschen Zellen der Scheide der degenerierten motorischen Nervenfasern blieb erhalten.

НАБЛЮДЕНИЯ НАД ШВАННОВСКИМИ ГЛИАЛЬНЫМИ ЭЛЕМЕНТАМИ В ИЗМЕНЕННЫХ МИОНЕВРАЛЬНЫХ СИНАПСАХ

ПОЛИКАРПОВА Г. А., СВАТКО Л. Г. и ШМЕЛЕВА Г. Н.

В настоящем исследовании изучалось состояние глиальной ядерноплазматической части двигательного нервного окончания при различных патологических процессах в организме (рак, нефрит, экспериментальная острая лучевая болезнь, лейкоз и др.). Материал обрабатовался импрегнацией серебром по методу Бильшовского—Грос, препараты изучались при помощи фазового-контрастного устройства. Изменения, наблюдаемые в мионевральных синапсах, касаются как нейрофибриллярного компонента окончаний, так и их ядерно-плазменных образований, в особенности глиальных шванновских элементов. В окончаниях с прогрессирующими реактивными изменениями со стороны нейрофибриллярного аппарата, в виде разрастания терминалей осевого цилиндра, наблюдается так-же увеличение глиальной части, которая наряду с увеличением массы начинает менять свою структуру. Растущие отделы глиоплазмы принимают синцитиальное строение, количество ядер во много раз увеличивается. Все части разросшегося терминального аппарата всегда погружены в глиальную протоплазму, которая является необходимой составной частью растущих терминалей. Таким образом существует параллелизм между характером реакции нейрофибриллярного аппарата и глиальной частью мионевронального синапса.

G. A. POLIKARPOVA
L. G. SVATKO
G. N. SHMELEVA

Казань, Кафедра гистогологии Медицинский Институт, СССР