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# Postembryonic Development of the Neural Lamella of the Hypocerebral Ganglion in *Melanogryllus desertus* Pall. (Orthoptera, Gryllidae)

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**Abstract:** The sheath of the hypocerebral ganglion was divided into two parts: an outer layer, the neural lamella and an inner layer, the perilemma. Dark and light types of glial cells were identified in the perilemma. In the 4th and 7th nymphal stages, some fibrous material related to the neural lamella accumulated in the light glial cells. It was surrounded by rough endoplasmic reticulum membranes and was then transported towards te outer parts of the sheath. Finally, it was bound to and intermingled with the lamella. These observations indicate that the 4th and 7th nymphal stages are critical for the development of the neural lamella. In the 9th nymphal and adult stages, the sheath of the ganglion was fully formed.

**Key Words:** Insect neuroendocrine system, Hypocerebral ganglion, Neural lamella, Perilemma, Glial cells.

## *Melanogryllus desertus* Pall. (Orthoptera, Gryllidae) Hiposerebral Gangliyonunda Neural Lamella'nın Postembriyonik Gelişimi

**Özet:** Hiposerebral ganglion kılıfı dışta nöral lamella ve içte de perilemma olarak iki kısma ayrılmaktadır. Perilemma'da koyu ve açık gliyal hücreler ayırt edilmiştir. 4. ve 7. nimfal dönemde, açık renkli gliyal hücreler içinde nöral lamella ile ilişkilendirilen fibrilli materyal birikir. Sözkonusu materyal granüllü endoplazmik retikulum membranlarıyla çevrelenir ve kılıfın dış kısmına doğru taşınır, sonunda nöral lamellaya bağlanarak birleşip kaynaşır. Gözlemler, 4. ve 7. nimfal dönemlerin nöral lamella gelişimi için kritik periyodu oluşturduğunu ortaya koymuştur, 9. nimfal dönem ve ergin dönemde gangliyon kılıfı tamamen şekillenmiş durumdadır.

**Anahtar Sözcükler:** Böcek nöroendokrin sistemi, Hiposerebral gangliyon, Nöral lamella, Perilemma, Gliyal hücreler.

## Introduction

As a component of the insect neuroendocrine system, the hypocerebral ganglion is located ventrally in the oesophagus on the pathway of neural impulses. Although there is some general information in the literature about the structure and function of the ganglion (1, 2, 3, 4, 5), there has been little detailed research. However, the fine structure and postembryonic development of the ganglion have been examined in a harmful orthopter, *Melanogryllus desertus* (6).

Like the other parts of the neuroendocrine system, the ganglion is covered by a neural lamella of connective tissues. Scharrer (7) and Hess (8) reported some early observations on the formation of the neural lamella, and postulated that the glial cells underlying it were probably involved in production. Despite these findings, they did not mention when or how the neural lamella is produced. The layers of connective tissue are usually developed after the organ they cover is almost fully formed (9). The neural lamella continues to increase in thickness during the postembryonic stages, but, as noted by Ashhurst (10), this process has not yet been investigated. Although there is some evidence for the role of glial cells in the development of the neural lamella (6), the percise relationship has not been clearly identified. In addition, the functions of glial cells in regeneration and neural repair of the insect central nervous system (11, 12, 13, 14, 15) strongly suggest that there are some parallels between glial repair and normal development. Furthermore, as suggested by Smith et al. (16), with more information on the normal development of the lamella of insects, it would be possible to make some comparisons with neural repair in mammals.

Thus, the aim of this study was to investigate the postembryonic development of the neural lamella and to discover whether, in terms of ultrastructure, the glial cells are the main component of the postembryonic development of the connective tissues.

## Materials and Methods

Specimens of adult female and male *Melanogryllus desertus* (Orthoptera, Gryllidae) were collected from the district of Bornova and cultured in laboratory conditions as described by Karakişi (6). Eggs were obtained from the cultured specimens.

Because of their morphological features, the 4th, 7th, and 9th nymphal and adult specimens were dissected. Hypocerebral ganglions were quickly removed and fixed in cacodylate–buffered paraformaldehyde and glutaraldehyde. Phosphate–buffered osmium tetraoxide was used for postfixation. The tissues were embedded in EPON 812. Thin sections were stained with uranyl acetate–lead cytrate and examined in a JEOL 100C electron microscope.

## Results

The structure of the fully developed ganglion sheath was first identified in the adult specimens.

The sheath covering the ganglion was identical to the sheath of the corpus cardiacum (Fig. 1). Exteriorly, the sheath had a homogenous non–cellular layer, the neural lamella. The cellular layer, the perilemma, was situated under the neural lamella. The neural lamella was infolded and penetrated to the inner part. It was fibrous or lamellar in appearance inside the cover (Fig. 2).

The main ultrastructural elements of the perilemma (Fig. 2) were the glial cell bodies and protrusions. The light and dark types of glial cells were easily distinguished due to their different cytoplasmic and nuclear densities. Moreover, the cells were organized in different

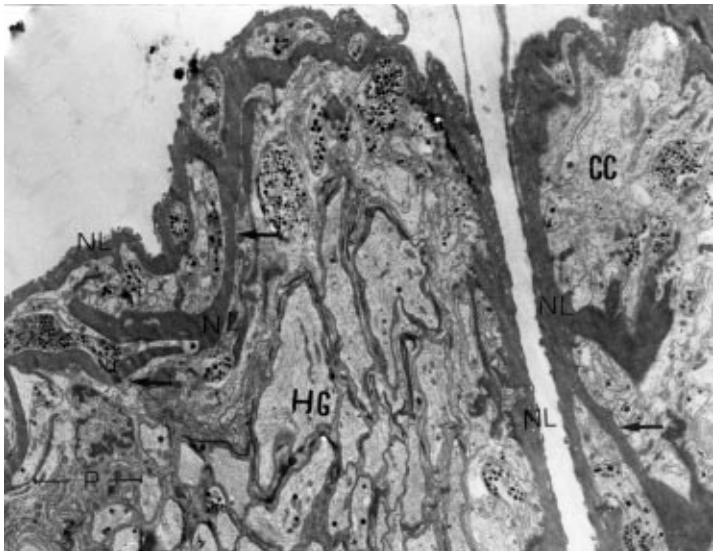


Figure 1. Adult stage. The sheath covered hypocerebral ganglion (HG) and corpus cardiacum (CC). The homogenous layer, neural lamella (NL), its infoldings (→) and the cellular layer, the perilemma (P)x4600.

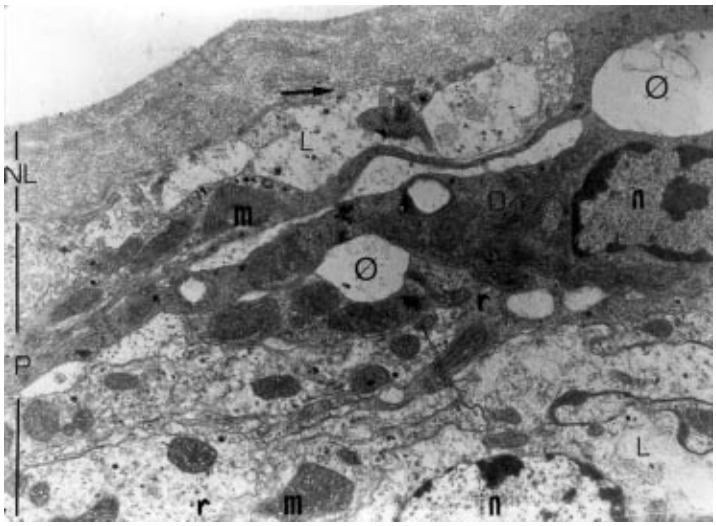


Figure 2. Adult stage. Fibrous or lamellar (→) appearances of neural lamella (NL). Light (L) and dark (D) types of glial cells contained nuclei (n), mitochondria (m) and free ribosomes (r). Note the widened extracellular area (Ø). x 11200.

localizations, the light ones situated immediately below the neural lamella, whereas the dark ones were located interiorly. Both types of cells were rich in mitochondria and free ribosomes. The extracellular areas were often widened.

In order to establish the relationships between the neural lamella and glial cells, the progressive formation of the ganglion sheath was investigated in the 4th, 7th and 9th nymphal stages.

In the 4th nymphal stage, because of the absence of the widened extracellular area, the cellular part of the sheath was compact in appearance. Dark glial cells with rough endoplasmic reticulum, well–developed mitochondria and free ribosomes were located below the protrusions of the light glial cells (Fig. 3). The axonal bundles surrounded by the protrusions were characterized by the presence of granules (Fig. 3, 4).

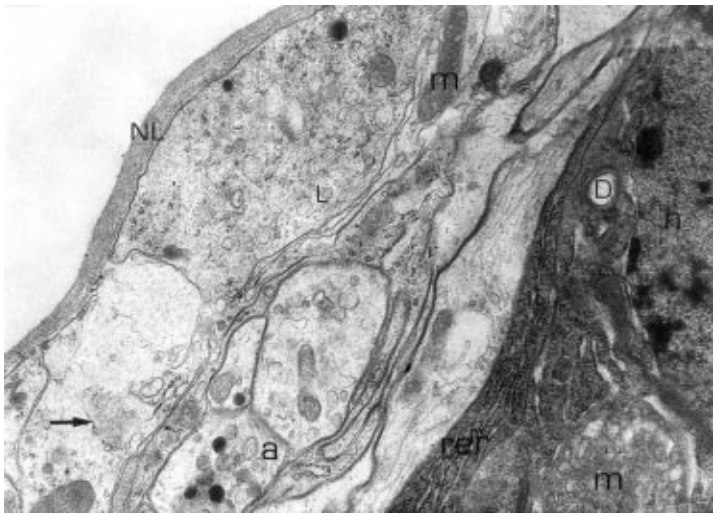


Figure 3. 4th nymphal stage. Compact appearance of the perilemma with some axonal bundles (a). Accumulation of the fibrous material (→) in light cells (L) situated immediately below the neural lamella (NL). Dark glial cells (D) with nucleus (n), rough endoplasmic reticulum (rer), and well–developed mitochondria (m). x 17000.

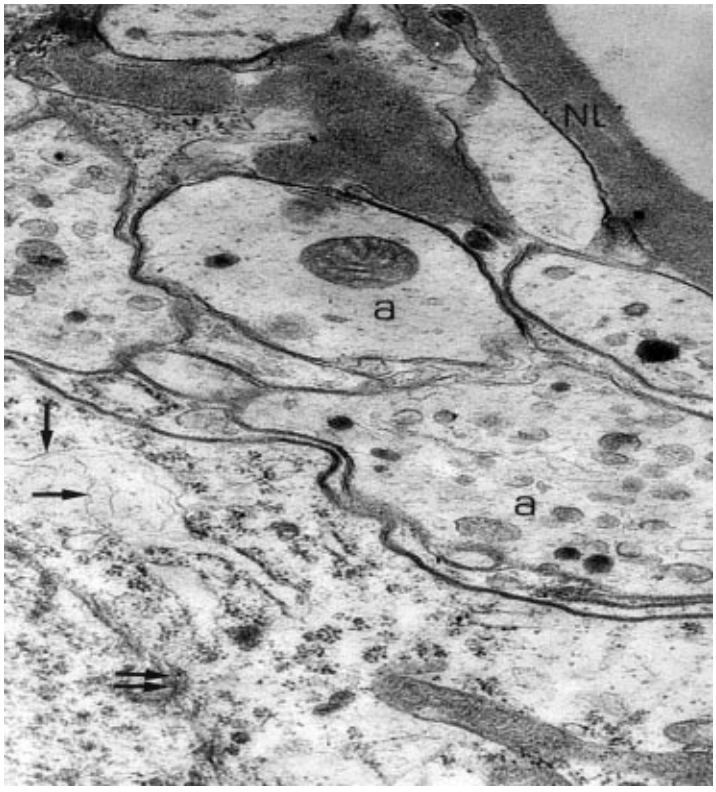


Figure 4. 4th nymphal stage. Longitidunally oriented (→) or irregularly cumulated (⇉) fibers in light glial cells. Axonal bundles (a) surrounded by the protrusions of the light cells and infoldings of the neural lamella (NL). x 17000.

An accumulation of some fibrous materials which resembled the neural lamella was the striking feature of the light cells durnig this stage (Fig. 3). No accumulation was observed in the dark cells.

The fibrous materials were composed of thin fibers which were oriented longitidunally, or cumulated irregularly (Fig. 4). Cumulations were also observed in widely dispersed clusters (Fig. 5). The clusters packed by the rough ER membranes were transported towards the neural lamella (Fig. 6) and finally, bound to and intermingled with it (Fig. 7).

The transportation, binding and intermixing of the fibrous materials were continuous processes. As shown in Fig. 6, it was possible to observe the pre–bound and still moving clusters together.

The definitive property demonstrated in the 7th nymphal stage was the presence of infoldings of the neural lamella. Rough endoplasmic reticulum membranes continued to bound the fibrous materials (Fig. 8). Some ultimate relationships between the membranes of the rough endoplasmic reticulum and Golgi apparatus were also observed (Fig. 9).

In the 9th nymphal stage, the neural lamella was very similar in structure to its formation in the adult stage; it was fully formed and well developed (Fig. 10). The widened extracellular areas mentioned above in respect of the adult stage were also seen in the perilemma (Fig. 11). Interestingly, no accumulation or transportation of fibrous materials was observedd in this stage. Furthermore, the existence of the small amount of rough endoplasmic reticulum indicated the completion of the postembryonic development of the lamella.

## Discussion

Because of the fact that there have been few reported studies of the structure of the hypocerebral ganglion, we

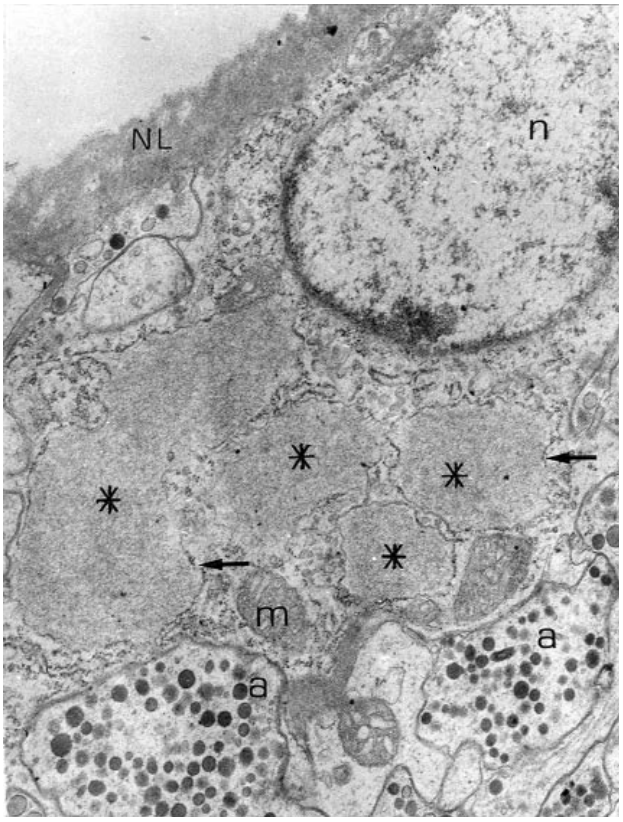


Figure 5. 4th nymphal stage. Light glial cells placed between the neural lamella (NL) and axonal bundles (a). Fibrous materials in widely dispersed clusters (*) surrounded by rough endoplasmic reticulum membranes (→). Nucleus (n) and mitochondria (m) can also be seen.

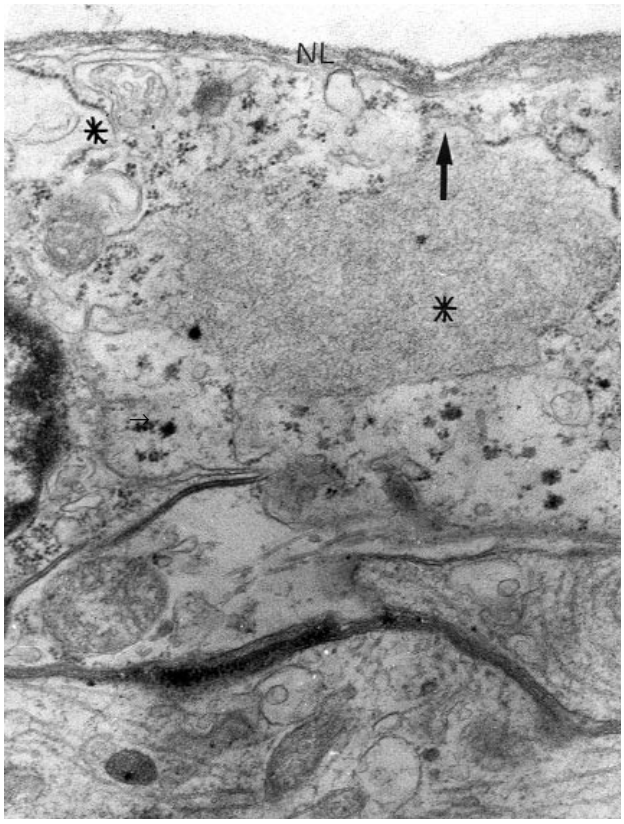


Figure 6. 4th nymphal stage. Transporting of the packet clusters ( * ) of fibrous materials towards the neural lamella (NL). Note the empty vacuoles of the bound material (*) . x 23000.

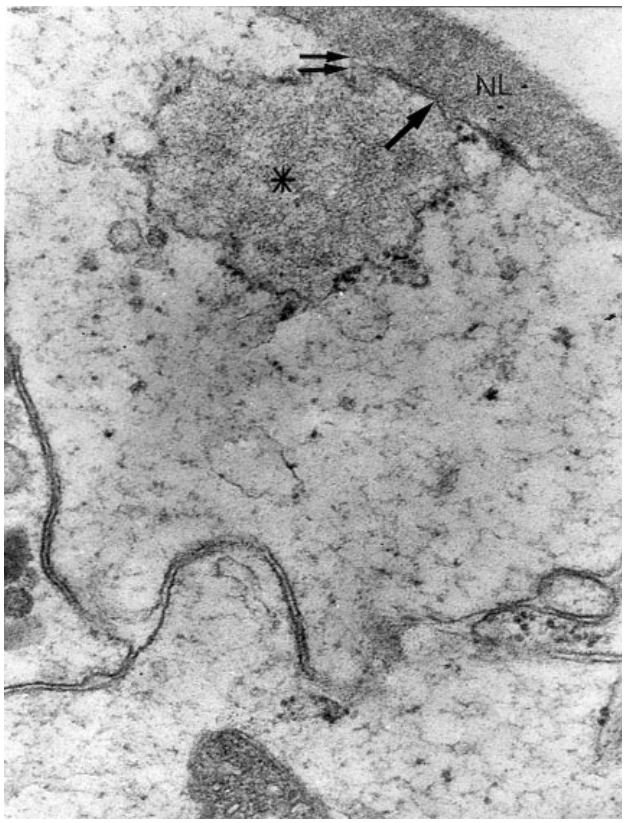


Figure 7. 4th. nymphal stage. The binding (→) and intermingling (⇉) of the packed clusters (*) with the neural lamella (NL).x 46000.

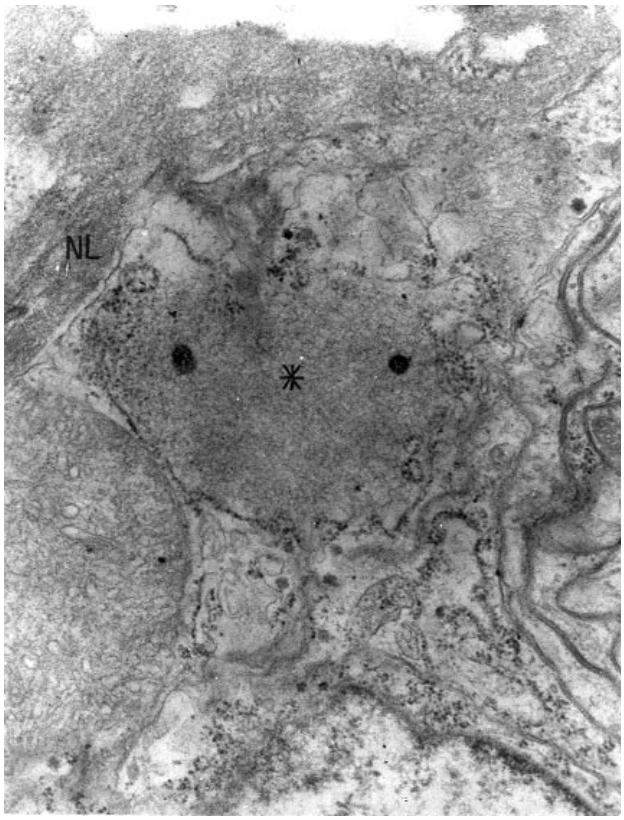


Figure 8. 7th nymphal stage. Packed clusters (*) exhibited near the infoldings of the neural lamella (NL), x 29000.

have had to restrict the discussion on the references given concerning other parts of the insect neuroendocrine system.

Since Scharrer's study (7), the terminology used for naming the layers of the sheath of the neuroendocrine system has been inconsistent. Scharrer called the sheath of the cockroach cerebral ganglion the "perilemma", and divided it into two parts: an outer, homogenous "neural lamella" and, an inner, cellular "perineurium". Although the term "perineurium" was discarded by Hess (8) since this word denoted a connective tissue layer in the nerves of other animals, there has not been agreement on the terminology. For example, the outer layer has been called the "basement membrane" or "connective tissue" (17); "acellular laminated sheath" (18), "tunica propria" (19) and "stroma" (1, 20). Moreover, the terms "perineurial and subperineurial lamella" have been used again by some authors (13, 15).

We have used the terms "neural lamella" and "perilemma" suggested by Hess because of their fine structural appearances. However, for the perilemma, the terms "sheath cells" and "barrier cells" used by Smith et al. (16) seem more useful since they indicate the different functions of the glial cells.

What ever the parts of the sheath are called, close relationships between the two layers of the sheath were observed in the present study. The light glial cells of the perilemma were the main component of the postembryonic developmental processes of the neural lamella. This provides definitive proof for the previous suggestions of Scharrer (7), Hess (8) and Odhiambo (17).

Buy why, some may ask, should only one type of glial cells have a natural ability to develop connective tissue? We strongly believe that, at least in *Melanogryllus desertus,* this is the case; fibrous materials were not observed in the dark type of glial cells. As a result, dark cells may be involved in other functions.

The diversity of neuroglia in the insect central nervous system has not been clearly identified. However, Smith et al. (16) adapted a classification system based on an analogy of the components to mammalian cells. The authors suggested that insects have a well–developed

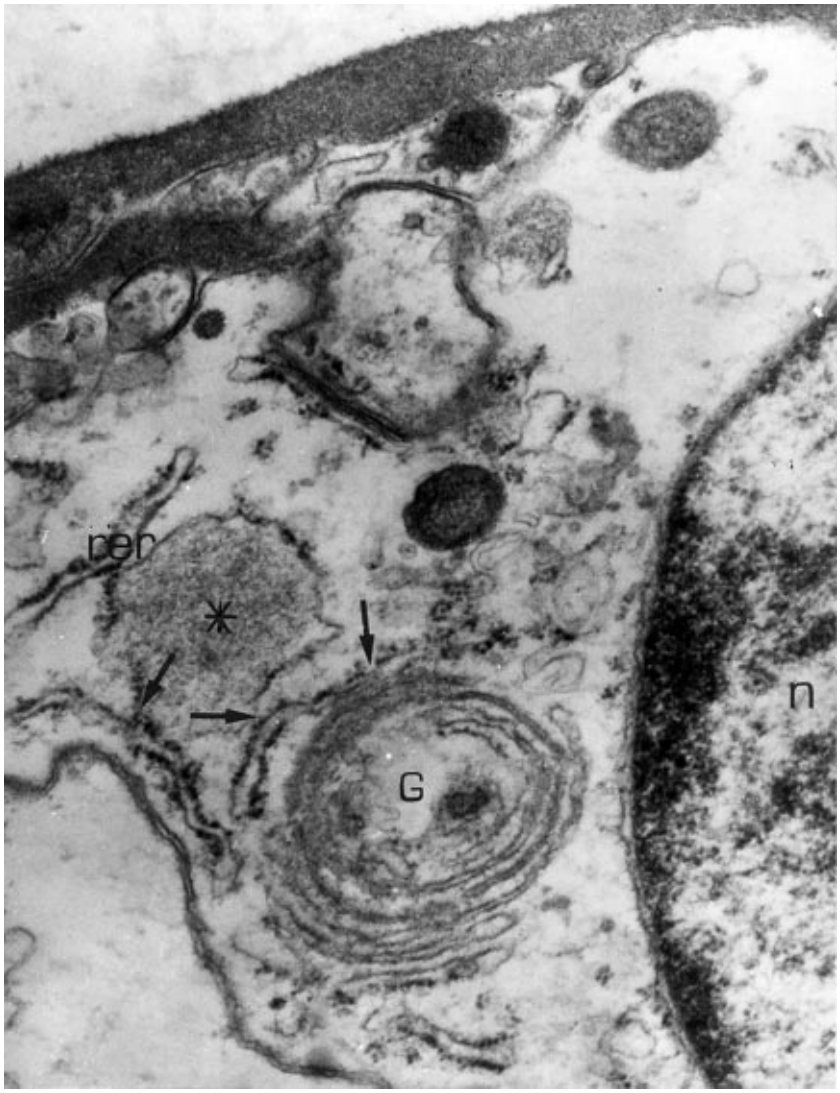


Figure 9. 7th nymphal stage. The close relationships (→) between the membranes of rough endoplasmic reticulum (rer) surrounding the clusters (*) and Golgi (G); nucleus (n) also can be seen. x 18000.

blood–brain barrier system. This system consists of two cell types: the sheath cells localized in the outer parts of the cellular layer, and the barrier cells situated immediately below them. In terms of ultrastructure, our conclusions are also in agreement with the classification of Smith et al.; the light glial cells may be identical to the sheath cells and it is very probable that the dark glial cells could correspond to the barrier cells of Smith et al.

Although some detailed investigations have been carried out to identify the embryonal development of the connective tessues (9, 21), as noted by Ashhurst (10), the postembryonic stages of development have not been firmly established. Ashhurst concluded that in embryos of *Schistocerca gregaria*, the perineurial cells formed a thick layer from days 9 to 11. On day 9, the nervous system was surrounded by a basement membrane, and, by day 12, the nymphal neural neural lamella was fully formed.

Our results indicate that the 4th and 7th nymphal stages are critical periods in the postembryonic development of the neural lamella of *Melanogryllus desertus*. In the 9th nymphal stage, the lamella was extensively formed. In *Schistocerca gregaria* embryos, Ashhurst reported only a small amount of rough endoplasmic reticulum on day 12. In the 4th and 7th nymphal stages of *Melanogryllus desertus*, fibrous materials were surrounded by large amounts of rough endoplasmic reticulum. However, in the 9th nymphal and adult stages, the endoplasmic reticulum was observed in only small amounts.

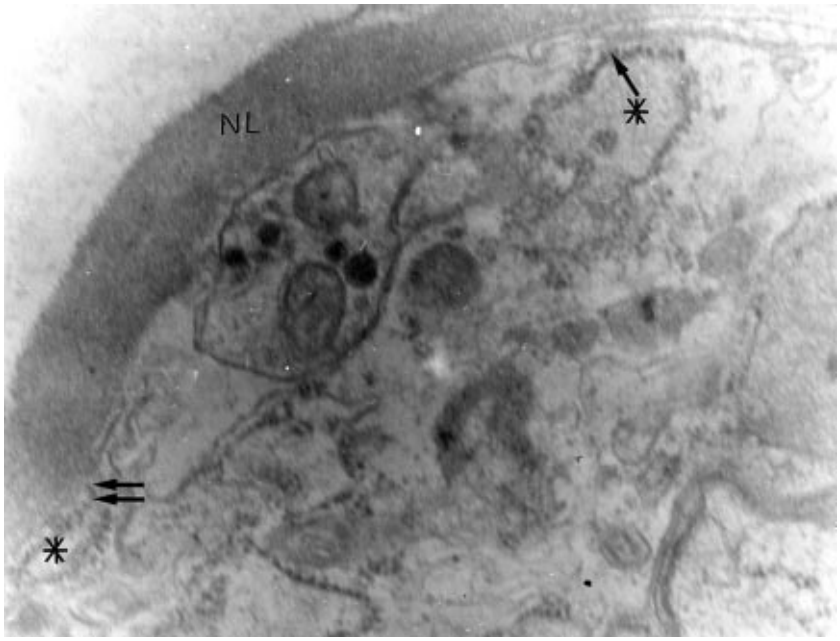


Figure 10. 7th nymphal stage. Note the still binding (→) and pre–bound (⇉) clusters (*). x 34000.

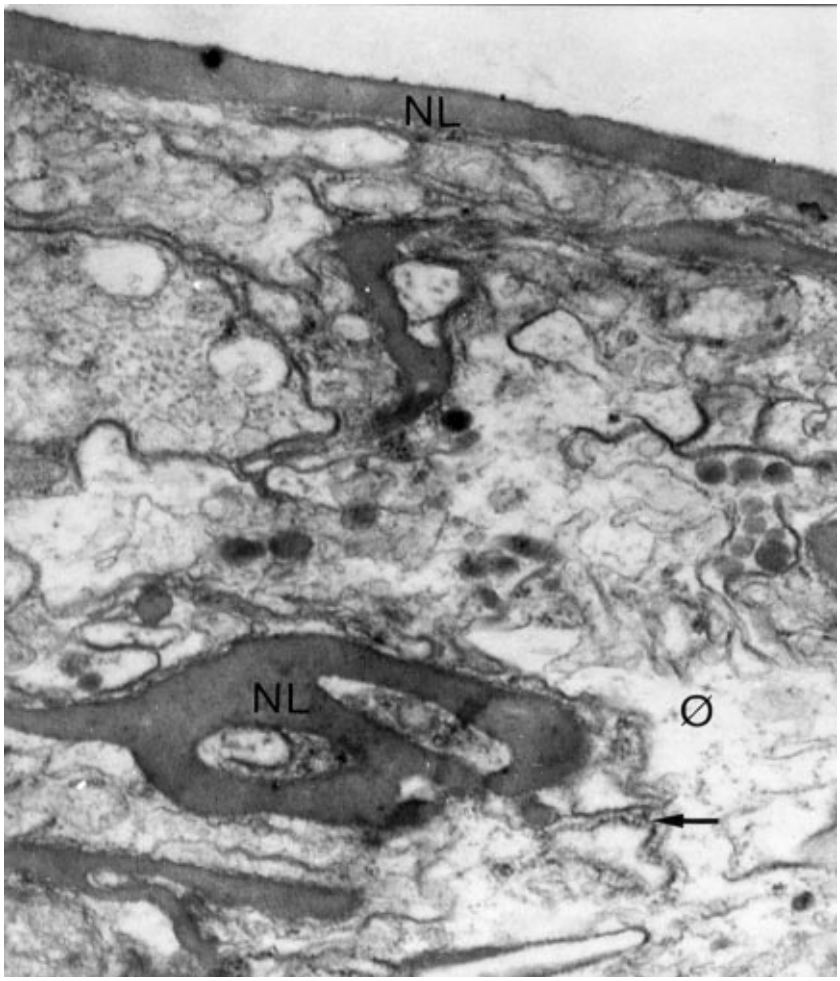


Figure 11. 9th nymphal stage. A well developed and infolded neural lamella (NL). There are widened extracellular areas in the perilemma (∅). Note the small amount of rough endoplasmic reticulum (→) in the light glial cells. x 26000.

The biochemical properties of the fibrous material forming the insect connective tissue have been discussed widely since Gray's (22) first report of the presence of collagen–like fibers in the insect tympanal membrane. Although Scharrer (18) reported that the connective tissue covering the corpus cardiacum of *Leucophae maderae* was collagenic, a non–collagenic structure in the sheath of the corpus allatum of *Schistocerca gregaria* was suggested by Odhiambo (17).

Ashhurst (10) collated and compared data obtained from histochemical studies, and concluded that the neural lamella of some species like *Locusta migratoria, Periplenata americana, Carausius morosus* and *Galleria sp.* contained glycosaminoglycans which are always associated with fibrous collagen.

We also observed regular, thin bands on some individual fibers (Fig. 5). Nevertheless, it is not easy at this stage to say that these were completely collagenic. However, general information about the bioysynthesis and formation of the collagen indicate that the precursor materials were synthesized in the rough endoplasmic reticulum and then transferred to Golgi membranes. In other words, the proteinic chains are formed as procollagen in the Golgi apparatus (23, 24). The ultimate relationships observed between the endoplasmic reticulum and Golgi membranes (Fig. 9) strongly support the general information.

In recent years, several studies focusing on the mechanisms of glial regeneration in the insect central nervous system have been carried out (11, 12, 13, 14, 15). In *Drosophila melanogaster*, Smith et al. (16) reported that in the pattern of events following selective lesions of neuroglia, haemocytes derived from the bathing haemolymph could be transformed into granule–containing cells which play an important role in neural repair. The sequences of glial repair mentioned in that report could bear a number of similarities to the equivalent processes of the vertebrate central nervous system. The authors also noted that the sheath cells occurred by redifferentiation of the circulating blood cells. In addition, direct evidence of the site of the origin of the components of neural repair has been obtained in studies performed on *Drosophila* mutants. These studies indicate that sheath cells and granule–containing cells have the same mesodermal origin. In light of this it is probable that the special function of the light glial cells in *Melanogryllus desertus* is related to the natural regeneration ability of these cells, at least in the 4th and 7th nymphal stages.

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