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Investigation of the histological development of the frontal ganglion in *Locusta migratoria* L. 1758 (Orthoptera, Acrididae)

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Abstract: The frontal ganglion plays a critical role in insect feeding and molting-related behaviors. It is present in all insect orders at all developmental stages. In this study, the histological development of the frontal ganglion of *Locusta migratoria*, the African migratory locust, was investigated. Ganglia were dissected at 3 distinct developmental stages and their histological structure was evaluated at the light microscope level. The development of the cells generating the ganglion in the first and fifth nymphal stages and in adults was determined using the variance analysis method. Total width and length of the ganglion cells as well as width and length of the nucleus were measured. A significant expansion of the cell and nucleus diameter was observed during development from the youngest stage toward adulthood.

Key words: *Locusta, Locusta migratoria*, African migratory locust, frontal ganglion, histology

Locusta migratoria L. 1758 (Orthoptera, Acrididae)'da frontal ganglionun histolojik gelişiminin incelenmesi

Özet: Frontal ganglion böceklerde beslenme ve deri değiştirmeye bağlı davranışlarda büyük bir rol oynar. Tüm gelişimsel evrelerde bütün böcek ordolarında mevcuttur. Bu çalışmada, Afrika göçmen çekirgesi *Locusta migratoria*'nın frontal ganglionu 3 farklı evrede dissekte edilip, ganglionun histolojik gelişimi incelenmiştir. Ganglionun histolojik yapısı ışık mikroskobu düzeyinde gösterilmiştir. Ganglionu oluşturan hücrelerdeki gelişme 3 farklı evrede ölçümler alınarak varyans analizi yöntemleriyle belirlenmiştir. Birinci, beşinci nimfal ve ergin evrelerdeki ganglion hücrelerinin total hücre en ve boyları ile nukleus en ve boylarına ait ölçümler alınmıştır. Gelişme sürecinde genç evreden ergine doğru hücre ve nukleus çaplarında bir genişleme olduğu belirlenmiştir.

Anahtar sözcükler: *Locusta*, *Locusta migratoria*, Afrika göçmen çekirgesi , frontal ganglion, histoloji

Introduction

The stomatogastric nervous system (SNS) forms a network of peripheral ganglia associated with the insect gut. The formation of the SNS presents many parallels to the development of the vertebrate peripheral nervous system. The SNS nerves innervate muscles of the mouth cavity, foregut, and midgut, and regulate food uptake and food transport (Hartenstein, 1997). The SNS originates at the dorsomedial surface of the foregut. In locusts, it consists of 4 ganglia located along the foregut. These are the unpaired frontal and hypocerebral ganglia, and the paired ingluvial ganglia (Ganfornina et al., 1996).

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The frontal ganglion (FG) is an essential component of the stomatogastric nervous system of insects (Willey, 1961; Penzlin, 1985; Ayali, 2004). In locusts, the FG lies in the forehead, on the dorsal side of pharynx, in front of the brain (Ayali et al, 2002). Anteriorly, the FG is connected to the tritocerebrum by the paired frontal connectives. Posteriorly, it gives rise to the unpaired recurrent nerve, running to the hypocerebral ganglion (Ude et al., 1978). The other 3 pairs of efferent nerves (the anterior, posterior, and median pharyngeal nerves) branch onto the dilator muscles of the gut in a rostrum to caudal order, making the FG the major source of foregut muscles innervation. The ganglion is characterized by a central neuropil surrounded dorsally and laterally by a single or double layer of neurons (Ayali and Zilberstein, 2003). Aubele and Klemm (1977) described 19 neurons located in the FG (20 μm in diameter) that send their axons to innervate foregut muscles via the frontal connectives.

The FG plays a critical role in locust molting (Hughes, 1980; Zilberstein et al., 2006). The role of the FG during molting has been reported for a number of insect species (Bounhiol, 1938; Clarke et al., 1963; Penzlin, 1971; Hughes, 1980; Carlson et al., 1983; Bell, 1986; Bestman et al., 1997; Miles et al., 1998). There are 2 stages during ecdysis in which an insect needs to exert pressure on the body wall (Reynolds, 1980). The first stage is splitting the old cuticle and the second is expanding the new cuticle and wings. The principal mechanism of this process is filling the gut with air (Zilberstein et al., 2006; Ayali, 2009). During the molt, the FG displays an air-swallowing behavior. A frontal ganglionectomy inhibits air swallowing and, as a result, eclosion and wing expansion fail to occur properly (Bell, 1986).

The FG is important for the control of food uptake throughout the gut, and crop emptying (Ayali and Zilberstein, 2003). A frontal ganglionectomy caused a decrease in feeding activity and food intake in *Schistocerca gregaria* (Hingham et al., 1966) and *Locusta migratoria* (Bignell, 1974).

The anatomical location of the ganglion has been shown in many species (Ayali, 2004), such as *Acheta domesticus* (Kirby et al., 1984), *Periplaneta americana* (Willey, 1961), *Schistocerca gregaria* (Dando et al., 1968), *Manduca sexta* (Borg et al., 1973), and *Apis mellifera* (Boleli et al., 1998). The details of the cellular structure of the insect FG were shown by electron microscopy studies of *P. americana* (Ude et al., 1978) and *Acheta domesticus* (Kirby et al., 1984).

The life cycle of all locust species comprises 3 stages: egg, larva, and adult. The larval stage of gregarious locusts is divided into 5 instars. At the first instar, their color is white when newly hatched, but in 1-2 h, it turns black. The second instar is distinguished by a much larger head compared to the first instar. At the third instar, 2 pairs of wing buds are easily recognizable. At the fourth instar, their color is black and yellow, while the wing buds are larger and more obvious, but they are shorter than the length of the pronotum. At the fifth instar, their color is bright yellow with a black pattern. Wing buds are larger than the length of the pronotum, but they cannot be used for flight. The final molt is from the fifth instar hopper to the adult stage.

This study was aimed at comparatively determining the cellular structure of the FG in *L. migratoria* in the first and fifth instars and in the adult stage.

Materials and methods

Animals

L. migratoria specimens were reared under crowded conditions in 60-L cages. The cages were kept at 30 °C under a 12-h light, 12-h dark lighting regime. Locusts were fed daily with fresh wheat grass and flaked oats. Both sexes of *L. migratoria* were used. For the histological investigation, adults and larvae of the first and fifth larval stages of both sexes were examined.

Histology

Locusts were anaesthetized in CO_2 and their wings and legs were removed. The FG and the nerves leaving it are easily accessible by cutting out the head cuticle (Zilberstein et al., 2006). The FG was dissected out from adult, first instar, and fifth instar locusts and was put into physiological saline solution. FGs were then fixed in Bouin's solution, embedded in paraffin, sectioned at 5 μm, and stained with Gill's hematoxylin & eosin (H&E) and toluidine blue. The whole mount preparations were photographed with an Olympus

BX51 with the Altra 20 Soft Imaging System and investigated under a light microscope.

Data analysis

The development of the cells generating the ganglion was determined in the 3 distinct stages by variance analysis. The cells were picked randomly and measurements included total width and length of the ganglion cells and width and length of the nucleus. The values measured in each distinct stage were summarized and the data were subjected to ANOVA (unilateral variance analysis) in order to detect any important changes in the cells. All statistical analysis was done with SPSS 17.0 and MS Office Excel.

Results

The FG lies in the frontal region, just anterior to the brain and over the dorsal part of the pharynx; it is connected to the tritocerebrum of the brain with a pair of frontal connectives (Figure 1). The total diameter of the ganglion is approximately 70 µm in the first instar stage, approximately 110 µm in the fifth instar stage, and approximately 140 µm in adults.

There are 2 layers identified in the structure of the sheath enveloping the ganglion. These are the outermost neural lamella, consisting of connective tissue without cells, and the inner perilemma layer, composed of sheath cells. The perilemma is more developed and has a thicker structure than the neural lamella (Figure 2). Although the principle structure of this sheath is similar in different phases throughout the development of the ganglion, an increase in the thickness of the sheath depending on development can be discerned. The ganglion sheath measured 3 µm in the first nymphal stage, 6.25 µm in the fifth nymphal stage, and 7 µm in the adult stage.

Glial cells have a role in protecting the neurons. Nuclei of these cells are frequently seen in the perilemma layer of the ganglion sheath and are abundant in the region between the neuropil and nerve cells (Figure 3). The neuropil region, which is devoid of any ganglion cells, is a neural plexus composed of large and small axons and dendrites and is located in the center of the FG. The neuropil can be subdivided into a central region of coarse neuropil and a peripheral finer region (Figure 4).

The nerve cells of the FG are located on the periphery of the ganglion (Figure 5). These cells are significantly larger than those located in the brain. The size of the ganglion cells, 80-100 in number, is quite variable. The diameters of the neurons range from 7 to 45 µm. The nuclei of the nerve cells are large and seem to be light in color under the light microscope (Figure 6). The homogeneously diffused nucleoplasm is located in the nucleus. The nerve cells

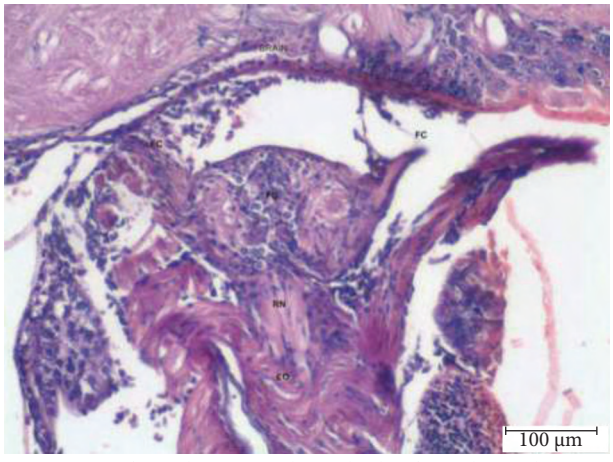

Figure 1. A frontal section through the frontal ganglion and brain where the ganglion is clearly seen and its orientation understood, in the adult stage. H&E stain. FG: frontal ganglion, FC: frontal connective, RN: recurrent nerve, EO: esophagus. Scale bar, 100 µm.

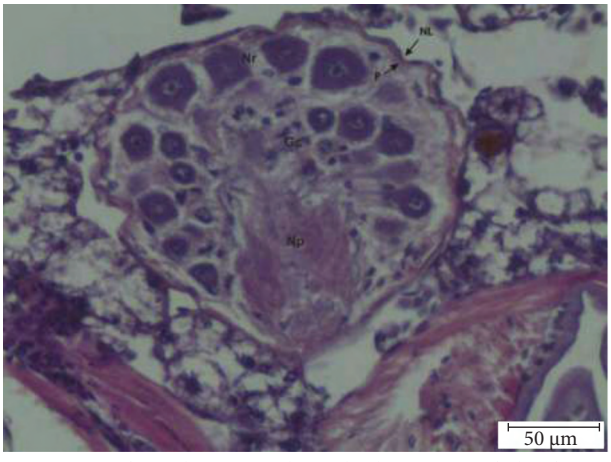

Figure 2. A frontal section through the center of the frontal ganglion, in the adult stage. H&E stain. Gc: glial cell, Nr: neuron, Np: neuropil, NL: neural lamella, P: perilemma. Scale bar, 50 µm.

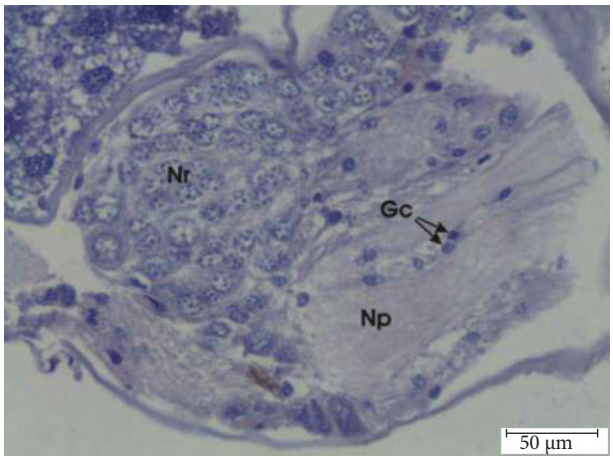

Figure 3. A frontal section through the frontal ganglion at the fifth instar stage. H&E stain. Gc: Glial cells, Nr: neuron, Np: neuropil. Scale bar, 50 µm.

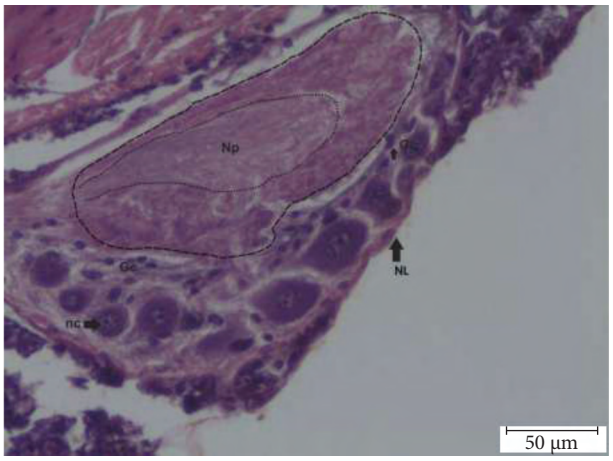

Figure 4. A sagittal section through the frontal ganglion in the adult stage. H&E stain. The neural cell bodies are in the peripheral zone, surrounding the neuropil (marked by a dashed line). The coarse neuropil in the central core of the ganglion (marked by dotted line) can be distinguished from the surrounding finer neuropil. Np: neuropil, NL: neural lamella. Scale bar, 50 µm.

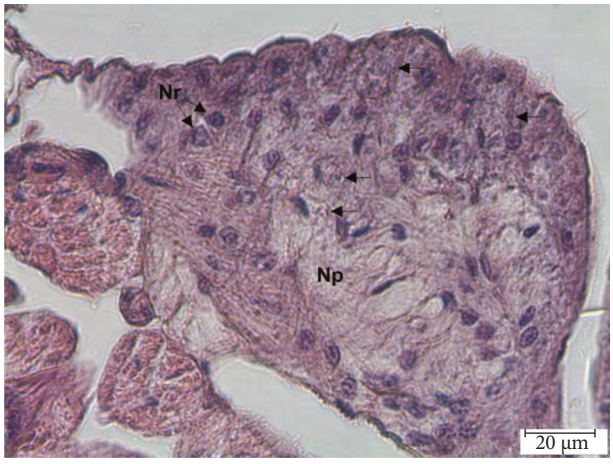

Figure 5. A frontal section through the frontal ganglion in the first instar stage. H&E stain. Np: neuropil, Nr: neuron, Arrows: glial cells. Scale bar, 20 µm.

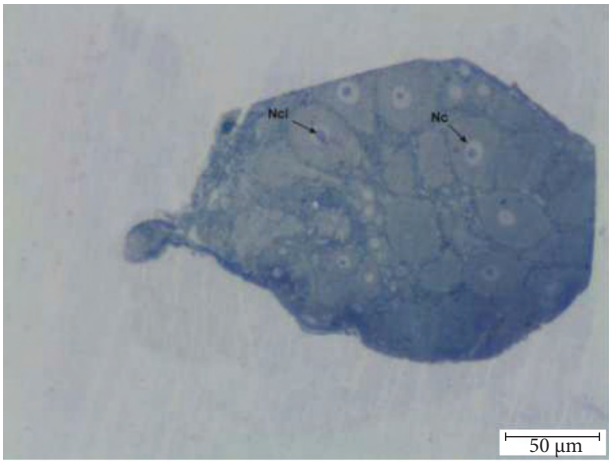

Figure 6. A transverse section through the nerve cells of the ganglion in the adult stage. Toluidine blue stain. The nuclei of the nerve cells are quite big and are seen to be light in color in the slides. Nc: nucleus, Ncl: nucleoplasm. Scale bar, 50 µm.

differ in size at the distinct developmental stages. The cell perikarya are largest in the adult stage.

The development of the cells generating the ganglion was determined using the variance analysis method. The summary statistics related to the values measured in each distinct stage are shown in Tables 1-3. The mean values of width and length of the total cell and nucleus and their 95% confidence intervals are also shown.

The P-value resulting from the unilateral variance analysis was found to be 0.00 for each character. Hence, the significance of the difference between the diameters of the whole cell and the nucleus in the unilateral variance analysis was

Table 1. The summary statistics related to values of diameters of the nucleus and total cell in the first nymphal stage. N: sample number, M: mean, SE: standard error of mean, SD: standard deviation, Min: minimum, Max: maximum.

	Nucleus width	Nucleus length	Total cell width	Total cell length
N	29	29	29	29
M ± SE	4.93 ± 0.17	5.21 ± 0.17	8.66 ± 0.40	10.85 ± 0.57
SD	0.94	0.91	2.16	3.05
Min	3.25	3.25	5.25	6.75
Max	6.50	7.50	15.75	18.75

Table 2. The summary statistics related to values of diameters of the nucleus and total cell in the fifth nymphal stage. N: sample number, M: mean, SE: standard error of mean, SD: standard deviation, Min: minimum, Max: maximum.

	Nucleus width	Nucleus length	Total cell width	Total cell length
N	30	30	30	30
M ± SE	6.25 ± 0.35	7.18 ± 0.36	16.96 ± 0.97	19.53 ± 1.18
SD	1.90	1.99	5.29	6.44
Min	2.75	3.75	7	10.75
Max	9.25	12.50	34	36.50

Table 3. The summary statistics related to values of diameters of nucleus and total cell in the adult stage. N: sample number, M: mean, SE: standard error of mean, SD: standard deviation, Min: minimum, Max: maximum.

	Nucleus width	Nucleus length	Total cell width	Total cell length
N	30	30	30	30
M ± SE	7.85 ± 0.28	8.73 ± 0.33	21.12 ± 1.46	23.45 ± 1.62
SD	1.56	1.78	7.98	8.87
Min	5.50	5.75	10	11
Max	10.25	12.50	39	41.50

confirmed by ANOVA (Table 4). The different measurements obtained in the 3 distinct stages were further subjected to Tukey's test to reveal that most of the distinct characters were significantly different ($P \leq 0.05$). The length and the width of the whole cell in the fifth stage and in adults were not different ($P > 0.05$). Considering all of these data, it is possible to conclude that the cell perikarya may enlarge due to an increase in the amount of cytoplasm from the first stage to the adult phase, and consequently the nerve cells become larger. It is clearly seen that the same enlargement throughout the life stages of the locust occurs in the nucleus, as well.

Table 4. Unilateral variance analysis of total cell and nucleus measurements in the first instar, fifth instar, and adult stages.

ANOVA						
		Sum of squares	**dF**	**Mean square**	**F**	**Significance**
Nucleus width	Between groups	126.18	2	63.09	27.12	0.00
	Within groups	200.06	86	2.33		
	Total	326.24	88			
Nucleus length	Between groups	183.15	2	91.58	34.08	0.00
	Within groups	231.07	86	2.69		
	Total	414.22	88			
Total cell width	Between groups	2366.78	2	1183.39	36.51	0.00
	Within groups	2787.28	86	32.41		
	Total	5154.06	88			
Total cell length	Between groups	2441.92	2	1220.96	28.25	0.00
	Within groups	3743.28	86	43.53		
	Total	6185.21	88			

Discussion

The anatomical location of the FG has been shown in many species (Ayali, 2004). It has been indicated that, histologically, the ganglion has a centrally placed neuropil, it is enveloped by an outermost neural lamella, and the nerve cells extend to the dorsal and lateral parts of the neuropil. In addition, in this study, both the histological structure and the anatomical localization of the ganglion are in accordance with previous results (Willey, 1961; Dando et al., 1968; Borg et al., 1973; Kirby et al., 1984; Boleli et al., 1998).

Aubele and Klemm (1977) reported that the adults of *L. migratoria* had approximately 100 ganglion cells and that the diameters of these cells ranged from 25 to 50 µm. Ude et al. (1978), in a study using an electron microscope on *Periplaneta americana*, showed that the ganglion had 60-80 neurons, the diameter of the neurons was 25-30 µm, and the cells were placed dorsally and laterally. Miles and Booker (1994), in a study on *Manduca sexta*, reported that the diameter of the FG was 160 µm in both the fifth nymphal stage and in adults, the FG had approximately 35 neurons, and the diameters of these neurons ranged from 20–to 45 µm. Ayali et al. (2002) demonstrated that the FG was 200-250 µm in diameter and cell bodies ranged in size from 10 to 50 µm in diameter in the adults of *Schistocerca gregaria*.

In the present study, we revealed that the number of the cells generating the ganglion was approximately 80-100, while the whole cell diameters varied between 5 and 18 µm in the first stage, 7 and 37 µm in the fifth stage, and 10 and 42 µm in adults. Regarding the total diameter of the ganglion, it was approximately 75 µm in the first stage, approximately 110 µm in the fifth stage, and approximately 140 µm in adults. It is possible to conclude that the cell perikarya may enlarge due to an increase in the amount of cytoplasm from the first stage to the adult phase and, consequently, the nerve cells become larger. It is clearly seen that the same enlargement may occur in the nucleus, as well, throughout the life stages of the locust.

Karakişi (1991), in a study on the hypocerebral ganglion of *Melanogryllus desertus* Pall., reported that the diameters of the ganglion cells were extended as the development proceeded but that the diameter

of the nucleus did not show considerable expansion throughout the growth period. In the current study with *L. migratoria*, we revealed that both the diameter of the cells and the diameter of the nucleus were considerably expanded throughout the development period. Our findings on nucleus development do not substantiate the findings of Karakişi.

The general structure of the FG of *L. migratoria* is entirely compatible with the structure of other ganglia, such as the central ganglion of *Rhodnius prolixus* (Wigglesworth, 1959), the hypocerebral ganglion of *Melanogryllus desertus* (Karakişi, 1991), and the thoracic and abdominal ganglia of *P. americana* (Hess, 1958).

The findings related to the structure of the sheath enveloping the ganglion in *L. migratoria* are similar to the findings of Karakişi (1991), Hess (1958), Ayali et al. (2002), and Wigglesworth (1959).

In the center of the ganglion, the neuropil, the part without the cells, through which the axons pass, was identified. Ayali et al. (2002) demonstrated that the neuropil can be subdivided into a central region of coarse neuropil and a peripheral finer region in *Schistocerca gregaria*. We also demonstrated these findings in our study.

The dark staining of the nerve cells and nuclei of the glial cells generating the FG and the light view of the nuclei of the cells in *L. migratoria* is similar to the structure in *P. americana* (Ude et al., 1978).

It was observed that in the FG, examined in 3 distinct stages of development, both cell and nucleus diameters expanded as the development proceeded. The number of ganglion cells was almost equal in all 3 stages. According to the measurements made, it is obvious that the expansion occurred in the cell perikarya due to an increased amount of cytoplasm, which was effective on the hypertrophy of the ganglion. There is no report on hypertrophy of the FG during the development period in other insects examined.

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