Neurosecretory Cells And Corpora Cardiaca During The Five Days Of The Second Instar Nymphs Of Albino Schistocerca Gregaria (Forsk.)

By

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Neurosecretory Cells And Corpora Cardiaca During The Five Days Of The Second Instar Nymphs Of Albino Schistocerca Gregaria (Forsk.)

Süheylâ KÜÇÜKEKŞİ*

Department of Zoology, Faculty of Science, University of Ankara

ABSTRACT

The neurosecretory cells of the brain, the suboesophageal ganglion and corpora cardiaca in nymphs of albino *S. gregaria* were studied at intervals of 24 hours during the five days of the second instar.

The neurosecretory cells in the brain are seen in the protocerebrum and tritocerebrum. In the protocerebrum they are especially located in the pars intercerebralis (median neurosecretory cells not in large number) and ventrally to the nerve of median ocellus (four and five pyriform neurosecretory cells). A few lateral neurosecretory cells can also be identified in the protocerebrum. The neurosecretory cells are scattered over the surface of the tritocerebrum. In the suboesophageal ganglion, they are situated at the both sides of the dorso and ventro-lateral parts and at the mid-lines.

One type of neurosecretory cell can be identified by its staining reactions, inclusions, and cytoplasmic differentiation. The histologic situation of the neurosecretory cells indicate that cyclic activity does not appear. The neurosecretory cells do not contain densely packed neurosecretory granules, but only some small granules in intermediate quantities.

The corpora cardiaca appear to be a storage organ only in the second instar nymph of albino *S. gregaria*.

INTRODUCTION

The importance of neurosecretory cells and corpora cardiaca in the insects life (in the embryonic, nymphal and adult stages) is very clear and there is no doubt as to their functions.

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Neurosecretory cells are found in the brain of various insect species belonging to different orders [17, 34, 2, 1, 5, 4, 24, 10 etc.] and also in the other ganglia [12, 13, 7, 23, 30 etc].

The neurosecretory cells can be classified as different cell types according to their staining reactions and various other properties [34, 11, 16]. However several studies have demonstrated that staining reaction varies with the physiological of the cell and the development stage of the insect [33, 31].

The functions of the neurosecretory cells are very numerous, and act on morphological as well as on physiological development. There is much evidence of their involvement in neurosecretory control of molting [36, 37], sclerotization of cuticle, colour change [18], behaviour, circadian rhythms [26], many metabolic processes – protein, carbohydrate and lipid metabolisms, cell respiration, excretion and neuroendocrine integration [31, 32] and in reproduction control [39, 25, 14, 36, 9].

In some species, cyclic activity can be seen in the brain neurosecretory cells which can be positively correlated with the onset of the molting cycle and with many major physiological events [31, 10], but in other insects such as Locusta migratoria migratorioides [27], there is a continuous production of neurosecretory material.

Corpora cardiaca as a neurohaemal organ receiving the brain hormone also have intrinsic secretory cells, so that they can not be considered to be a storage organ only in the usual sense. It should be recognised that many species differences exist in the cytol -ogy of the c. cardiaca. The functions of the c. cardiaca are complex. Many physiological and metabolic processes such as fat body, respiration, and carbohydrate metabolism can be attributed to the brain c. cardiaca system [31, 32].

The present paper describes the histology of neurosecretory cells of the brain, the suboesophageal ganglion and the corpora cardiaca during the five days of the second instar in nymphs of albino Schistocerca gregaria (Forskal). The aim of this research was to study whether variations occur in the histology of neuro-
secretory cells if so, whether cyclic activation in the neurosecretory cells is possible.

**MATERIAL AND METHODS**

Eggs of albino *S. gregaria* were taken from the stock culture of the Anti-Locust Research Centre in London. After hatching, first instar nymphs of albino *S. gregaria* were taken from the main stock and reared in separate cages under normal laboratory conditions at a temperature ranging from 25°C to 31°C and a relative humidity of 65%. After the first molt, specimens of the second nymphal stage kept in individual jars according to age. They were fed upon freshly cut grass and bran. At intervals of 24 hours through the five days of the second instar at least 7 specimens were killed, and their heads were fixed in Bouin solution under reduced pressure to eliminate the large air-sac, and to obtain quick and proper fixation of the neurosecretory centres. Fixation was made at the same time of the day (about 10 a.m.) to eliminate possible diurnal alterations in the neurosecretory activity. After fixation, the cuticle of the head capsule was dissected. The organs were dehydrated by passing through the alcohols (30%, 50, 70, 90) and supercedrol (as a clearing agent), supercedrol-paraffin wax or paraplast, and finally embedded in paraffin wax or paraplast and blocked.

Some of the sections were made in the laboratory of the Anti-Locust Research Centre and the remainder was completed in our laboratory in Turkey. Serial sections were taken at 8 or 10 μ thicknesses and stained with Gomori's chrome–haematoxyline–phloxin (CHP) [6, 20, 22] with paraaldehyde–fuchsine (PF) [22, 21] and with Heidenhain's azan [35].

Two diameters of the nuclei and of the bodies of the neurosecretory cells were measured with a micrometer eye piece. The means and standard deviations of the measurements were calculated for statistical analyses and to have an approximate idea of any differentiation in the size of the neurosecretory cells.
OBSERVATIONS AND RESULTS

Neurosecretory cells can be seen in the protocerebral and tritocerebral regions of the brain and in the suboesophageal ganglion when examining the heads of second instar nymphs of albino *S. gregaria*.

The neurosecretory cells in the protocerebrum can be identified as follows: some neurosecretory cells are present in the dorsal part of the pars intercerebralis as two closely placed small groups of cells, a few are present in the lateral part, four or five cells lie in two groups at the mid-line of the antero-ventral region, and one or two neurosecretory cells are situated at the bottom of the optic lobes on both sides of the protocerebral lobes.

Neurosecretory cells are scattered through the tritocerebrum at the peripheral surface (Fig. 1).

The neurosecretory cells in the suboesophageal ganglion are more obvious than in the brain. They are observed at the both sides of the dorso and ventro lateral and the dorso and ventromid-line of the suboesophageal ganglion (Fig. 5, 6).

The neurosecretory cells can be distinguished from normal neurons by their larger nuclei and cytoplasm. They contain some inclusions which stain dark purple (on the light violet cytoplasm) or reddish purple (on the green cytoplasm) with PF, dark bluish purple or reddish (on the pink or pink-violet cytoplasm) with CHP, dark red or reddish-violet (on the light red or violet cytoplasm) with HA (Fig. 2, 3, 4).

The neurosecretory cells are in different shapes of irregular polygons and pyriforms.

The classification of neurosecretory cells into different groups according to their cytoplasmic inclusions and differentiations and staining reactions is not possible. Although some different colour inclusions and cytoplasm could sometimes be seen with the same stains (i.e dark purple and reddish purple inclusions and light violet and green cytoplasm with PF), they were in the same neurosecretory cells, or in sections that were stained at different times. There is also no clear variation according to the
size and differentiation of the cytoplasmic inclusions in the neurosecretory cells during the five days of the second nymphal stage. Small inclusions are scattered in the cell bodies. There are no densely packed granules in the neurosecretory cells.

The measurements of the diameters of the nuclei and the bodies of the neurosecretory cells of the protocerebrum, the tritocerebrum, the suboesophageal ganglion and the frontal ganglion with their means, standard deviations and P. values are given in the table 1, 2, 3. When the short and long diameters of the nuclei of neurosecretory cells of the protocerebrum are examined, it is seen that P. values are significant (P < 0.05, between the 2nd

<table>
<thead>
<tr>
<th>TABLE I.</th>
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<tbody>
<tr>
<td>The measurements of the diameters of nuclei and bodies of N.S.C.</td>
</tr>
<tr>
<td>(in microns) in the brain of second instar nymphs</td>
</tr>
<tr>
<td>of albino S. gregaria (Forsk.)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Protocerebrum</th>
<th>Nuclei of N.S.C.</th>
<th>Neurosecretory C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAYS</td>
<td>Mean and S.D.</td>
<td>Mean and S.D.</td>
</tr>
<tr>
<td>1</td>
<td>12.65±2.20</td>
<td>16.50±2.80</td>
</tr>
<tr>
<td>2</td>
<td>13.21±3.53</td>
<td>17.96±2.20</td>
</tr>
<tr>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>3</td>
<td>11.66±2.10</td>
<td>16.43±2.15</td>
</tr>
<tr>
<td>4</td>
<td>11.76±2.10</td>
<td>16.15±2.28</td>
</tr>
<tr>
<td>5</td>
<td>13.65±2.60</td>
<td>16.66±2.36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tritocerebrum</th>
<th>Nuclei of N.S.C.</th>
<th>Neurosecretory C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAYS</td>
<td>Mean and S.D.</td>
<td>Mean and S.D.</td>
</tr>
<tr>
<td>1</td>
<td>12.00±2.16</td>
<td>18.00±2.16</td>
</tr>
<tr>
<td>2</td>
<td>10.11±1.21</td>
<td>14.76±2.90</td>
</tr>
<tr>
<td>3</td>
<td>13.01±2.43</td>
<td>17.71±1.70</td>
</tr>
<tr>
<td>4</td>
<td>12.61±2.41</td>
<td>17.71±2.01</td>
</tr>
<tr>
<td>5</td>
<td>13.41±2.98</td>
<td>17.08±3.13</td>
</tr>
</tbody>
</table>

* P value is significant, (P < (0.05) between 2–3 days for short and long diameters of nuclei of N.S.C. and not for the other days).
TABLE II.

The measurements of the diameters of nuclei and bodies of N.S.C. (in microns) in the S.O.G. of second instar nymphs of albino S. gregaria (Forsk.)

<table>
<thead>
<tr>
<th>DAYS</th>
<th>Nuclei of N.S.C.</th>
<th>Neurosecretory C.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean and S.D.</td>
<td>Mean and S.D.</td>
</tr>
<tr>
<td>1</td>
<td>13.75±2.63</td>
<td>20.41±3.36</td>
</tr>
<tr>
<td>2</td>
<td>12.15±2.86</td>
<td>16.41±4.53</td>
</tr>
<tr>
<td>3</td>
<td>14.41±3.10</td>
<td>17.85±3.61</td>
</tr>
<tr>
<td>4</td>
<td>14.16±3.48</td>
<td>17.83±3.90</td>
</tr>
<tr>
<td>5</td>
<td>14.91±4.30</td>
<td>18.56±4.18</td>
</tr>
</tbody>
</table>

* P value is significant (P < (0.05)) between 1–2 days for long diameter of nuclei and short and long diameter of cell and not for the other days.

TABLE III.

The measurements of the diameters of nuclei and bodies of cells in the frontal G. of the second instar nymphs of albino S. gregaria (Forsk.)

<table>
<thead>
<tr>
<th>DAYS</th>
<th>Nuclei of C.</th>
<th>Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean and S.D.</td>
<td>Mean and S.D.</td>
</tr>
<tr>
<td>1</td>
<td>8.11±1.38</td>
<td>11.66±1.25</td>
</tr>
<tr>
<td>2</td>
<td>7.50±1.16</td>
<td>10.83±0.83</td>
</tr>
<tr>
<td>3</td>
<td>8.95±0.53</td>
<td>12.28±1.96</td>
</tr>
<tr>
<td>4</td>
<td>9.45±1.29</td>
<td>12.80±2.19</td>
</tr>
<tr>
<td>5</td>
<td>9.45±1.91</td>
<td>12.21±3.46</td>
</tr>
</tbody>
</table>

and 3rd days). This means that the size of the nuclei of neurosecretory cells of the protocerebrum at the second day is bigger than at the third day. But there is no clear variation in the amount of neurosecretory inclusions and cytoplasmic differentiation at the examining days (1 st, 2 nd, 3 rd, 4 th and 5 th days). Cyclic changes in the neurosecretory cells are correlated with changes in the volume of cells and their nuclei and in the amount
of neurosecretory inclusions [10, 24]. More densely packed inclusions in the neurosecretory cells (larger amounts of material) than in the others may therefore indicate not an increased rate of synthesis, but a decreased rate of discharge, so that the material is accumulated within the cell [24, 14]. In these observations, the neurosecretory cells did not contain larger amounts of material, but small inclusions appeared scattered in cell bodies. This means that the rate of synthesis and release of neurosecretory material seemed to be the same in the second instar nymphs examined at different ages and that there was no apparent change in their activity.

The situation of the neurosecretory cells of the tritocerebrum and of the suboesophageal ganglion seemed to be the same as in the protocerebrum. Although, the P. values of long diameters of the nuclei and of short and long diameters of the neurosecretory cells of the suboesophageal ganglion are significant (P < 0.05, between 1–2 days), no variation in the cytoplasmic inclusions appeared during the five days. The activity of cells seems to continue at the same level.

Some cells around the frontal ganglion show an affinity for the neurosecretory stains (PF, CHP). They stained like brain neurosecretory cells with some small inclusions but were smaller than brain neurosecretory cells (Fig. 7). The measurements of the nuclei and cell diameters are given at the table 3.

However it is not clear whether they are neurosecretory cells or not and it would be necessary to observe them in the successive instars before a decision could be made.

The corpora cardiaca lie behind the brain dorsal to the hypocerebral ganglion (Fig. 8). They consist of anterior paired and unpaired, and posterior lobes. Anterior paired lobes are projected into the lumen of the aorta and fuse ventrally to form an unpaired lobe which limits the ventral wall of the aorta (Fig. 9, 10, 11, 12). The posterior lobes form a narrower portion of the gland, dorsal to the large anterior lobes, and their outlines are undulated. (Fig. 11).
It is possible to see the cell boundary, but the cytoplasm is generally syncytial. The nuclei are ellipsoid or circular, with diameters between 6.66 μ and 10.00 μ.

Over the five days during which the albino nymphs were examined there were no variations in the histology of the corpora cardiaeca. Some small inclusions which stained deep purple with PF as in the neurosecretory cells appeared scattered in the cytoplasm of the paired anterior lobes, especially near the aorta. In some sections taken from the second and fifth days of the second instar nymphs, the coarse deep purple granules were seen as collected in the border of the aorta in the anterior paired lobes (Fig. 10, 11). It is clear that the stainable neurosecretory material (carrier protein for brain hormones) coming from the brain neurosecretory cells is collected in the anterior lobes, which are neurohaemal organs and is periodically released into the aorta. There was no indication about the secretion of the posterior corpora cardiaeca (Which may be the glandular portion of the structure) during the days of the second nymphal instar.

DISCUSSION

The location of the neurosecretory cells of protocerebrum during the five days of the second instar in nymphs of albino S. gregaria is similar to that of the other locusts, such as in the adults of S. gregaria [24], Anacridium aegyptium, Acheta domesticus and Melanogryllus desertus [14, 17], and in the embryos of S. gregaria and A. aegyptium [28, 29].

The pars intercerebralis region of the protocerebrum in the second instar nymph of albino S. gregaria does not have a large number of neurosecretory cells (Median neurosecretory cells) as does the adult and the fifth instar nymph of S. gregaria (not albino) [24, 10] or the adult of Anacridium aegyptium [14]. A few lateral neurosecretory cells are located in each half of the protocerebrum in the second instar nymph of albino S. gregaria, as in the adult of S. gregaria and in the other insects [24, 26].

Generally, a few ptyriform neurosecretory cells are located in the anterio-ventral surface of the protocerebrum, just below the
nerve of the median ocellus, as in the embryo of *S. gregaria* and *A. aegyptium* [27, 28], and in the adults of *Locusta migratoria*, *Schistocerca gregaria* [19], *Melanoplus sanguinipes* [8], *Anacridium aegyptium*, *Acheta domesticus* and *Melanogryllus desertus* [17]. These neurosecretory cells are named the third neurosecretory centre in the protocerebrum.

According to the inclusions of the neurosecretory cells in the protocerebrum, tritocerebrum and suboesophageal ganglion in the second instar nymphs of albino *S. gregaria*, one type of cell was identified. These stained purple with AF (sometimes reddish green in the same cell), blue violet with CHP (sometimes reddish in the same cell), reddish violet with HA. This situation is similar to that in adult *melanoplus* [8] and *Metocheus uniguttatus* [3] and in the embryos of *S. gregaria* and *A. aegyptium* [28, 29]. However a different type of neurosecretory cell can be observed in the adult and in the fifth instar nymph (not albino) of *S. gregaria* [24, 10], in the adult of *Anacridium* [14], *Acheta domesticus* [16] *Dermacentor variabilis* [34] and many other insects.

Cyclic activation does not occur in the neurosecretory cells (brain and suboesophageal ganglion during the second instar of the nymph of albino *S. gregaria* as it does in *Locusta migratoria migratorioides* [27] and in the nymphs of *Rhodnius* (3 rd, 4 th and 5 th instars) and *Oncopeltus fasciatus* (5 th instar) [10]. On the contrary, in the 5 th instar nymph of *S. gregaria* the neurosecretory cells in the pars intercerebralis of the brain and suboesophageal ganglion show cyclic activity [10].

It is not clear that the cells in the frontal ganglion of the second nymphs of albino *S. gregaria* which show affinity for the neurosecretory stains are neurosecretory cells. Kenneth [27] in *Locusta migratoria migratorioides* and Delphin [7] in the adult *S. gregaria*, do not accept as neurosecretory the cells of the frontal ganglion which stain with PF and CHP (neurosecretory stains), because their axons neither contain neurosecretory material nor show cyclic activity.

Corpora cardiaca in the second instar nymphs of albino *S. gregaria* appear to be only a storage organ which contains neuro-
secretory material in its anterior lobes. It was not possible to identify some intrinsic secretion of the posterior lobes which may be glandular portions of the c. cardiaca [24, 14, 31].

ACKNOWLEDGMENTS

I am grateful to The General Director of the Anti-Locust Research Centre. Dr. P.T. Haskell and the Chief of Biology Department Dr. R.F. Chapman and to the other staff in the Centre for their generous hospitality, providing facilities and supplying materials. I am much indebted to Mr. Ramsdale (Entomologist who works at The W.H.O. in Turkey) for reading the manuscript.

EXPLANATION OF FIGURES

Photomicrographs of sections of brain, frontal ganglion, suboesophageal ganglion (for neurosecretory cells) and corpora cardiaca.

Fixing: Bouin; staining: paraaldehyde-fuchsin, Gomori’s chrome-haematoxyline-phloxin and Heidenhain’s azan.

Figure 1. Section through the parts of brain. 6.7 X 4 X 2.

Figure 2. Median neurosecretory cells of protocerebrum. 6.7 X 40 X 2.

Figure 3. A median neurosecretory cell of protocerebrum. 6.7 X 100 X 2.

Figure 4. Anterior-ventral neurosecretory cells of protocerebrum. 6.7 X 100 X 2.

Figure 5. Neurosecretory cells of suboesophageal ganglion. 6.7 X 40 X 2.

Figure 6. At higher magnification, a neurosecretory cell of suboesophageal ganglion. 6.7 X 100 X 2.

Figure 7. Frontal ganglion. 6.7 X 20 X 2.

Figure 8. Section through protocerebrum and corpora cardiaca. 6.7 X 4 X 2.

Figure 9. Anterior paired corpora cardiaca (neurosecretory material is collected around the aorta) 6.7 X 20 X 2.

Figure 10. Anterior corpus cardiaca. 6.7 X 40 X 2.

Figure 11. Section through anterior and posterior corpora cardiaca. 6.7 X 20 X 2.

Figure 12. Anterior corpus cardiaca (no neurosecretory material). 6.7 X 40 X 2.

a) aorta; acc- anterior corpora cardiaca (Paired) fr- frontal ganglion; gr- granule; hp- hypocerebral ganglion; n- nucleus; ne- neurosecretory cell; oe- oesophagus; pce) Posterior corpora cardiaca; pin- pars intercerebralis; tr- tritocerebrum.
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ÖZET

Albino S. gregaria (Forsk.)'ın ikinci nişin evresinde beyin ve sub oesophagus ganglion'unda nörosekresyon hücreleri ve corpora cardiaca histolojik olarak incelemiĢ ve periyodik bir faaliyetin olup olmadığını araĢtırılmıştır.

Protocerebrum'da nörosekresyon hücreleri bilhassa şu bölgelerde görülmuştur: a) Pars intercerebralis'te birbirine yakun iki grup halinde bulunan ve çok sayıda olmayan median nörosekresyon hücreleri, b) Protocerebral lobun lateralinde bulunan ve birkaç hücreden ibaret lateral grup, c) Median ocellus'un ventralinde görülen ve genellikle ar- mut şeklinde 4-5 hücreden ibaret ventral grup, d) Optik lobun kadesinde histolojik bakımdan nörosekresyon hücreleri karakterinde 2-3 hücre daha görülmemektedir.

Tritocerebrum'da görülen nörosekresyon hücreleri bütün çevreye dağılmış vaziyettedir.

Suboesophagus ganglion'undaki nörosekresyon hücreleri ise ganglion'un dorsal ve ventral bölgelerinin lateralleri ve orta çizgilerine gelecek şekilde dizilmiştir.

Nörosekresyon hücresi içerişinde görülen granüller ince ve muazzam bir şekilde dağılmıştır. İri granüller ve yoğun toplanmalara rastlanmamıştır. Granüllerine ve hücre farklılaşmasına göre bir tipten fazla nörosekresyon hücre tipi ayrı edilememiĢtir. Histolojik duruma göre periyodik bir faaliyet saptanamamıştır.

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