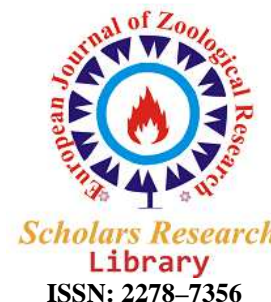




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Neurosecretion and peptides in *Locusta migratoria migratoria*: Comparative analysis in relation to phase polyphenism

Amel Ben Hamouda^{1,2*}, Mounir Trabelsi¹, Arnold De loof², Abderrahmen Bouain³ and
Mohamed Habib Ben Hamouda⁴

¹ Department of Embryology and Histology, Faculty of Medicine of Sousse, Tunisia,

² Laboratory for Developmental Physiology, Genomics and Proteomics, Zoological Institute, Leuven, Belgium

³ Department of Life Sciences, Faculty of Sciences of Sfax, Tunisia

⁴ Department of Insect Physiology, National Institute of Agronomy of Tunis, Tunisia

ABSTRACT

An HPLC analysis of hemolymph and accessory gland extracts followed by histological study of neurosecretory cells was undertaken to uncover differences between solitary and gregarious phases of migratory locusts, *Locusta migratoria migratoria*, reared under either isolated or crowded conditions respectively. Some differences in the chromatographic pattern could be detected. One of the major peaks in the hemolymph of isolated-reared adults was found to be a minor one in the crowd-reared individuals whereas two other peaks seemed to be more pronounced in the gregarious phase. The dominant chromatographic peak in accessory glands extracts of the crowd-reared animals was found to be a minor one in isolated-reared females. In the case of solitary accessory glands, the major peaks seem to be specific to the solitary phase. Histological analysis of the neurosecretory cells shows that solitary female was characterized by the presence of 'A' cell cluster in the postero-lateral median region of the pars intercerebralis and it seems that these cells are not active in the gregarious phase. The significance of these gregarious phase specific peptides with respect to neurosecretory cell activity has been discussed.

Keywords: Locusts, neurosecretory cells, peptides, polyphenism.

INTRODUCTION

Acridids respond to crowding in a variety of ways that are usually exemplified by rapid changes in behaviour and culminate in enduring long-term morphological and/or chromatic responses. A common feature of both short-term and long-term effects is that they are graded, dependent not only on density but also on the duration and on the phase history of the maternal generation [1]. Locusts demonstrate an extreme form of this density-dependent polyphenism known as 'phase change' or 'phase transition'. Local population density induces graded changes in coloration, morphometry, food selection, metabolism, oocyte number, egg mass, reproductive physiology, neurophysiology, endocrine physiology, molecular biology, immune responses, longevity, pheromone production, and behaviour [2,9]. At low density, individuals are generally scattered, cryptically coloured, relatively inactive and avoid one another, except for mating. At high densities, they become brightly coloured and actively aggregate forming cohesive bands of nymphs (hopper bands) or swarms of adults.

The neuro-hormonal mechanisms that drive and accompany this transition are still far from completely understood, however, it has been proved that several classical neurotransmitters/modulators [10], pheromones [11] and also neuropeptides [12,13], play an important role. These hormonal factors are released by neurosecretory cells in the central nervous system that control many major physiological events in the post-embryonic life of insects.

Epigenetic transfer of phase state depends upon low molecular mass, water-soluble chemicals within foam secreted by the reproductive accessory glands at the time of oviposition [14, 15, 16, 17, 5, 8, 9]. Although attempts to correlate egg foam chemistry with behaviour have been made [18] and some preliminary inquiries provided promising results [19,15], bioactive foam components have yet to be identified [8]. This specific chemical agent responsible for transmission of gregarious behaviour between locust generations is under the control of neurosecretory cells [19].

The aim of the present work was to establish a relationship between endocrinological activity and phase change. Therefore, we examined histological differences in the *pars intercerebralis* and peptide differences in the hemolymph and accessory glands of solitary and gregarious females of *L. migratoria migratoria*.

MATERIALS AND METHODS

Locusts: Female Asian migratory locusts (*Locusta migratoria migratoria*) were reared under crowded and isolated conditions at 30 ± 2 °C with a 12 h: 12 h light-dark cycle. They were fed fresh sorghum leaves or grass supplemented with wheat bran. Gregarious adults were kept in groups of 100-200 individuals in wood-framed cages or isolated in transparent containers according to Ben Hamouda et al. [9].

Preparation of hemolymph and accessory gland extracts: The hemolymph of mature females (20 days old), reared under crowded as well as isolated conditions, was collected in glass capillaries from a small puncture made in the coxal membrane of the hind leg. Twenty microliter haemolymph was taken from each female. Accessory glands were dissected in Ringer's solution (8.77 g/L NaCl, 0.19g/L CaCl₂, 0.75 g/L KCl, 0.41 g/L MgCl₂, 0.34 g/L NaHCO₃, 30.81 g/L sucrose, and 1.89 g/L trehalose, pH 7.2) and immediately placed in an ice-cold methanol/water/acetic acid (90:9:1) solution. These were then homogenized, sonicated and centrifuged for 30 min (10.000 g; 4°C).

Capillary high pressure liquid chromatography (CapLC) analysis: Hemolymph and accessory gland extracts were mixed with 200 µl of an extraction medium containing methanol, Milli-Q water and acetic acid (90:9:1v/v/v). The samples were then centrifuged at 13,000 rpm for 5 min at 4 °C. The supernatant was removed and combined with 400 µl of 0.1% aqueous trifluoroacetic acid (TFA). The methanol was evaporated in a speedvac. The aqueous solution was then extracted with 200 µl of n-hexane to remove the bulk of lipids. The remaining traces of solvents were evaporated in vacuo. The watery layer was again diluted with 0.1% TFA, filtered through a Millipore PVDF filter (0.45 µm pore size) and used for HPLC analysis. After reconstitution of the sample in 15 µl acetonitrile/water (4:96) solution, 10 µl was injected by the CapLC Autosampler (Waters Associates) onto the Symmetry C-18 column (5 µm, 0.32 mm×150 mm). Column conditions were as follows: solvent A: milli-Q water with 0.1% trifluoroacetic acid, solvent B: acetonitrile (HPLC gradient grade, Riedel-de Haën, Germany) with 0.1% trifluoroacetic acid. Gradient elution was performed using a linear gradient from 15% B up to 70% B in 90 min at a constant flow rate of 5 µl/min. The eluting peptides were detected with the CapLC Photo Diode Array detector (Waters Associates) within an absorbance area from 200 to 400 nm, with a resolution of 1.2 nm and a sampling rate of 1 spectrum per second. A chromatogram was generated as a function of the highest absorbance during the chromatographic run (100%).

Histology of the neurosecretory system: Brain, was dissected out from females into physiological saline solution under binocular microscope and fixed in Bouin's solution for 48 hours, washed in 70% alcohol, dehydrated in graded alcohol baths and then embedded in paraffin. Serial sections were cut in paraffin at 5 µm.

The sections were stained in paraldehyde-fuchsin and Heidenhain's azan trichrome to show different types of neurosecretory cells. The method used was as described by Martoja and Martoja-Pierson [20].

RESULTS AND DISCUSSION

Phase differences in hemolymph and accessory gland peptides: Acidic methanolic extracts of hemolymph samples of individuals *L. migratoria* were analyzed by C18 reversed-phase HPLC. The chromatographic pattern of locusts reared under different conditions revealed some striking differences (Figure 1). The most remarkable one was two peaks with a retention time of 36 min and 39 min (named HP2 and HP3) that were found to be quite dominant in the hemolymph of most of the crowd-reared females. These peaks were much lower when the locusts had been reared in isolated-reared conditions. On other side, peak eluted at 26 min (named HP1) was higher in isolated-reared females. These findings suggest a phase-related dependency. Clynen et al. [13] have compared peptide profiles in the hemolymph of solitary- and crowd-reared male and female *S. gregaria* adults. One peak, comprised of several masses, was detected only in solitary-reared adults of both sexes. There were quantitative

differences between the phases in other peaks, which were found in higher concentrations in gregarious hemolymph. Rahman et al. [21] who have also explored hemolymph peptide profiles in adult *S. gregaria*, and reported somewhat different phase-related patterns in hemolymph peptide profiles to those documented by Clynen et al. [13]. However, he did find a peptide of molecular mass 6.08 kD that occurred in substantially higher concentrations in the hemolymph of gregarious insects of both sexes (up to 0.1 mM). This was later confirmed by Rahman et al. [22] to be the same compound as the 6075-kD peptide originally reported by Clynen et al. [13] to be in higher concentrations in the hemolymph of gregarious than solitary insects. In the case of *L. migratoria*, the comparison of the absorption pattern of the hemolymph indicates that there are some peptides, which are differentially released. These ones differ from those identified in *S. gregaria* in the number of peaks and their elution time. Further purification will be necessary to determine firstly their identity and next their similarity with those determined in *S. gregaria*.

The chromatographic pattern of accessory gland samples of females reared under the different conditions revealed the appearance of two permanent peaks in isolated-reared females (Figure 2). One of them, named AGP2 with a retention time of 36 min, seems specific to solitary phase: it doesn't appear in females reared under crowded conditions. On the other hand, the first peak, named AGP1, eluted at 28 min was quite dominant in gregarious phase. These phase differences were hypothesized to reflect the presence of a gregarizing factor in accessory glands that contributed by the crowded mother to the transfer of phase to the offspring. The role of these glands has been demonstrated in the case of *S. gregaria*: A detailed series of experiments by McCaffery et al. [15] showed that gregarizing activity is found in the foam secreted by female accessory glands which is deposited with the eggs during oviposition [14]. It was reported that behavioural gregarization could be reinstated if eggs separated early from crowd-reared parents were topically treated with aqueous extracts of female accessory glands [14]. Ligaturing of the accessory glands in crowd-reared females resulted in behaviourally solitarized hatchlings, suggesting that the accessory glands are a source of the gregarizing agent [14]. Furthermore, it was confirmed by Miller et al. [23] that behavioural gregarizing activity was found in aqueous extracts of gregarious egg foam in *S. gregaria*. The bioactive compound was found to have characteristics consistent with an alkylated L-dopa analogue.

Phase differences in neurosecretory cells: Three closely apposed groups of neurosecretory cells are present in the dorsal part of the pars intercerebralis region of the protocerebrum: they are A, B and C cells (Figure 3). These neurosecretory cells revealed a difference between crowd-reared (gregarious phase) and isolated-reared (solitary phase) locust adults. In the median region of the pars intercerebralis, a group of about eight A-cells loaded with neurosecretions were observed in isolated-reared females (Figure 4). However, it was absent in crowded locusts. Some serial and successive sections allowed studying the activity of these cells depending on the phase state of females (Figure 5). Using the same technique, Highnam [24] shows that the neurosecretory system of females of *S. gregaria* isolated from males contain large amounts of paraldehyde-fuchsin-stainable material. These findings favour the hypothesis that the cluster of A-cells contains some factors that inhibit gregarization phenomenon via accessory glands with a direct effect on inhibitor neurohormones or indirectly through endocrine glands. Highnam and Haskell [25] reported that the release of neurosecretory material is prevented in isolated *S. gregaria* by the absence of adequate stimulation and in crowded *Locusta* by the action of some inhibiting agent.

It was demonstrated phase-dependent quantitative differences in neuroparsin of *Locusta m. migratorioides*, released from median neurosecretory cells of the pars-intercerebralis into the *corpora cardiaca* [26]. Moreover, relevant studies confirmed that neuroparsins are peptides secreted by neurones which have their cell bodies in the pars-intercerebralis of the brain and project to the storage lobes of *corpora cardiaca*, from which they are released into the hemolymph and serve multiple physiological functions such as anti-juvenile, anti-diuretic, hyper-glycaemic, hyper-lypaemic and neurotogenic effects [26, 29]. So, the presence of neurosecretions in the A-cells of the *pars intercerebralis* of isolated-reared locust has been thought to indicate their physiological function on phase polymorphism. Moreover, it has been shown that neuroparsin A content of the *corpora cardiaca* (CC) was higher in crowded than in isolated adults of *L. m. migratorioides* [4]. This study is preliminary and hypothetical and requires further research.

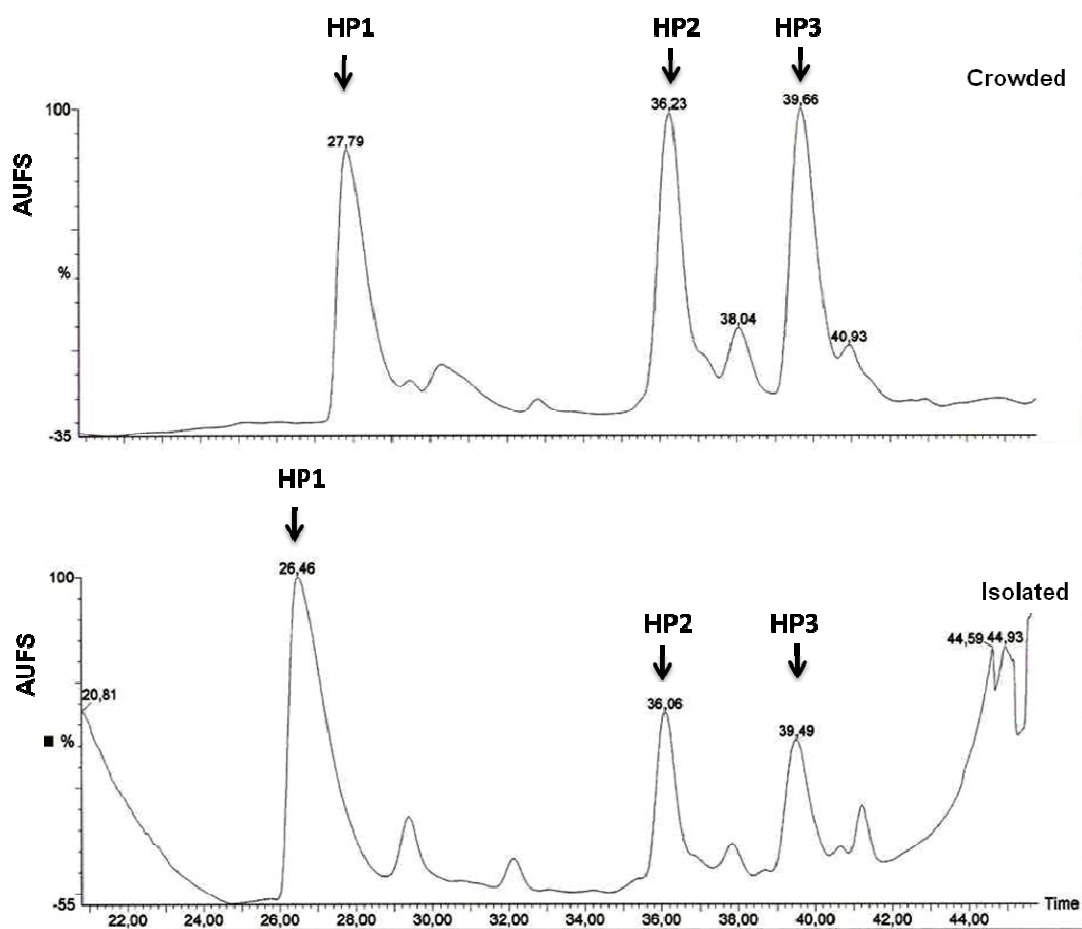


Figure 1: HPLC profile (214 nm) of hemolymph of 20-days-old female gregarious and solitary *Locusta migratoria migratoria*. The differential peaks are marked.

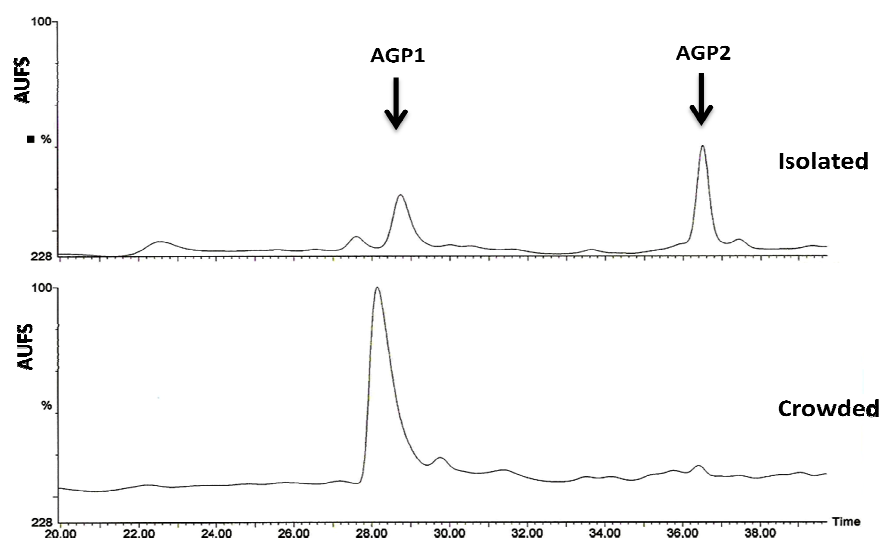


Figure 2: HPLC profile (214 nm) of the accessory glands of female 20-days-old gregarious and solitary *Locusta migratoria migratoria*. The differential peaks are marked.

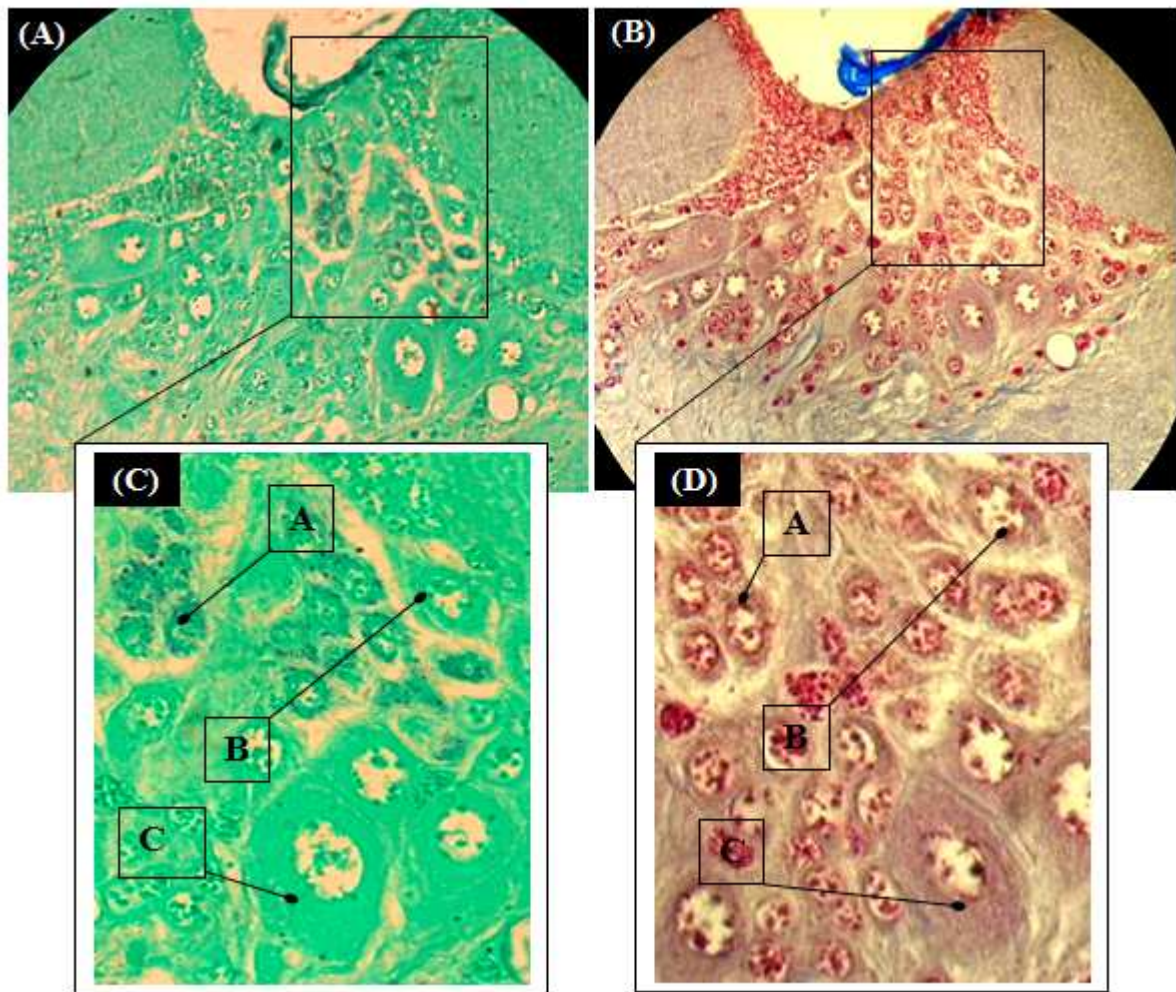


Figure 3: Serial sections of the same brain of *Locusta migratoria migratoria* stained with paraldehyde fuchsin (A) and Heidenhain azan (B) at 400× magnification showing 'A', 'B' and 'C' neurosecretory cells : (C) and (D).

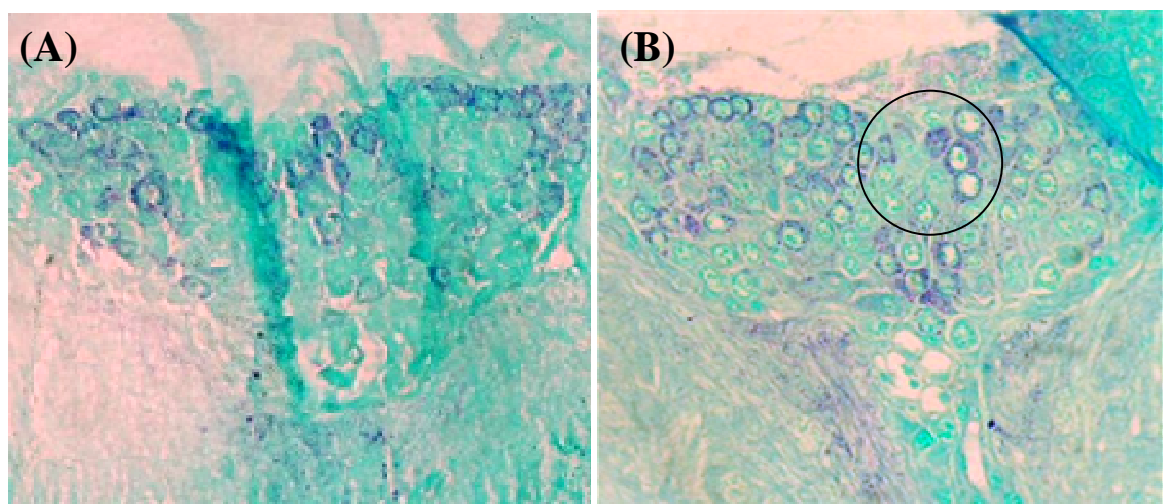


Figure 4: Vertical sections through the pars intercerebralis region of brain of a mature female *Locusta migratoria migratoria* at 400× magnification. (A): crowded-reared females; (B): isolated-reared females.

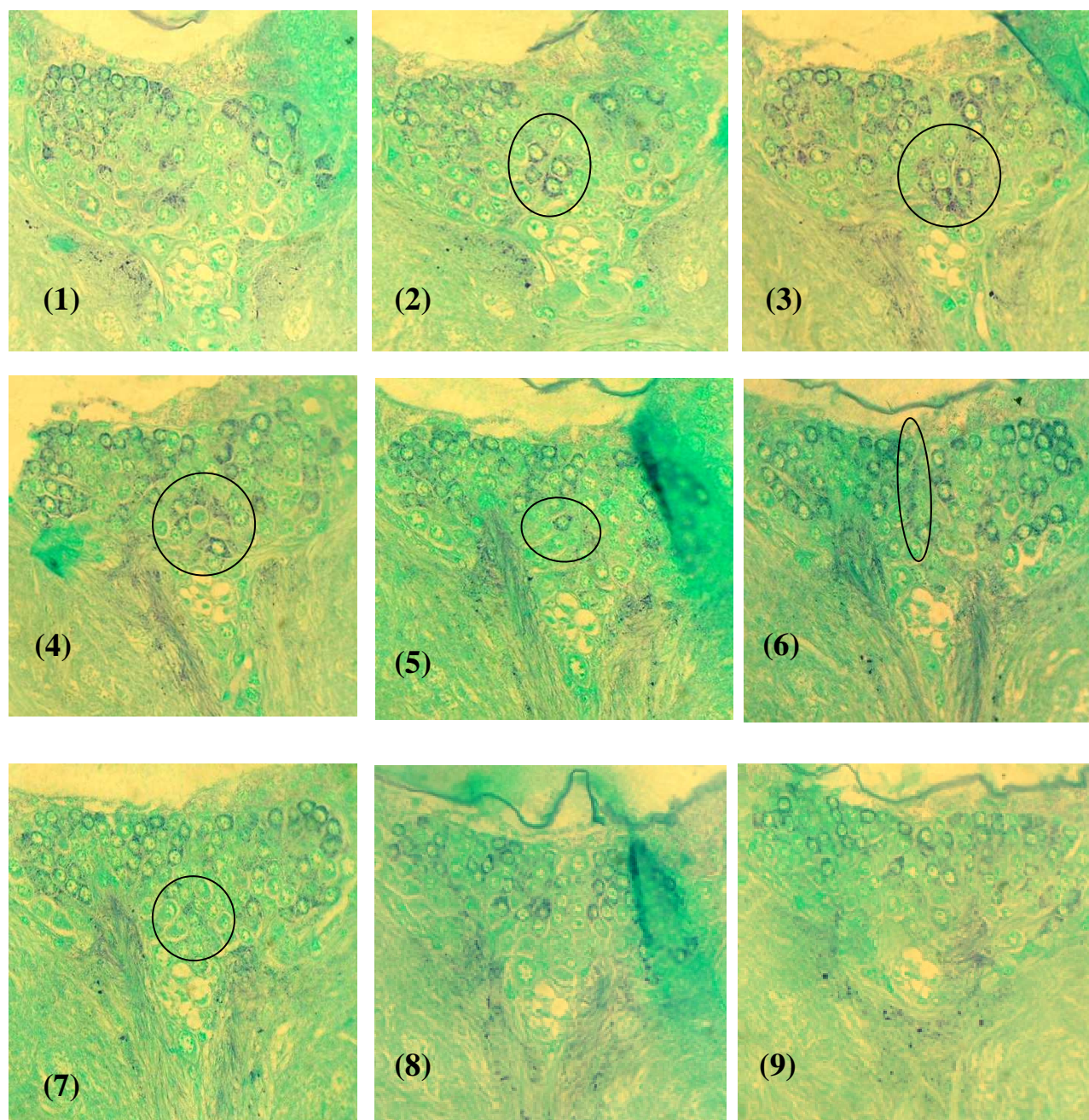


Figure 5: Successive sections through the pars intercerebralis region of the brain of isolated-reared females *Locusta migratoria migratoria*, showing A-cells type topography from the anterior (1) to posterior region (9) of the brain at 400× magnification.

REFERENCES

- [1] SW Applebaum; E Avisar; Heifetz Y. *Archives of Insect Biochemistry and Physiology*, **1997**, 35, 4, 375–391.
- [2] BP Uvarov. *Grasshoppers and Locusts. A Handbook of General Acridology*, London, New York, vol. 1, **1966**, 481.
- [3] MP Pener. *Advances in Insect physiology*, **1991**, 23, 1-79.
- [4] MP Pener; Yerushalmi Y. *Journal of Insect Physiology*, **1998**, 44, 5-6, 365–377,.
- [5] SJ Simpson; RA McCaffery; Hägele BF. *Biological Reviews of the Cambridge Philosophical Society, Cambridge University Press*, **1999**, 74, 4, 461-480,.
- [6] A Hassanali; PGN Njagi, basher MO. *Annual Review of Entomology*, **2005**, 50, 223-245.
- [7] K Seidelmann, *Biology Letters*, **2006**, 2, 4, 525-527.
- [8] SJ Simpson; Miller GA. *Journal of Insect Physiology*, **2007**, 53, 9, 869-876.

- [9] A Ben Hamouda; M Ammar; MH Ben Hamouda; Bouain A. *Journal of Insect Physiology*, **2009**, 55, 7, 617-623.
- [10] SM Rogers; T Matheson; K Sasaki; K. Kendrick; SJ Simpson; Burrows M. *Journal of Experimental Biology*, **2004**, 207, 3603-3617,.
- [11] HJ Ferenz; Seidelmann K. *Physiological Entomology*, **2003**, 28, 1, 11-18.
- [12] M Breuer; B Hoste; De Loof A. *Physiological Entomology*, **2003**, 28, 1, 3-10.
- [13] E Clynen; D Stubbe; A De Loof; Schoofs L. *Comparative biochemistry and physiology. Part B, Biochemistry & molecular biology*, **2002**, 132, 1, 107-115.
- [14] BF Hägele; Simpson SJ. *Journal of Insect Physiology*, **2000**, 46, 9, 1295-1301.
- [15] AR McCaffery; SJ Simpson; MS Islam; Roessingh P. *Journal of Experimental Biology*, **1998**, 201, 347-363.
- [16] K Moumène; M Ammar; N Barbouche; B Hajjem; Ben Hamouda MH. *Annale de l'INRAT*, **2001**, 74, 99-111.
- [17] MM Rahman; B Hoste; A. De Loof; Breuer M. *Entomologia Generalis*, **2002**, 26, 3, 161-172.
- [18] AG Malual; A Hassanali; B Torto; YOH Assad, Njagi PGN. *Journal of Chemical Ecology*, **2001**, 27, 7, 1423-1435.
- [19] MS Islam, *Journal of the Asiatic Society of Bangladesh: Science*, **1997**, 23, 111-122.
- [20] R Martoja; M Martoja-Pierson, *Initiation aux techniques de l'histologie animale*. Masson et Cie, Paris, **1967**, 345.
- [21] MM Rahman; L Vanden Bosch; G Baggerman; E Clynen; K Hens; B Hoste; K Meylaers; T Vercammen; L Schoofs; A De Loof; Breuer M. *Peptides*, **2002**, 23, 11, 1907-1914.
- [22] MM Rahman; G Baggerman; M Begum; A De Loof, Breuer M. *Physiological Entomology*, **2003**, 28, 1, 39-45.
- [23] GA Miller; MS Islam; DW Claridge; T Dodgson; Simpson SJ. *Journal of Experimental Biology*, **2008**, 211, 3, 370-376.
- [24] KC Highnam, *Quarterly Journal of Microscopical Science*, **1962**, 103, 57-72.
- [25] KC Highnam; Haskell PT. *Journal of Insect Physiology*, **1964**, 10, 6, 849-864.
- [26] A Ayali; MP Pener; Girardie J. *Archives of Insect Biochemistry and Physiology*, **1996**, 31, 4, 439-450.
- [27] E Clynen; J Huybrechts; P Verleyen; A De Loof; Schoofs L. *BMC Genomics*, **2006**, 7, 201, 1-15.
- [28] A De Loof; I Claeys; G Simonet; P Verleyen; T Vandersmissen; F Sas; Huybrechts J. *Insect Science*, **2006**, 13, 1, 3-12.
- [29] L Badisco; I Claeys; T Van Loy; M Van Hiel; V Franssens; G Simonet; Vanden Broeck J. *General and Comparative Endocrinology*, **2007**, 153, 1-3, 64-71,.