

Histological study of neurosecretory cells in the brain of migratory locust, *Locusta migratoria*, Linnaeus, 1758 (Orthoptera: Acrididae)

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Abstract

Migratory locust (*Locusta migratoria*) is a significant agricultural pest known for its devastating impact on crops across Asia and Africa. This study investigates the neurosecretory cells (NSCs) within the locust's brain, focusing on their morphology, distribution, and potential roles in regulating physiological processes. Locust brain is divided into three main regions: protocerebrum, deutocerebrum, and tritocerebrum, covering distinct types of NSCs. Specifically, medial neurosecretory cells (MNCs) contain A, B, and C cell types, while lateral neurosecretory cells (LNCs) and ventral neurosecretory cells (VNCs) exhibit variations in cell type presence. The study employed by Bargmann's chrome-alum haematoxylin-phloxine (CHP) staining, to visualize and analyze the neurosecretory structures. The findings reveal a well-defined and integrated neuroendocrine system that plays a crucial role in the locust's adaptation and behavior.

Keywords: Migratory locust, *Locusta migratoria*, neurosecretory cells.

Introduction

Order Orthoptera encompasses a diverse group of insects, including crickets, bush crickets, grasshoppers, and locusts, comprising approximately 17,000 species (Tembhare, 2012). This order is further classified into two suborders: Ensifera, which includes three superfamilies and eight families, and Caelifera, consisting of two superfamilies and nine families (Tembhare, 2012). Among the various species of locusts, four are found in India: Desert locust (*Schistocerca gregaria*), Tree locust (*Anacridium sp.*), Bombay locust (*Nomadacris succincta*), and Migratory locust (*Locusta migratoria*). Migratory locust is particularly noteworthy due to its significant impact on agriculture, as it can cause extensive damage to crops and grazing lands during its gregarious phase, leading to substantial economic losses across numerous countries in Asia and Africa (Pener and Simpson, 2009 [22]; Tanaka and Nishide, 2013) [28].

Insects possess specialized neurosecretory cells (NSCs), which are defined as specific neurons that release chemical messengers to target tissues, playing a crucial role in various physiological processes (Stay and Tobe, 2003) [27]. The supraesophageal ganglion serves as the primary neurosecretory center in insects, comprising three distinct regions: the protocerebrum (anterior), deutocerebrum (middle), and tritocerebrum (posterior). Within these regions, different types of NSCs are identified, including medial neurosecretory cells (MNCs) and lateral neurosecretory cells (LNCs) in the protocerebrum (Tembhare, 2012; Hadeln *et al.*, 2018) [13], posterior neurosecretory cell (PNCs) of deutocerebrum (Barsagade and Jiwtode, 2020) [3] and ventral neurosecretory cells (VNCs) in the tritocerebrum (Tembhare, 2012; Hadeln *et al.*, 2018) [13]. The classification of NSCs into A, B, and C cells is based on their morphology and staining affinities, with MNCs located mid-dorsally in the pars intercerebralis and LNCs situated laterally (Highnam, 1961 [14]; Highnam and Haskell, 1964) [15].

The NSCs across various species within the Orthoptera order has revealed the presence of MNC, LNC, and VNC in the brains of these insects, as well as in the ventral and

abdominal ganglia (Ibrahim and Ismail, 1979) [17]. These neurosecretory cells are also found in other organs, such as the corpora cardiaca (CC) and corpora allata (CA), which secrete neurohormones that play vital roles in processes such as metamorphosis and reproduction. Understanding the structure and function of NSCs in locusts and other Orthopteran species is essential for developing effective pest management strategies and mitigating the agricultural impacts of these critical pests (Ibrahim and Ismail, 1979) [17].

The current study aims to investigate the neurosecretory cells (NSCs) in the migratory locust, *Locusta migratoria* Linnaeus, 1758 (Orthoptera: Acrididae) and their implications for understanding the physiological processes that influence the insect's behavior.

Materials and methods

Collection of Specimens: Male and female migratory locusts, *Locusta migratoria* were collected from the campus of Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, using a hand-picking net trapping method. The collection period spanned from December to February. The collected locusts were anaesthetized using chloroform. Following anaesthetization, the brains of the locusts were carefully dissected out for further analysis.

Tissue Preparation: The dissected brain tissues were placed in Bouin's fixative solution for a duration of 12 to 15 hours to preserve the cellular structure. After fixation, the tissues underwent dehydration through ascending grades of alcohol, followed by clearing in xylene and take sections of 5-7 μm thickness by using a rotary microtome.

Staining: The tissue sections were stained using Bargmann's Chrome-Alum Haematoxylin-Phloxine (CHP) technique, as described by Bergman (Pearse, 1968). First, the slides were placed in xylene for 40 minutes to remove the wax, followed by dehydration through descending alcohol grades and immersion in distilled water. The sections were

then mordanted in 3% chrome alum solution in Bouin's fixative at 40°C for 24 hours. After washing in running water for 30 minutes until colorless, the slides were oxidized in a freshly prepared mixture of 0.6% potassium permanganate and 0.6% sulfuric acid for 5 minutes, then bleached in 2.5% potassium metabisulphite solution and washed in running water. The slides were stained with chrome haematoxylin for 20–30 minutes, differentiated in 1% hydrochloric acid for 3–5 minutes, washed in running water until dark blue, and rinsed in distilled water. The slides were then stained in 0.5% aqueous phloxine for 5–10 minutes, rinsed in distilled water, and immersed in 5% phosphotungstic acid for 1–2 minutes before washing in running water. Differentiation in 90% alcohol for 25–30 minutes was followed by dehydration with absolute alcohol twice (20 minutes each), clearing in xylene, and finally mounting in DPX for microscopic observation.

Observations and result

Insect neuroendocrine is structurally as well as functionally well- defined and integrated with the nervous system. The cephalic neuroendocrine system in insect is ectodermal in origin.

Nervous system of migratory locust, *L. migratoria* consists of brain, sub-oesophageal ganglia and the ventral nerve cord. Brain is bilobed in structure and it is divided into three regions: protocerebrum, deutocerebrum, tritocerebrum (Fig 1a) and optic lobe (Fig 1c). Protocerebrum consists of cell groups MNCs and LNCs while PNC and VNC are observed in deutocerebrum and tritocerebrum part of the brain respectively (Fig 1b, 1d). The brain of *L. migratoria* have all cell groups present in it with different cell types.

The neurosecretory cells in insects are classified into three

types on the basis of their histology as well as morphological characters, i.e., - A cell, B cell and C cell types. This cell types according to their affinities, present in different groups of NSCs. In medial group of protocerebrum which is situated in antero-dorsal part of the pars intercerebralis (PI), the MNCs lie closed to each other. All three types of cells i.e., A cell, B cell and C cells are observed in MNC (Fig. 2a, 2b) and consists large amount of NSCs as compare to other groups. C cell (green arrow) is larger than B cell (yellow arrow) and A cell (dark arrow) (Fig 2c, 2d)

LNCs are found in each lateral side of protocerebrum and it also consists of all cell type similar to the MNC i.e., A cell, B cell and C cell (Fig. 3a,3b). C cell (dark arrow) of LNC are larger than A cell (yellow arrow) and B cell (green arrow) which show granular cytoplasm (Fig 3c, 3d). In deutocerebrum, the pair of PNC group (Fig 4a, 4b) consists of cell type A and B while cell type C is not observed (4c,4d). In PNC, also A cell (dark arrow) is smaller and more frequent than C cell (Fig 4c, 4d). A pair of VNC situated in tritocerebrum (Fig 5a,5b) show cell type A and B but C cell is lacking (Fig. 5c,5d). Thus MNC, LNC, PNC, and VNC show differential distribution of A, B and C cell (Table 1) in the brain of *L. migratoria*.

Table 1: Identified cell types present in different region of brain of *L. migratoria*

Cell Groups	Cells Present	A Cells	B cells	C cells
MNC	✓	✓	✓	✓
LNC	✓	✓	✓	✓
PNC	✓	✓	✗	✓
VNC	✓	✓	✓	✗

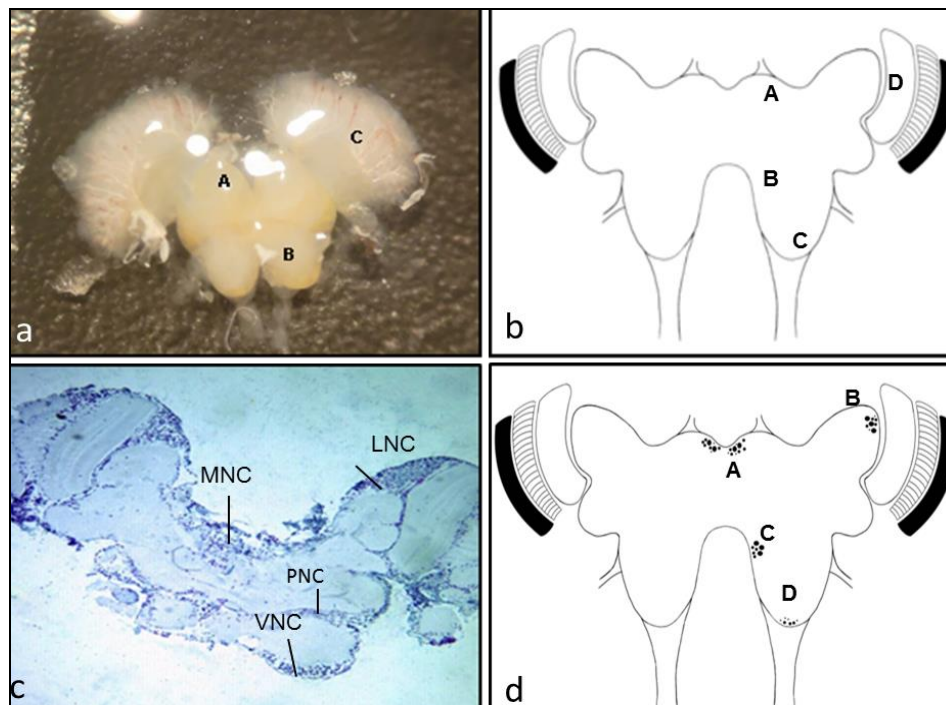


Fig. 1: Brain of *L. migratoria* showing different neurosecretory cell groups.

1a – Dissected brain of *L. migratoria* showing protocerebrum (A), tritocerebrum (B) and optic lobe (C); **1b** - Schematic diagram of brain showing protocerebrum (A), deutocerebrum (B), tritocerebrum (C) and optic lobe (D); **1c**- Frontal section of brain of *L. migratoria* showing

position of neurosecretory cells shows medial neurosecretory cells (MNC), lateral neurosecretory cells (LNC), posterior neurosecretory cells (PNC) and ventral neurosecretory cells (VNC); **1d** – Schematic diagram showing MNC (A), LNC (B), VNC (C) and PNC (D).

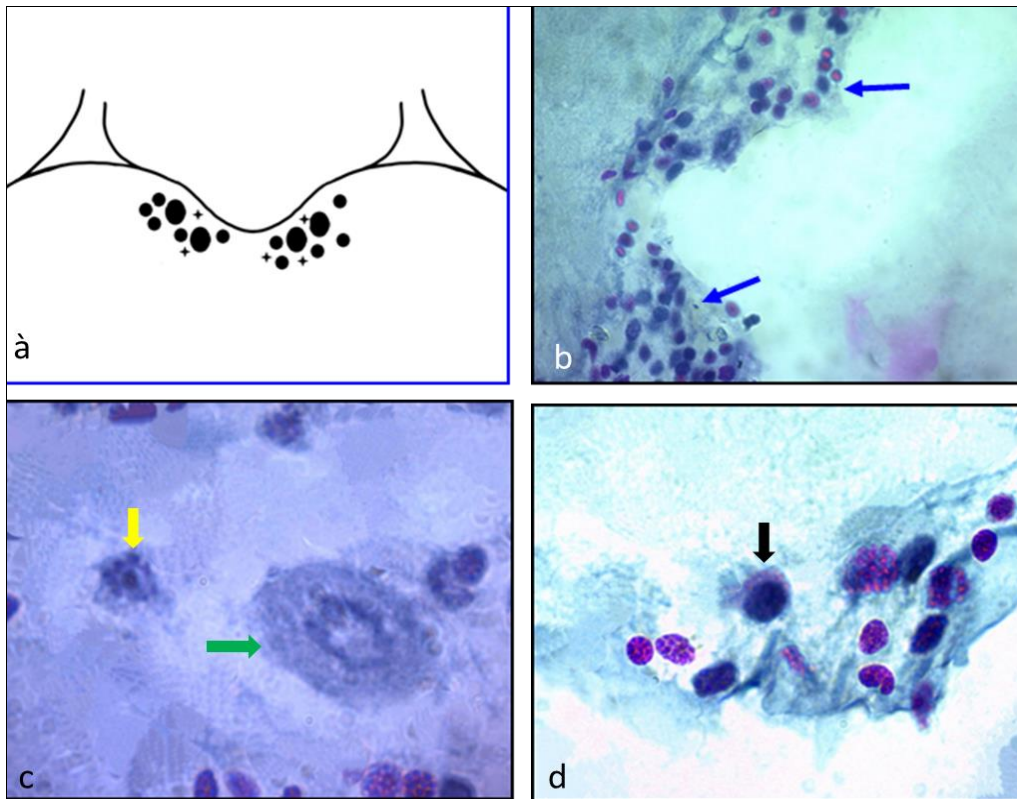


Fig. 2: MNC of pars intercerebralis of protocerebrum.

2a – Schematic diagram through MNC, showing A cell (●), B cell (✦) and C cell (●); **2b** – T.S. showing medial neurosecretory cells (MNC) (arrow) 40X; **2c** – T.S. through medial neurosecretory cells (MNCs) showing C cell (green arrow) and B cell (yellow arrow) 100X; **2d** – T.S. through medial neurosecretory cells (MNCs) showing A cell (dark arrow) 100X.

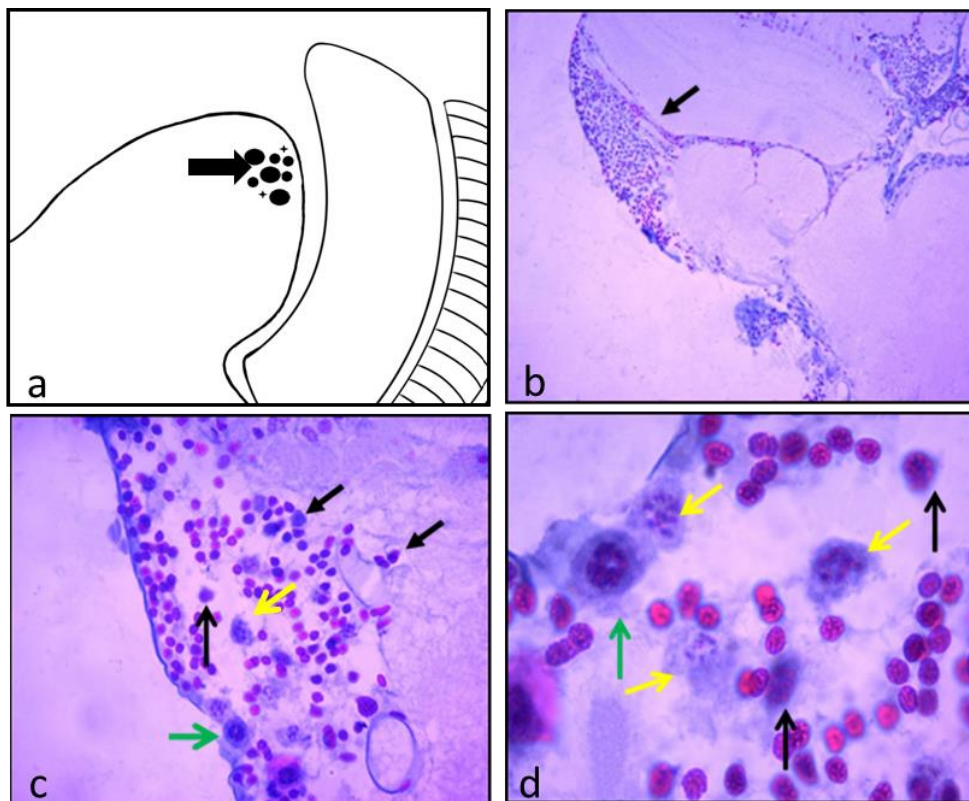


Fig. 3: LNC group of protocerebrum.

3a – Schematic diagram through LNC, showing A cell (●), B cell (✦) and C cell (●); **3b** – T.S. showing LNC (arrow) 10X; **3c** –LNCs showing C cell (green arrow), B cell (yellow arrow) and A cells (dark arrow) 40X; **3d** – T.S. through LNCs showing C cell (green arrow), B cell (yellow arrow) and A cell (dark arrow) 100X.

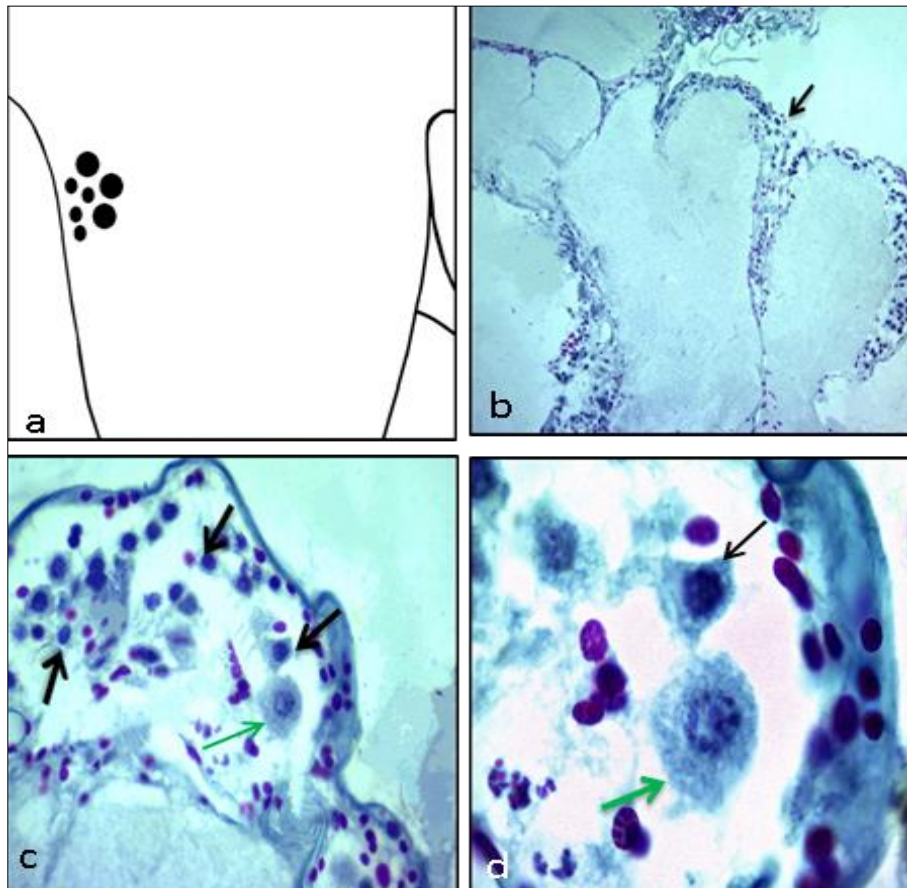


Fig. 4: PNC of deutocerebrum

4a – Schematic diagram through PNC, showing A cell (●) and C cell (●); **4b** – T.S. showing posterior neurosecretory cells (PNC) (arrow) 10X; **4c** –PNCs showing A cell (dark arrow) and C cell (green arrow) 40X; **4d** –PNCs showing A cell (dark arrow) and C cell (green arrow) 100X,

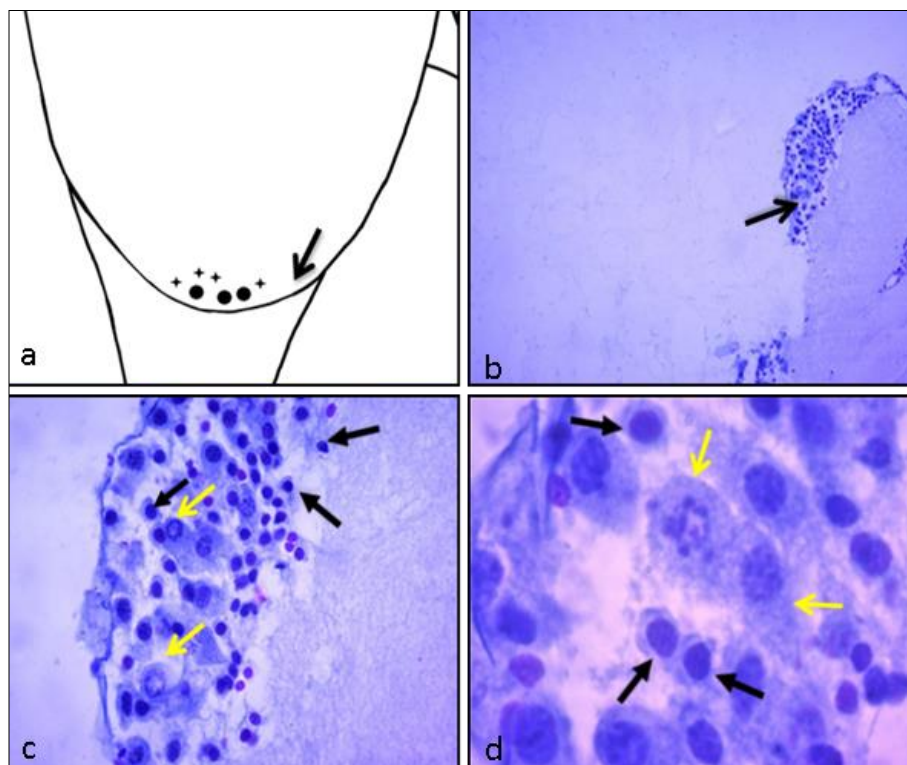


Fig. 5: VNC of tritocerebrum showing A and B cell.

5a – Schematic diagram through VNC showing A cell (●) and B cell (✦); **5b** –VNC (arrow) 10X; **5c** –VNCs showing A cell (dark arrow) and B cell (yellow arrow) 40X; **5d** –T.S. through ventral neurosecretory cells (VNCs) showing A cell (black arrow) and B cell (yellow arrow) 100X.

Discussion

Neuroendocrine system in insects consist of different organs like brain (frontal ganglion), corpora cardiaca, corpora allata, subesophageal ganglion, thoracic and abdominal ganglion. Nervous system classified into following types (a): Central nervous system, (b): Stomodaeal nervous system and (c): Peripheral nervous system. Insects have relatively simple central nervous system. Brain of insects is bilobed structure and both the lobes are well differentiated from each other. It is cream whitish in color similar to the other group of insects. Insect brain is divided into three dorso-ventral regions, forebrain or protocerebrum, midbrain or deutocerebrum and hind or tritocerebrum (Eichendorf and Kalmring, 1980^[10]; Tembhare, 2012; Hadeln *et al.*, 2018).

On the basis of staining affinity and other cytomorphological characteristics, earlier workers (Thomsen 1952^[31], 1954; Nayar, 1955^[21]; Highnam, 1961^[14]; Delphin, 1965^[6]; Hinks, 1971^[16]; Thakare and Tembhare, 1975)^[30] classified the NSC of various groups into the cell types like, A, B, C. During the study, MNC group of cells lies close to each other in PI region of the protocerebrum. It consist large amount of cells as compare to other groups of the cells. MNC have all three kinds of neurosecretory cells present in it. Similarly, LNC also consist of cell type A, B and C cell. The posterior group of cells located in deutocerebrum PNC, A cell and C cell types have been observed. Were VNCs consists of cell type A and B.

Pars intercerbralis (PI) of protocerebrum consist of the group of cells called medial neurosecretory cells (MNC) (Dogra, 1966)^[7]. Protocerebrum also consist of lateral neurosecretory cells (LNC) on the lateral side of the brain near to optic lobe (OL). Tritocerebral region the brain consist of ventral neurosecretory cells (VNC) group (Ibrahim and Sayed, 1979)^[17]. In some insects, studies revealed that fourth group of cells have been identified in the region of deutocerebrum of the brain called as posterior neurosecretory cells (PNC) (Barsagade and Jiwatode, 2020)^[3].

In *S. gregaria*, neurosecretory cells are reported in PI as well as in corpora cardiac (Highnam, 1961) but present work give description of neurosecretory cells in PI of protocerebrum as well as in deutocerebrum and tritocerebrum of brain of *S. gregaria*.

The different orthopterans species different groups of cells have been identified similarly to MNC, LNC, PNC and VNC. Ibrahim and Sayed (1979) describe the medial neurosecretory cells (MNCs) in *Gryllus bimaculatis* in two parts, Anterior MNC, near to the anterior portion of PI which includes cell type B and C while posterior MNC, located close to the posterior portion of PI includes the A and C cells. This study also revealed the lateral group of cells called as LNC similar to the LNC group observed in *L. migratoria*. LNC consist of cell type A, B, C and D cells. The VNC group of cells in *L. migratoria* is found similar to the tritocerebral neurosecretory cells (TNSCs) which is similar to VNC group shows B, C and D cells. Similarly, such types of NSCs A, B, and C are also described in VNC of *Acheta domesticus* by Geldiay and Edward (1973)^[12].

According to Delphin (1965)^[6], Karacali and Geldiay (1980)^[18], Awasthi and Singh (1981)^[1] and Barsagade and Jiwatode (2020)^[3], MNC consist of different cell type such as A, B and C cells. When embryo of *S. gregaria* and *Anacridium aegyptium* were studied, protocerebrum exhibits

NSC in small number forming the MNC, LNC and ventro-medial region (VMNC) (Kucukeksi, 1969^[19]; Kucukeksi, 1973)^[20].

LNC have similar A, B and C cell types as MNC. In ventral group VNC consist of cell types A and B while cell type C is not observed, but the existence of C cell is cannot be denied. The posterior part of the brain deutocerebrum consist of PNC group where cell type A and C is observed (Cymborowski and Dutkowski, 1969^[5]; Kucukeksi, 1969^[19]; Dutkowski *et al.*, 1971^[9]; Barsagade and Jiwatode, 2020).

The A cell which is observed is similar to the cell type found in the ventral ganglion of *Clitumnus extradentatus* and *Carausius morosus*, Raabe (1965), in the thoracic and abdominal ganglion of *L. migratoria migratorioides*, Chalaye (1967)^[4] and in subesophageal ganglion of *Eristalis taphicus* (Pathak and Ghosh, 1990 and 1991)^[24].

B cell observed in brain of *L. migratoria* is similar to previous findings of diifferent workers, thoracic and abdominal ganglion of *L. migratoria migratorioides*, Chalaye (1967)^[4], *Poekilocerus pictus*, Sing and Arif (1981)^[26] and *Melanogryllus desertus* (Aydemir and Ergen, 2001)^[2]. The C cell are very large in size with large nucleus similar to the D type cell of *S. gregaria*, Delphin (1965)^[6], B cell type found in *Blaberus craniifer* (Geldiay, 1959)^[11] and *Melanogryllus desertus* (Aydemir and Ergen, 2001).

Conclusion

Neurosecretory cell in solitary phase of *L. migratoria* is observed in 4 regions of the brain i.e. MNC, LNC, PNC and VNC. These regions includes different cell types such A cell, B cell and C cell, differentiate on their staining affinities and cytomorphological characters.

References

1. Awasthi VB, Singh UV. An analytical study of the neurosecretory cells of the brain in *Philosamia ricini* (Hutt.) (Lepidoptera: Saturniidae) during post-embryonic development. *Experientia*,1981;37:372-375.
2. Aydemir EM, Ergen G. Histological investigations on the neurosecretory cells in thoracic and abdominal ganglia of *Melanogryllus desertus* (Orthoptera: Gryllidae). *Turkish Journal of Zoology*,2001;25(2):83-86.
3. Barsagade DD, Jiwatode VR. Histomorphological study of cephalic neuroendocrine system during pupal metamorphosis in Indian worker honeybee *Apis cerana indica* (F.) (Hymenoptera: Apidae). *Vidyabharati International Interdisciplinary Research Journal*,2020;11(1):131-137.
4. Chalaye D. Neurosécration au niveau de la chaîne nerveuse ventrale de *Locusta migratoria migratorioides*. *Bulletin de la Societe Zoologique de France*,1967;92:87-107.
5. Cymborowski B, Dutkowski A. Circadian changes in protein synthesis in the neurosecretory cells of the central nervous system of *Acheta domesticus*. *Journal of Insect Physiology*,1970;16:341-348.
6. Delphin F. The histology and possible functions of neurosecretory cells in the ventral ganglia of *Schistocerca gregaria* Forskal (Orthoptera: Acrididae). *Transactions of the Entomological Society of London*,1965;117(6):167-214.

7. Dogra GS. Studies on the neurosecretory system of the female mole cricket *Gryllotalpa africana* (Orthoptera: Gryllotalpidae). *Journal of Zoology*, London,1966:152:163-178.
8. Dogra CS, Ewen B. Histology of the neurosecretory system and the retrocerebral endocrine glands of the adult migratory grasshopper, *Melanoplus sanguinipes* (Fab.) (Orthoptera: Acrididae). *Journal of Morphology*,1970:130:451-466.
9. Dutkowski AB, Cymborowski B, Przelecka A. Circadian changes in the ultrastructure of neurosecretory cells of the pars intercerebralis of the house cricket. *Journal of Insect Physiology*,1971:17:1763-1772.
10. Eichendorf A, Kalmring K. Projections of auditory ventral-cord neurons in the supraesophageal ganglion of *Locusta migratoria*. *Zoomorphologie*,1980:94:133-149.
11. Geldiay S. Neurosecretory cells in ganglia of the Roach *Blaberus craniifer*. *Biology Bulletin*,1959:117(2):267-274.
12. Geldiay S, Edwards J. The protocerebral neurosecretory system and associated cerebral neurohaemal area of *Acheta domesticus*. *Zeitschrift fur Zellforschung und Mikroskopische Anatomie*,1973:145:1-22.
13. Hadeln J, Althaus V, Häger L, Homberg U. Anatomical organization of the cerebrum of the desert locust *Schistocerca gregaria*. *Cell and Tissue Research*,2018:374:39-62.
14. Highnam KC. The histology of the neurosecretory system of the adult female desert locust, *Schistocerca gregaria*. *Journal of Microscope Science*,1961:102:27-38.
15. Highnam KC, Haskell PT. The endocrine systems of isolated and crowded *Locusta* and *Schistocerca* in relation to oocyte growth and the effects of flying upon maturation. *Journal of Insect Physiology*,1964:10:849-864.
16. Hinks CF. A comparative survey of the neurosecretory cells occurring in the adult brain of several species of *Lepidoptera*. *Journal of Entomology (A)*,1971:46(1):13-26.
17. Ibrahim A, Sayed I. Neurosecretory centers in the brain of adult *Gryllus bimaculatus* (De Geer) (Orthoptera: Gryllidae). *International Journal of Insect Morphology and Embryology*,1979:8:265-275.
18. Karacali S, Geldiay S. The neurosecretory system of the adult *Melanogryllus desertus* Pall. (Orthoptera, Gryllidae). *Cell and Tissue Research*,1980:211:223-234.
19. Kucukeksi S. Some observations of the endocrine system in the embryo of the gregarious and solitary phases of *Schistocerca gregaria* (Orthoptera: Acrididae) I- the ventral head glands. *Journal of Sciences Naturelles*,1969:14(C):1-20.
20. Kucukeksi S. Some observations of endocrine system in the embryo of *Anacridium aegyptium*, L. (Orthoptera: Acrididae) II. The neurosecretory cells and corpora cardiaca and corpora allata. *Journal of Sciences Naturelles*,1973:17(C):21-36.
21. Nayar KK. Studies on the neurosecretory system of *Iphita limbata* Stal. I. Distribution and structure of the neurosecretory cells of the nerve ring. *Biological Bulletin*,1955:108(3):296-307.
22. Pener MP, Simpson SJ. Locust Phase Polyphenism: An update. *Advances in Insect Physiology*,2009:36:163-171.
23. Pathak SC, Ghosh S. Neurosecretory cells in *Diptera*. *Folia Morphologica*,1990:38:174-185.
24. Pathak SC, Ghosh S. Some new observations on the neuroendocrine system in *Diptera*. *Journal of Entomon*,1991:16(1):17-21.
25. Raabe M. Recherches sur la neurosécrétion dans la chaîne nerveuse ventrale du phasme, *Clitumnus extradentatus* les éléments neurosécréteurs. *Comptes Rendus de Académie des Sciences Paris*,1965:260:6710-6713.
26. Sing YN, Arif M. Morphology and secretory activities of the brain and ventral nerve cord neurosecretory cells during the post-embryonic development of *Poekilocerus pictus*, Fabr. (Acridoidea: Pyrgomorphidae). *Archives of Biological Sciences*,1981:92:35-52.
27. Stay B, Tobe SS. Insects endocrine system. In: *Encyclopedia of Hormones*,2003:316-328. Academic Press.
28. Tanaka S, Nishide Y. Behavioral phase shift in nymphs of the desert locust, *Schistocerca gregaria*: Special attention to attraction/avoidance behaviors and the role of serotonin. *Journal of Insect Physiology*,2013:59:101-112.
29. Tembhare DB. *Textbook of Modern Entomology*,2nd ed. Nagpur: Himalaya Publishing House: 2012. p. 502.
30. Thakare VK, Tembhare DB. Histochemistry of the neurosecretory cells of the pars intercerebralis of the dragonfly, *Orthetrum chrysis* (Selys) (Anisoptera: Libellulidae). *Odonatologica*,1975:4(4):225-235.
31. Thomsen E. Functional significance of the neurosecretory brain cells and the corpus cardiacum in the female blow-fly, *Calliphora erythrocephala* Meig. *Journal of Experimental Biology*,1952:29:137-172.
32. Thomsen E. Studies on the transport of neurosecretory material in *Calliphora erythrocephala* by means of ligaturing experiments. *Journal of Experimental Biology*,1954:31(3):322-330.