

NEUROSECRETION IN *ORNITHODOROS SAVIGNYI* (AUDOUIN) (IXODOIDEA: ARGASIDAE). THE DISTRIBUTION OF NEUROSECRETORY CELLS IN THE BRAIN

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ABSTRACT

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The arrangement of the brain and peripheral nerves in *Ornithodoros savignyi* (Audouin) is similar to that of other argasid and ixodid ticks. Histological studies, using a specialized staining technique (aldehyde fuchsin), have shown 15 groups of neurosecretory cells in the cortex of the brain.

Résumé

LA NEUROSECRETION CHEZ *ORNITHODOROS SAVIGNYI* (AUDOUIN) (IXODOIDEA: ARGASIDAE). DISTRIBUTION DES CELLULES NEUROSECRETORIESES DANS LE CERVEAU

La disposition du cerveau et des nerfs périphériques chez *Ornithodoros savignyi* (Audouin) est semblable à celle que présentent les autres tiques chez les Argasidés et les Ixodidés. Par l'usage d'une technique de coloration spéciale (Fuchsine aldéhyde), des études histologiques ont mis en évidence 15 groupes de cellules neurosécrétrices dans le cortex cérébral.

INTRODUCTION

The hormonal control of insect life cycles has been studied extensively (Wigglesworth, 1970; Wyatt, 1972) and this knowledge has been valuable during the development of insect control methods using hormone analogues (Sláma, Romaňuk & Šorm, 1974). Very little, however, is known about the hormonal control of tick life cycles and it was felt that such information would provide a useful background to work on the effects of hormone analogues on ticks.

Neurosecretory cells in the brain have been described in a number of ixodid ticks. Ioffe (1964) found 18 groups of these cells and compared their distribution in *Dermacentor pictus* with that in *Hyalomma asiatica* and *Ixodes ricinus*. Later she found that the amount of neurosecretory material in most of the cell groups increased from spring until autumn and she correlated this with the pattern of seasonal diapause (Ioffe, 1965). Dhanda (1967) reported the same groups of cells in *Hyalomma dromedarii* and showed that the pattern of secretion in the different groups was such that one could be implicated in moulting processes and another in oviposition. Binnington & Tatchell (1973), working on *Boophilus microplus*, described 15 groups of neurosecretory cells in the brain. They correlated neurosecretory activity in 2 groups with post-moult development, whereas another group was possibly involved in the storage and release of hormone products. In *Rhipicephalus sanguineus* there are 15 groups of neurosecretory cells which can be divided into 2 types, α and β (Chow & Wang, 1974). Cyclic changes in the quantities of neurosecretory material present in various groups of cells were described, and different groups were associated with 4 separate functions: normal physiology, ecdysis, digestion and maturation of the reproductive system. The latest and most comprehensive studies are those of Obenchain (1974b) and Obenchain & Oliver (1975) on *Dermacentor variabilis*. Obenchain (1974b) described 13 neurosecretory cell types in the brain of *D. variabilis*, differentiated on the basis of their staining affinities, sizes and shapes, and cytoplasmic contents. Obenchain & Oliver (1975) described the

distribution of the 13 cell types in 18 neurosecretory centres in the brain, each group or subgroup containing cells of a single type.

Work on neurosecretion in the Argasidae is less extensive. Gabe (1955) described neurosecretory cells in *Ornithodoros lahorensis* and *Ornithodoros erraticus*, in which he found 6 pairs of protocerebral groups and other neurosecretory cells near the pedal ganglia. Cox (1960) found a correlation between neurosecretory activity in the brain of *Ornithodoros turicata* and the moulting of the nymphs. Eichenberger (1970), describing the brain anatomy of *Ornithodoros moubata*, showed that both *A* and *B* type neurosecretory cells were present. *A* type cells, arranged in 15 paired bilateral groups, secreted actively after feeding. *B* cells were loosely distributed and secreted continuously. Studies correlating feeding, oviposition, and neurosecretion were carried out in *Argas persicus* by Eisen, Warburg & Galun (1973) and in *Argas (Persicargas) arboreus* by Shanbaky & Khalil (1975).

Despite all the information already available, the pattern of hormonal control of development and physiology in ticks is still incompletely known. In this paper the general anatomy of the brain of *O. savignyi* and the distribution of neurosecretory cells are described. This will provide a basis for studies on the activities of the various groups of neurosecretory cells and their involvement in hormone-mediated events in the life cycle.

MATERIALS AND METHODS

Maintenance of ticks

Adult *O. savignyi* used in this work were collected in the Vryburg area of the north-western Cape Province by using CO₂ as an attractant (Nevill, 1964).

The ticks were maintained in an incubator under conditions of a 15-h day at 35,7 \pm 1 °C and a 9-h night at 25,0 \pm 1 °C. The ticks were kept in glass dishes containing approximately 5 cm of sand from the north-western Cape Province. The temperature of this sand varied from 30,5 °C by day-19,0 °C at night, which is approximately the same as the temperature under 7,5 cm of sand in the Kalahari in summer (J. D. Bezuidenhout, personal communication). The humidity in the incubator ranged from 71%-87% during the day and from 65%-97% at night; the humidity under the sand was not measured, but can be expected to show less fluctuation.

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Ticks were held under these conditions for at least 6 weeks prior to any sampling to ensure that they were all entrained to the same rhythm.

Feeding of ticks

Adult ticks were fed on sheep. A double bag was glued onto the animals' shaven backs, 200-250 ticks were placed inside and both bags were then tied off to prevent escape. Most ticks engorged and detached within 40 minutes.

Sampling methods

Adult ticks were sampled at different times of the day and at different intervals after feeding, mating, and oviposition, to locate all the neurosecretory cells. Information from all the brains sectioned was combined and used to build up a map of the distribution of these cells. Male and female ticks were compared and, as no differences were found between them, much of the work was done using females because more of them were available.

Histological methods

The ticks were dissected under 0.9% saline. The brains were removed, either in isolation or together with neighbouring organs such as the aorta, pharynx and gut, and the reproductive organs. They were placed in Bouin's solution or in formol-saline for fixation, depending on the staining technique to be followed. The brains were embedded in Paraplast, and sectioned at 5-7 μm.

Sections were stained in aldehyde-fuchsin (Ewen, 1962), azan (Hubschmann, 1962), or chromium-haematoxylin-phloxine (Gomori, 1941) following fixation in Bouin's solution, or in Victoria blue (Dogra & Tandan, 1964) following fixation in formol-saline. The gross anatomy of the brain was also studied, both *in situ* and in whole mounts stained with methylene blue.

RESULTS

Brain morphology

The brain of *O. savignyi* is formed by the condensation of the central nervous system into a single mass, the synganglion surrounding the oesophagus. Its external anatomy in dorsal and ventral views is shown in Fig. 1 and 2. The dimensions of the adult brain (based on measurements from 20 specimens) are as follows:

- Mean width 580 μm (Range 385-750 μm)
- Mean length 550 μm (Range 450-675 μm)
- Mean depth 335 μm (Range 280-370 μm)

The brain and nerves are closely sheathed by the neurilemma, a thin layer 1-2 μm thick, which is continuous with the outer covering of the tracheae as they enter the brain. The brain, the nerves leading from it, and the oesophagus are all surrounded by the periganglionic blood sinus. The dorsal aorta leading from the blood sinus on the dorsal surface of the protocerebrum connects the sinus with the dorsally-situated heart, immediately under the cuticle and above the viscera. The haemolymph circulates through the blood sinus and can thus distribute hormones released by neurosecretory cells.

Internally, the brain consists of an outer cellular cortex composed of the cell bodies of the neurons, and an inner neuropile. The neuropile consists of nerve fibres and axons and forms a series of ganglia, with which the major nerves can be associated.

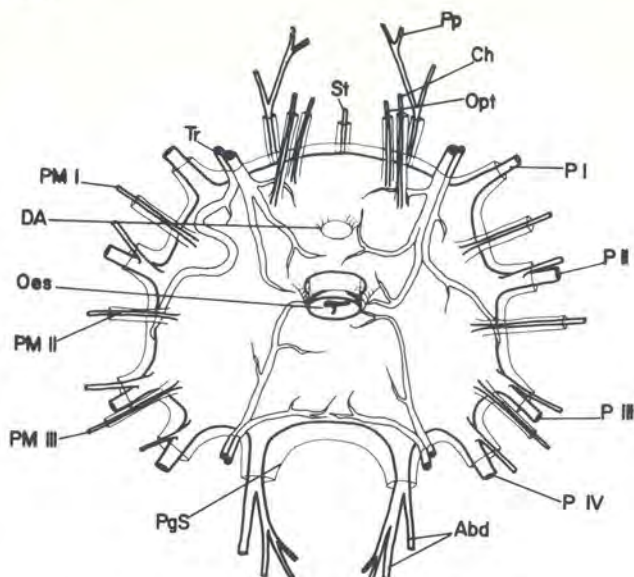


FIG. 1 *Ornithodoros savignyi*. Dorsal view of brain

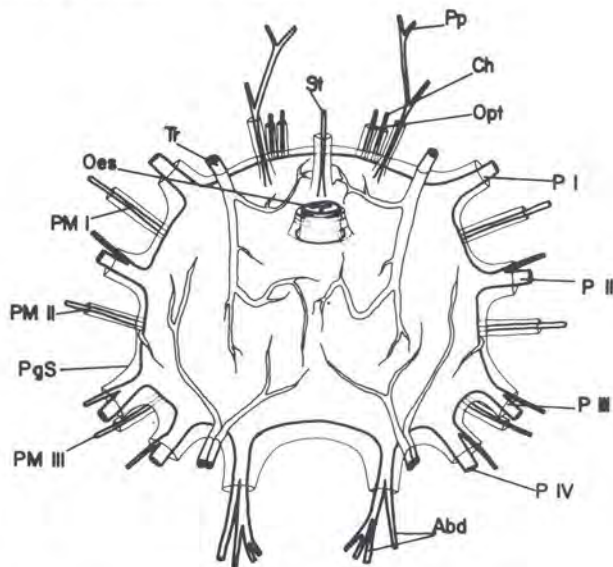


FIG. 2 *Ornithodoros savignyi*. Ventral view of brain

Distribution of neurosecretory cells in the brain

Staining with aldehyde-fuchsin has revealed 15 groups of neurosecretory cells in the brain of *O. savignyi* (Fig. 3). The use of different stains will probably reveal additional groups, but only aldehyde-fuchsin has proved successful for staining neurosecretory cells to date.

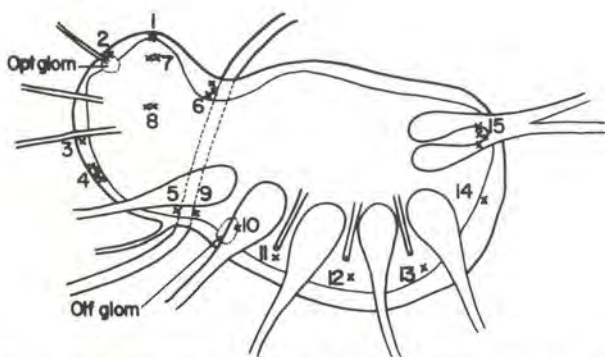


FIG. 3 *Ornithodoros savignyi*. Map of neurosecretory cell groups in the brain

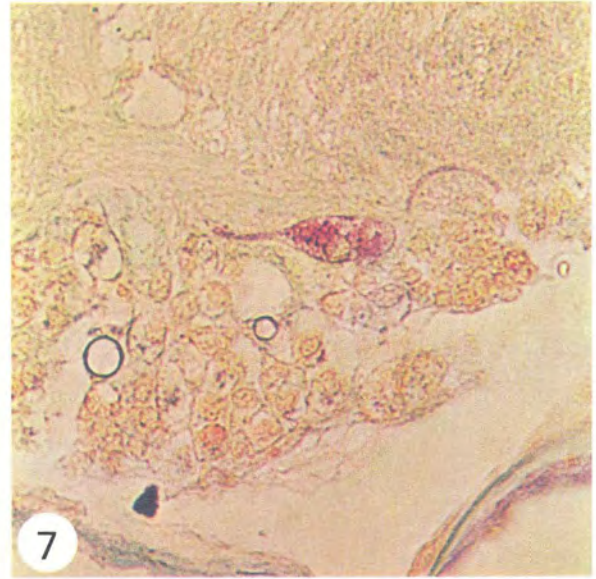
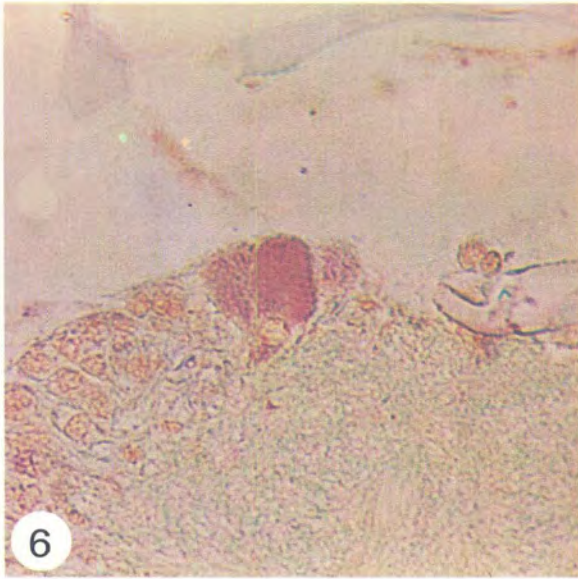
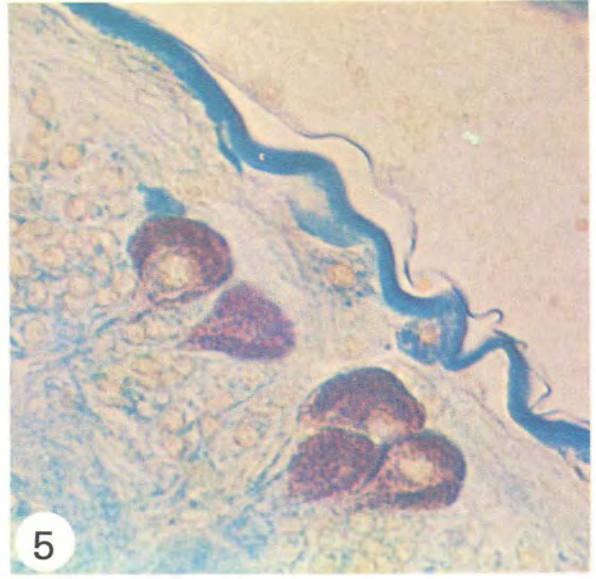
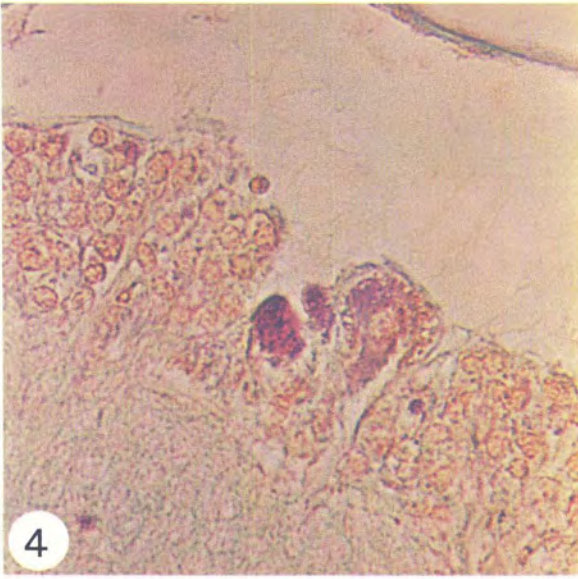


FIG. 4 *Ornithodoros savignyi*. Transverse section through Group 1. 600 \times
FIG. 5 *Ornithodoros savignyi*. Horizontal section through Group 4. 600 \times
FIG. 6 *Ornithodoros savignyi*. Transverse section through Group 7. 600 \times
FIG. 7 *Ornithodoros savignyi*. Transverse section through Group 10. 600 \times
FIG. 8 *Ornithodoros savignyi*. Transverse section through Group 15. 600 \times

With the exception of the medial abdominal group (Group 15), all groups are made up of a pair of bilaterally-arranged structures consisting of 1 to 4 cells. The mean dimensions of the cells in these groups (based on measurements from 10 specimens) are as follows: length 7 μm , breadth 5 μm , and depth 6 μm . The medial abdominal group, however, is made up of 2-4 much larger, darkly staining cells (mean length 30 μm , breadth 25 μm , and depth 16 μm).

The names given to the groups and the number of cells making up the individual structures in them are as follows:

1. Dorsal protocerebral: 1-2 cells
2. Dorsal protocerebral (mid-optic): 1 cell
3. Frontal: 1 cell
4. Dorsolateral palpal: 3-4 cells
5. Ventral cell of stomodeal bridge: 1 cell
6. Postero-dorsal cheliceral: 2-3 cells
7. Dorso-lateral cheliceral: 2-3 cells
8. Antero-ventral cheliceral: 1-2 cells
9. Ventral oesophageal: 1 cell
10. Ventral cell associated with olfactory glomerulus: 1 cell
11. Cells associated with pedal ganglion I: 1-2 cells
12. Cells associated with pedal ganglion II: 1-2 cells
13. Cells associated with pedal ganglion III: 1-2 cells
14. Cells associated with pedal ganglion IV: 1-2 cells
15. Medial abdominal: 2-4 cells

DISCUSSION

The anatomy and arrangement of peripheral nerves in the brain of *O. savignyi* are very similar to those of other species of ticks (Robinson & Davidson, 1914; Ioffe, 1963; Tsvileneva, 1965; Obenchain, 1974a). Neurohaemal organs, associated with tick brains and believed to be implicated in the storage and transport of hormone products, have been described for many species (Gabe, 1955; Eichenberger, 1970; Binnington & Tatchell, 1973; Roshdy, Shoukrey & Coons, 1973; Obenchain & Oliver, 1975), but such structures have not so far been found in *O. savignyi*. Eisen *et al.* (1973) were unable to find a neurohaemal organ in *A. persicus*, and suggested that the neurosecretory material drains out of the organ at such a rate that it is difficult to stain. The organ consists of a few cells near the oesophagus, and may easily be damaged or overlooked if it does not contain neurosecretion.

The distribution of neurosecretory cells in *O. savignyi* is similar to that in other ticks, especially the argasid *O. moubata* (Eichenberger, 1970). A noticeable difference, however, lies in the absence of a dorsal, post-oesophageal group, which is present in all other ticks for which the distribution of neurosecretory cells is described. Ioffe (1965) has found that there is a build-up of neurosecretory substance in these cells in the winter months and that the level of neurosecretion decreases in spring. This is, however, one of the cell groups showing the "least rigid seasonal character" (Ioffe, 1965), and it may be involved in normal physiological processes, a suggestion also made by Chow & Wang (1974). Dhanda (1967) and Binnington & Tatchell (1973) have implicated cells

of this group in the process of oviposition. It may be because stains other than aldehyde-fuchsin have failed to stain neurosecretory material that this group has not been found in *O. savignyi*. Attempts to demonstrate it and the neurohaemal organ will continue.

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LIST OF ABBREVIATIONS USED IN FIGURES

Abd	Abdominal nerve
Ch	Cheliceral nerve
DA	Dorsal aorta
Oes	Oesophagus
Olf glom	Olfactory glomerulus
Opt glom	Optic glomerulus
Opt	Optic nerve
PI, PII, PIII, PIV	Pedal nerves
PMI, PMII, PMIII	Motor nerves
PgS	Periganglionic sinus
Pp	Pedipalpal nerve
St	Stomodeal nerve
Tr	Tracheae

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