Odonatologica 29(4): 359-364 *December 1, 2000*

INNERVATION OF MOUTHPART SENSILLA IN THE DRAGONFLY *BRACHYTHEMIS CONTAMINATA* (FABRICIUS) (ANISOPTERA: LIBELLULIDAE)

S.M. WAZALWAR and D.B. TEMBHARE

Department of Zoology, Nagpur University Campus, Amravati Road, Nagpur 440 010, India

Received September 6, 1999 / Revised and Accepted March 22, 2000

Neuroanatomical studies demonstrate single dendritic innervation of trichoid sensilla, basiconic sensilla and microtrichia, and multidendritic innervation of the papillae and sensory pegs. These sensilla can therefore be considered as mechano- and chemoreceptors respectively. The campaniform sensilla are innervated by several dendrites and may function as proprioceptors. No innervations of the spines, teeth, hooks and acanthae was observed suggesting non-sensory nature.

INTRODUCTION

Various types of cuticular protuberances are found on the external surfaces of arthropods and RICHARDS & RICHARDS (1979) classified these structures in insects into four major types, i.e. spines, setae, acanthae and microtrichia. Those innervated by a single neuron are considered to be mechanoreceptors (THURM, 1964, 1965).

CHAPMAN (1982) reviewed earlier work on the sensilla on the mouthparts of Odonata (ZAWARZIN, 1912; PRITCHARD, 1965; PETRYSZAK, 1977 and BASSEMIR & HANSEN, 1980) and emphasized that the trichoid sensilla are innervated by a single neuron, whereas papillae are innervated by two to five neurons. Hence these can be considered as mechano- and chemoreceptors, respectively.

Recently, WAZALWAR & TEMBHARE (1999) noticed trichoid sensilla, acanthae, basiconic sensilla, campaniform sensilla, microtrichia, papillae, spines, teeth and hook-like cuticular protuberances on the mouthparts of the larva and adult *Brachythemis contaminata* with the help of scanning electron microscopy. The present neuroanatomical studies were undertaken to demonstrate neuronal innervation of various mouthpart sensilla with the help of some intravital neuroanatomical staining techniques.

MATERIAL AND METHODS

The last instar larvae and adults of *Brachythemis contaminata* were collected. Three groups, each consisting of 5 larvae and 5 adults, were made. Insects in one group were injected with 0.2 ml of 0.25% Methylene Blue in 0.7 NaCl aqueous solution. After 30 minutes, the insects were taken out and the head was separated from the body and fixed in 8% ammonium molybdate at 4°C overnight (GURR, 1962).

The mouthparts of the larvae and adults of a second group were dissected gently and kept in 0.1% $AgNO_3$ in 70% ethanol for 2 days at room temperature (BLEST, 1980).

In the third group of larvae and adults the mouthparts were dissected immediately and immersed in 1% Toluidine Blue solution at 50°C for 1 hour before fixing in 5% formalin in ethanol (ALTMAN, 1980).

The mouthparts stained with the above three methods were washed, dehydrated, cleared in xylene and kept in cedar-wood oil till they became completely transparent.

For histological studies, freshly dissected mouthparts were fixed in aqueous Bouin's fluid for 18--24 hours and directly transferred into dioxane (diethyl dioxide) for 1 hour changing the solution 3 times. Thereafter the material was transferred into a paraffin-dioxane mixture (1:1) for 4 hours and kept in liquid paraffin overnight. The material was embedded in wax (M.P. 60°C) and the sections were cut at 5 μm thickness. The paraffin sections were stained with Heidenhain's Iron-Haematoxylin--Orange G (HUMASON, 1962).

OBSERVATIONS

The trichoid sensilla and microtrichia of the larval labrum are innervated with distinctly stained dendrites (Fig. 1). Each trichoid sensillum and microtrichium is innervated by a single dendrite. The axonal fibres of the bipolar neurons form a well-defined nerve on each side running antero-posteriorly. The presence of nerve cells inside the socket of trichoid sensilla is evident in the stained preparations.

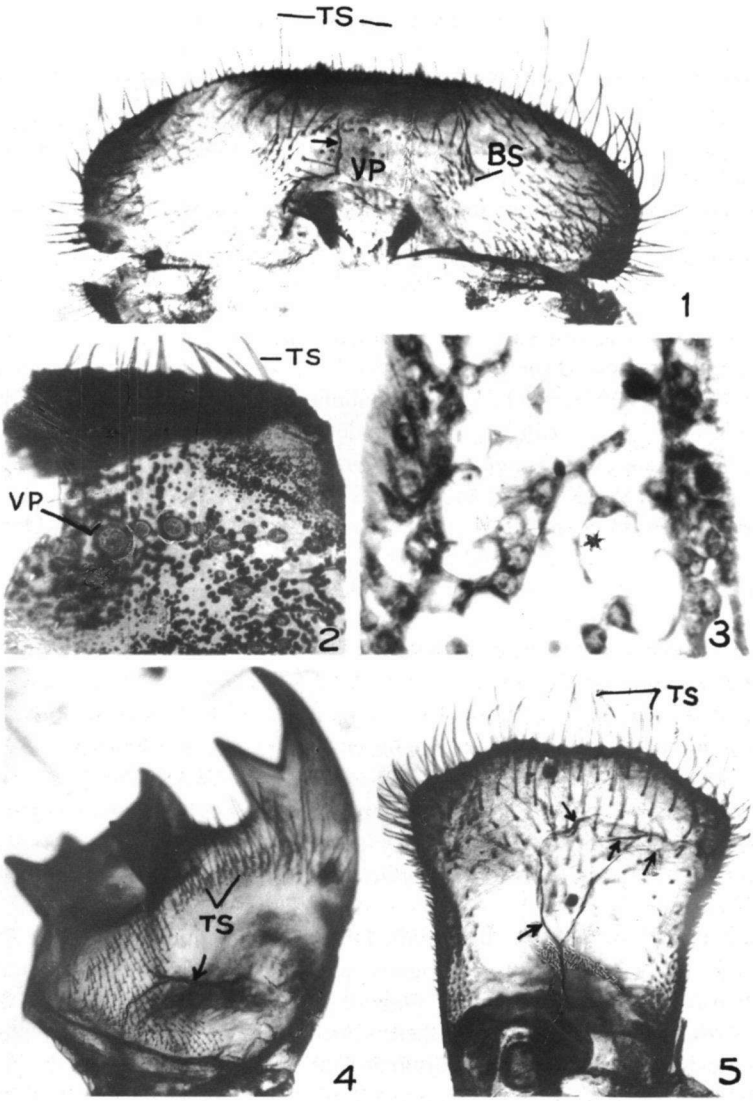
The dendrite runs the entire length of each trichoid sensillum. The basiconic sensilla and papillae situated on the ventral surface of the adult labrum are innervated by bipolar neurons. Each ventral papilla contains a large number of dendrite tips (Fig. 2). Similar to that of the larva, the dendrite runs to the tip of the trichoid sensilla of the adult labrum. The axonal fibres forming labral nerves are evident (Fig. 3).

The neuronal innervation of the trichoid sensilla of the mandibles in the larva and adult, and the formation of the sensory nerve by the axonal fibres can be seen clearly. The teeth are devoid of dendrite innervation (Fig. 4).

The trichoid sensilla on the hypopharynx of the larva and adult, as well as microtrichia on that of the adult, are innervated by dendrites. Each of them bears only a single dendrite. Their axonal fibres form fine nerves (Fig. 5).

The trichoid sensilla of the maxilla and maxillary palps of the larva and adult are innervated whereas the spines are without neuronal innervation (Figs 6, 7). The campaniform sensilla on the stipes are innervated by dendrites while the microtrichia in both, the larva and adult are also innervated (Fig. 8).

The trichoid sensilla of the prementum and labial palps of the larva and those distributed all over the surface of the labium and median lobe of the adult are each



Figs. 1-5. Innervation of the labrum, mandible and hypopharynx in *Brachythemis contaminata*: (1) larval labrum showing dendrites in trichoid sensilla (TS) and basiconic sensilla (BS) and darkly stained ventral papillae (VP); Methylene Blue; – (2) adult labrum showing a single dendrite in each trichoid sensillum (TS) and large numbers of dendrite tips in ventral papillae (VP); $AgNO_3$; – (3) cross section of adult labrum showing neuronal fibres (*) innervating the marginal trichoid sensilla; Heidenhain's Iron-Haematoxylin and Orange G; – (4) adult mandible showing a deeply stained dendrite in each trichoid sensillum (TS) and the sensory nerve (→); Methylene Blue; – (5) adult hypopharynx showing a well-stained dendrite in each trichoid sensillum (TS) and basal socket. The neuronal fibres form two branches which merge into the sensory nerve (→); Methylene Blue. – [Approximate magnifications: Fig. 1 (8x), 2 (12x), 3 (160x), 4 (12x), 5 (16x)]

provided with a dendrite. The movable hooks of the palps are devoid of neuronal innervation. The spines on the distal margin of the median premental lobe and that on the inner margin of the larval palps are also without dendritic processes. The campaniform sensilla on the middle lobe and squames of the labium of the adult and the sensory pegs on the ventral side of the middle lobe and submentum are innervated by a group of dendrites (Figs 9-12).

The labral and mandibular acanthae do not show neuronal innervation.

DISCUSSION

Neuroanatomical studies on the mouthpart sensilla in the larva and adult revealed that the trichoid sensilla of variable size, observed on various mouthparts, possess a large basal socket and a long tubular hair-like process with an internal single dendrite. They can thus be considered as mechanoreceptors (THURM, 1964, 1965), and at the same time support ZAWARZIN (1912); PRITCHARD (1965); PETRYSZAK (1977) and BASSEMIR & HANSEN (1980).

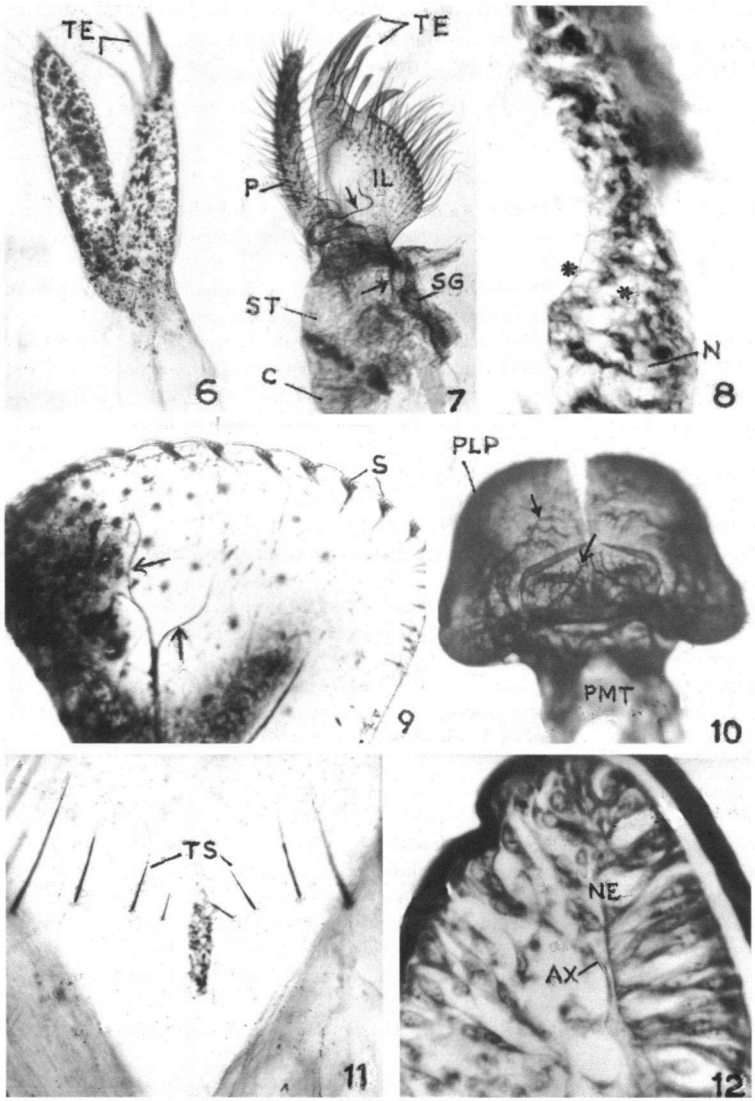
The papillae observed inside the labrum or epipharyngeal region are innervated by a group of dendrites and thus these sensilla are probably chemoreceptors (DETHIER, 1963). According to CHAPMAN (1982), the labral papillae described by ZAWARZIN (1912) and PRITCHARD (1965) and those on the maxillar palps described by BASSEMIR & HANSEN (1980) can confidently be regarded as chemoreceptors.

The campaniform sensilla are observed on the stipes (maxilla) and middle lobe and squames (labium) of *B. contaminata*. BULLOCK & HORRIDGE (1965) considered campaniform sensilla to be proprioceptors. ZAWARZIN (1912) regarded the campaniform sensilla on the mouthparts of *Aeshna* as chemoreceptors but according to CHAPMAN (1982) they are most likely the pressure receptors described in the larva of beetle *Speophyes lucidulus* by CORBIERE-TICHANE (1971).

The sensory pegs lying on the middle lobe and submentum (labium) in *B. contaminata* are structurally homologous with the sensory pores reported in *Coenagrion puella* and *Ischnura elegans* (BASSEMIR & HANSEN, 1980). According to CHAPMAN (1982) these sensilla are chemoreceptors.

The basiconic sensilla on the labrum and the microtrichia found on the labrum, hypopharynx and maxillae are innervated by a single dendrite and are thus presumed to be themselves, the mechanoreceptors (RICHARDS & RICHARDS, 1979).

Neuronal innervation of the mandibular teeth, maxillary spines and teeth, labial hooks and spines and labral and mandibular acanthae has not been observed in the present study. RICHARDS & RICHARDS (1979) unequivocally stated that the acanthae and spines are nonsensory cuticular protuberances. These cuticular processes may be primarily concerned with the mechanism of feeding in larva and adult *B. contaminata*.



Figs 6-12. Innervation of the maxilla and labium in *Brachythemis contaminata*: (6) larval maxilla indicating no dendritic innervations of the teeth; Methylene Blue; – (7) adult maxilla showing dendrites in each trichoid sensillum of the inner lobe and palp; Methylene Blue; – (8) cross section of larval maxilla showing neurons (N) and fibers (*) innervating the marginal trichoid sensilla; Heidenhain's Iron-Haematoxylin and Orange G; – (9) larval labial palp showing a distinct nerve of axonal fibres innervating sensilla (→); Toluidine Blue; – (10) adult labium showing a pair of nerves (→); Toluidine Blue; – (11) larval prementum showing stained dendrites innervating trichoid sensilla; Toluidine Blue; – (12) cross section of larval labium showing neurons (NE) and their axonal fibres (AX) forming a nerve; Heidenhain's Iron-Haematoxylin and Orange G. – [Approximate magnifications: Fig. 6 (10x), 7 (10x), 8 (160x), 9 (8x), 10 (10x), 11 (20x), 12 (160x)]

The nerve innervation pattern of the mouthparts in the larva and adult *B. contaminata* seems quite similar to that described in various orthopteroid insects (MORDUE, 1975; PETRYSZAK, 1975; UROVOY et al., 1978; LOUVEAUX, 1972; CARLINE et al., 1984).

REFERENCES

ALTMAN, J.S., 1980. Toluidine blue as a rapid stain for nerve cell bodies in intact ganglia. *In*: N.J. Strausfeld & T.A. Miller, [Eds], Neuroanatomical techniques, Insect nervous system. Springer, New York.

BASSEMIR, U. & K. HANSEN, 1980. Single-pore sensilla of damselfly larva: Representatives of phylogenetically old contact chemoreceptors? *Cell Tiss. Res.* 207: 307-320.

BLEST, A.S., 1980. Reduced silver impregnation of the ungewitter type. *In*: N.J. Strausfeld & T.A. Miller, [Eds], Neuroanatomical techniques, Insect nervous system. Springer, New York.

BULLOCK, T.H. & G.A. HORRIDGE, 1965. *Structure and function in the nervous system of invertebrates*, Vols 1 & 2. Freeman, San Francisco-London.

CARLINE, T., K. KUBRA, V.K. BROWN & R. BECK, 1984. Comparison of the distribution and nervous innervation of the sensilla on the labrum of Gryllus bimaculatus (De Geer) and Acheta domesticus (L.) (Orthoptera: Gryllidae) and an account of their development in A. domesticus. *Int. J. Insect Morphol.* 13: 81-103.

CHAPMAN, R.F., 1982. Chemoreception: the significance of receptor numbers. *Adv. Insect Physiol.* 16: 247-356.

CORBIERE-TICHANE, G., 1971. Ultrastructure de l'équipement sensoriel de la mandibule chez la larve du Speophyes lucidulus Delar (Coleoptera cavernicule de la sous-famille des Bathyscinae). *Z. Zellforsch.* 112: 129-138.

DETHIER, V.G., 1963. *The physiology of insect senses*. Methuen, London.

GURR, E., 1962. *Staining animal tissues, practical and theoretical*. Leonard Hill, London.

HUMASON, G.L., 1962. *Animal tissue techniques*. Freemen, San Francisco-London.

LOUVEAUX, P.A., 1972. Equipement sensoriel de système nerveux périphérique des pièces buccales de Locusta migratoria L. *Insectes soc.* 19: 359-368.

MORDUE, A.J., 1975. *Studies on the role of mouthpart and antennal receptors of Schistocerca gregaria (Forskal)*. PhD thesis, Univ. London.

PETRYSZAK, A., 1975. The sensory peripheric nervous system of Periplaneta americana (L.) (Blattoidea). 1. Mouthparts. *Zesz. Nauk Univ. jagiellonsk.* (Zool.) 20: 41-84.

PETRYSZAK, A., 1977. The sense organs of the mouthparts in Libellula depressa L. and L. quadrimaculata L. (Odonata). *Acta biol crac.* (Zool.) 20: 87-100.

PRITCHARD, G., 1965. Sense organs in the labrum of Aeshna interrupta lineata, Walker. (Odonata: Anisoptera). *Can. J. Zool.* 43: 333-336.

RICHARDS, A.G. & P.A. RICHARDS, 1979. The cuticular protuberances of insects. *Int. J. Insect Morphol.* 8: 143-157.

THURM, U., 1964. Mechanoreceptors in the cuticle of the honey bee: fine structure and stimulus mechanism. *Science* 145: 1063-1065.

THURM, U., 1965. An insect mechanoreceptor, Part I: Fine structure and adequate stimulus. *Cold Spring Harb. Symp. quant. Biol.* 30: 75-82.

UROVOY, J., W. FUNDALEWICZ-NEIMCZYK & M. ROSCISZEWSKA, 1978. Contribution à l'étude des organs sensoriels de clypeo-labrum chez Gryllus domesticus L. Orthoptera. *Acta biol. crac.* (Zool.) 21: 57-67.

WAZALWAR, S.M. & D.B. TEMBHARE, 1999. Mouthpart sensilla in the dragonfly, Brachythemis contaminata (Fabricius) (Anisoptera: Libellulidae). *Odonatologica* 28(3): 257-271.

ZAWARZIN, A., 1912. Histologische Studien über Insekten. 2. Das sensible Nervensystem der Aeschnalarven. *Z. wiss. Zool.* (A) 100: 245-286.