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ULTRASTRUCTURAL AND ELECTROPHYSIOLOGICAL STUDY OF THE SENSILLA ON THE PEDIPALPS AND FORELEGS OF *OXYOPES RUFISTERNUM* (ARANEAE: OXYOPIDAE).

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ABSTRACT

Spiders are found throughout the surface of earth right from Artic to dry desert region, particularly they are found abundant in areas rich in vegetation. The evolutionary success of spiders is primarily due to their highly developed sensory system. Although there can be little doubt about the spider's ability to smell, the location of the actual olfactory organs has been under debate for long time. While several behavioral studies have emphasized the role of gustation and olfaction in spiders, very few attempts have been made to depict the functional role and structure of the chemosensilla involved. Ultrastructural studies reveal a predominance of gustatory sensilla with a characteristic S-shape, an apical pore and arising steeply on the axis of the pedipalps and legs of spiders. The location of chemoreceptors is suitable for contacting substrates, prey or conspecifics. *Oxyopes rufisternum* is a potential predator in the agro ecosystem and thus of particular interest from the point of biological control. The present study was conducted to identify the olfactory receptors and gustatory receptors of the spider which help them to adapt and help in prey capture. There are about eight different sensilla found during the study. Their distribution patterns of individual sensillar types provides evidence for the importance of gustation and olfaction by sensilla on the pedipalps and forelegs as reported in behavioral studies in several species of spiders. Multicellular activity of individual sensilla in *O. rufisternum* is similar to that described in insects but it has a greater number of active units. The activation of groups of neurons from which the individual sensitivity spectra remains unknown.

KEY WORDS: ***Oxyopes rufisternum,*** **sensilla, pedipalp, legs, olfaction, gustation**

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INTRODUCTION

The evolutionary success of spiders is primarily due to their highly developed sensory system. Besides vision and mechanoreception, behavioral studies have demonstrated a chemical sense in spiders reflecting their ability to smell and taste. This enables them to rely on chemical cues for inter and intra-specific communication[1]. Ultrastructural studies reveal a predominance of gustatory sensilla with a characteristic S-shape, an apical pore and arising steeply on the axis of the pedipalps and legs of spiders[2- 5]. The location of chemoreceptors is suitable for contacting substrates, prey or conspecifics. Kronstedt[6] observed the occurrence of chemosensitive hairs on the cymbium of adult males and suggested them to be involved in the detection of presumed contact sex pheromones produced by the females. Studies on dragline following behavior emphasized the perception of chemical cues by the palps[7] and intraspecific differences observed were related to differences in the number of chemosensitive hairs on the palps[8]. In addition, electrophysiological studies have confirmed their gustatory capabilities. Harris and Mill[9] recorded the electrophysiological responses of curved blunt tipped hairs on the legs of *Ciniflo sp* and *Tegenaria sp* to monovalent salts and hydrochloric acid while Drewes and Bernard[10] observed responses from single pore sensillum on the pedipalps of the wolf spider, *Lycosa frondicola* to NaCl. Vallet, et. al.,[11] studied the electrophysiological responses of tarsal chemoreceptors of *Tegenaria atrica* to salts and amino acids and observed them to be multicellular. Ultrastructural and behavioral studies on chemosensory aspects have so far focused on the perception of contact sex pheromones. Although the location of the actual olfactory organs has been under debate for a long time except for the demonstration of olfactory capabilities of the tarsal organs described by Blumenthal[12], several behavioral studies have provided indirect evidence for the presence of other olfactory sensilla. The present study was undertaken to identify the gustatory and olfactory sensilla on the pedipalps and forelegs of male and female *O. rufisternum.* Such studies coupled with those on behavior and sensory physiology would contribute to a better understanding of the functional basis of predation in adults of *O. rufisternum*. It will also provide data for comparison with information on analogous structures in other species of spiders.

MATERIALS AND METHODS

Spiders

Adults of *O. rufisternum* were collected from the Animal Farm in the Loyola College Campus, Chennai. They were reared in plastic containers on grasshopper nymphs collected from the field.

Scanning electron microscopy

For SEM, the pedipalps and forelegs were fixed in glutaraldehyde and dehydrated through a graded series of ethanol. Subsequently, they were cleaned in tetrachloromethane as described by Cuperus[13]. Specimens were air-dried, mounted on aluminium stubs using double sided sticky tape and gold coated using a sputter coater. A Leica Stereoscan 440 electron microscope operated at 10-20 kV was used to observe the specimens.

Electrophysiology

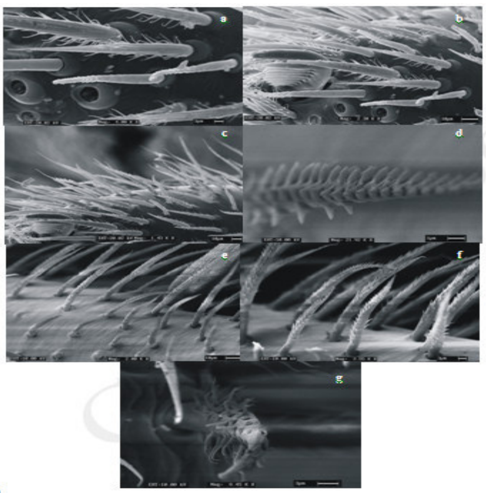
Adults of *O. rufisternum* were fixed on small Styrofoam blocks with adhesive tape such that their forelegs and pedipalps were exposed and oriented properly for electrode manipulation. A fine tip capillary electrode filled with 0.01M KCl was inserted close to the recording site and served as the indifferent electrode. The recording electrode contained different concentrations of the test substances dissolved in 0.001 M KCl serving as the electrolyte. Using a micromanipulator, the recording electrode was carefully slipped over a particular sensillum, following the method according to Hodgson, et. al., [14] taking care not to bend the sensillum which otherwise triggered responses from the mechanosensory neurons. The tip of the electrode was cleared regularly with a filter paper to prevent clogging. Initial contact with the stimulus containing recording electrode triggered a large shift in potential. Each stimulus lasted 3-5 secs and a 1 minute interval was given between recordings of different stimuli. Since responses from individual sensilla declined after 4-5 stimulations with different stimuli suggesting damage to the cells by the electrolyte being used, recordings were taken from different hairs. The signals were amplified 10 times using a differential amplifier (Syntech, IDAC-4-USB), which analysed, filtered with a pass band (10 Hz-3000 Hz) and digitized the signals with a built-in A/d converter. Using the software Autospike 32 (Syntech, The Netherlands), the signals were recorded as digitized files on a computer. Three replicates of individual compounds were tested at each concentration and 7 males and 6 females were used in the study.

RESULTS

Gustatory chemoreceptors on the pedipalps and forelegs of males and females of *O.rufisternum* were identified on the basis of their external morphology as well as electrophysiological responses which correlated well with ultrastructural observations. The gustatory sensilla are present on the lateral sides and tips of the pedipalps in both males and females, while on the foreleg they are present on the ventral surface thereby allowing them to contact substrates. On both the pedipalps and forelegs, they are characteristically lined in rows and arise steeply from the cuticular surface. On the pedipalps and forelegs of *O. rufisternum*, slightly curved blunt tipped hairs occur which are arranged serially between the less regular rows of mechanosensitive hairs which are straight with a pointed apical tip (Plate 1-4). Characteristic features which serve as the basis for identification of these group of sensilla include a) a steep angle relative to the surface, b) a slight S-shape ending in a blunt tip and c) spiral surface striations primarily along the apical region. These spiral striations are present only on the inner surface while on the outside, the surface is smooth. The density of these sensilla is primarily on the distal regions of the pedipalps and forelegs and concentrated on the ventro-lateral regions. In addition, the apical portion with or without a spur, is relative smooth. The spine-like projections appear to be opening into individual pores. On the pedipalps of both males and females of *O. rufisternum*, three types of chemosensilla were differentiated based on their size and also apical tip characters. Type A sensilla have a characteristic curved spur on the apical tip apparently covering a pore at the tip. These sensilla are 30±1.2 µm long and have a basal diameter of 5±0.05 µm and are arranged primarily in the distal end of the pedipalps in both male and female spiders. Types B, C and D differ from type A in lacking a spur at the apical tip. Type B sensilla are 25±0.9 µm long with a basal diameter of 4±0.04 µm and a blunt apical tip of 2±0.03 µm diameter opening into a pore. The sensillum is smooth at the basal end (15±0.75 µm) beyond which the cuticle is modified into serially arranged bulbous structures. Type C sensilla are 35±1.4 µm in length with a basal diameter of 5±0.06 µm and an apical diameter of 2±0.04 µm. The smooth basal region is longer is this sensillum and extends upto 22±1.2 µm. Type D sensillum are 70±2.3 µm in length with a basal diameter of 6±0.03 µm and an apical tip diameter of 2±0.05 µm. The smooth basal region extends 45±1.5 µm along its length. On the forelegs, type B and type C sensilla are present arranged in rows. Gustatory chemosensilla with spurs are absent on the forelegs of *O. rufisternum.* Type E sensilla present on the forelegs of both males and females of *O. rufisternum* are 12±0.8 µm in length, have a pointed apical region extending 2±0.06 µm. The basal diameter is 1±0.03 µm while the apical tip diameter is 0.5±0.01 µm. Numerous spine like projections firmly clasped are observed along majority of its length. Types F, G and H are olfactory sensilla, which, except for its length are similar with spine like projections along its entire length (Plate 1-4). These sensilla are concentrated on the apical regions of the pedipalps and forelegs and it appears that spine-like projections have a small pore like opening. Type F are 45±1.2 µm long with a basal diameter of 3.5±0.04 µm, type G are 55±1.4 µm with a basal diameter of 4±0.03 µm and type H are 85±2.5 µm with a basal diameter of 5±0.04 µm. The density of spine like projections appear to be higher in types F and G and high magnifications indicate possible openings as pores. A unique sensilla on the apical region of the pedipalp whose functional significance is unknown was found. Gustatory sensilla on the pedipalps and forelegs of *O. rufisternum* exhibit amplitude of 0.4-0.7 mv on stimulation with 0.01M KCl. Spike shapes were generally biphasic with an initial fast depolarization. Stimulation with an individual compound resulted in responses being multicellular, *ie*., the stimulus activated more than one sensory neuron and included 2-4 classes of spikes as judged by their amplitudes and shapes. These sensilla were sensitive to a variety of chemicals tested *viz*., salts such as NaCl, KCl; amino acids – phenyl alanine, hydroxyl proline, tryptophan, proline and glycine, sugar- fructose and to sugar alcohol, inositol (Figs. 1 & 2). The types of sensillum and its function are tabulated below.

Type	Length (μm)	Basal Diameters (μm)	Function
A.	30±1.2	5 ± 0.05	Gustatory
B.	25 ± 0.9	4 ± 0.04	Gustatory
C.	35 ± 1.4	5 ± 0.06	Gustatory
D	70 ± 2.3	6 ± 0.03	Gustatory
E	12 ± 0.8	1 ± 0.03	Unknown
F	55 ± 1.4	4 ± 0.03	Olfactory
G.	55 ± 1.4	4 ± 0.03	Olfactory
H	85 ± 2.5	5 ± 0.04	Olfactory

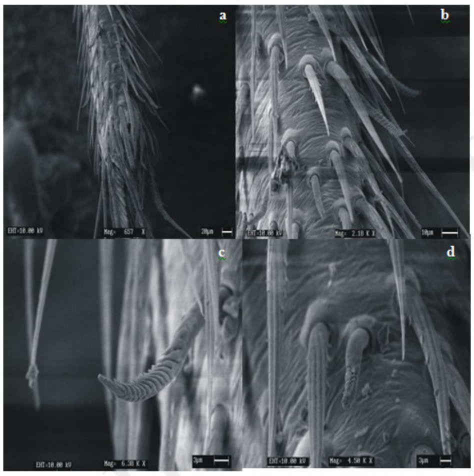
Plate – 1
Scanning electron micrographs of sensilla on pedipalps of female O. rufisternum



a)Gustatory sensillar types B and C along with mechanosensory sensilla; b)Gustatory sensillar types B,C and D; c)Distribution of various types of sensilla on the lateral surface of pedipalps; d)Higher magnification of spine-like striations on sensillar types B,C and D; e)Distribution of olfactory sensilla; f)Higher magnification of olfactory sensillar types F,G and H and g)Unique sensilla on the apical region of the pedipalps whose functional significance is unknown.

Plate – 2

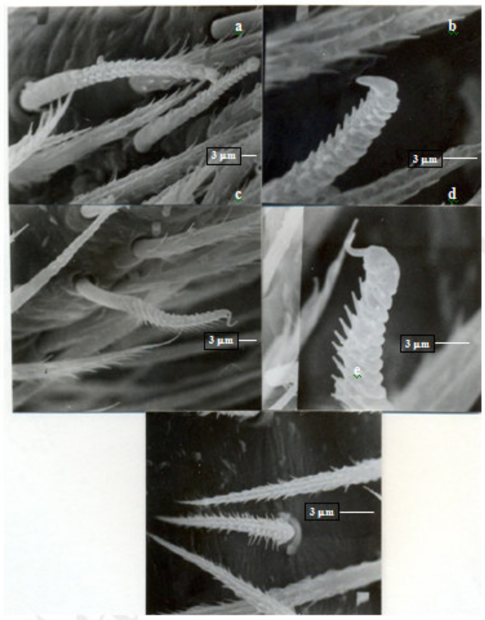
Scanning electron micrographs of sensilla on the forelegs of female *O. rufisternum*



a)Distribution of various types of sensilla on the distal region of the foreleg; b)Gustatory sensilla type C and the sharp pointed mechanosensory sensilla on the lateral surface of the foreleg; c)Type E sensilla with a smooth apical tip and d)Gustatory sensilla type D.

Plate - 3

Scanning electron micrographs of sensilla on pedipalps of male *O. rufisternum*



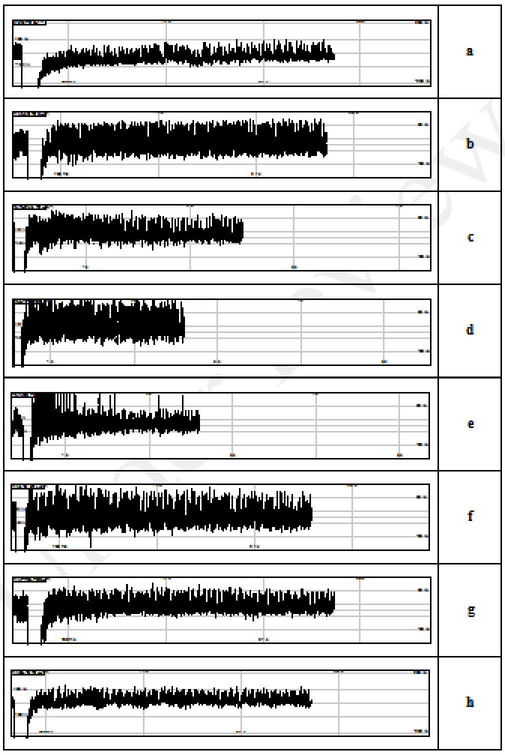
a)Gustatory sensilla type B; b)Modification of the apical region in type E sensillum; c)Type A sensilla with a spur on the apical tip; d)Higher magnification of the apical region of type A sensillum and e)Olfactory sensilla types G and H

Plate – 4

Scanning electron micrographs of sensilla on forelegs of male *O. rufisternum*

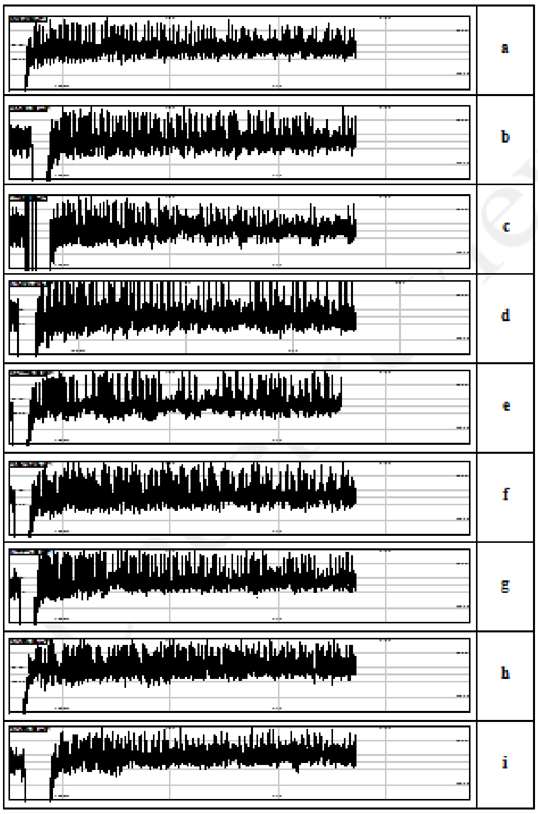
a)Distal region of the forelegs showing distribution of various types of sensilla; b)Type C gustatory sensillum; c)Type G olfactory sensillum; d)Higher magnification of type C gustatory sensillum showing probable pores on the spirally arranged structures and e) Type B gustatory sensillum.

Figure 1
Representative traces showing the electrophysiological responses from a type B gustatory sensillum on the pedipalps of female O. rufisternum to different stimuli –



a)0.01 M KCl; b)0.01 M NaCl; c)0.01 M Phenyl alanine; d)0.01 M Hydroxy proline; e)0.01M Inositol; f)0.01 M Lysine; g)0.01 M Proline and h)0.01 M Glycine

Figure 2
Representative traces showing the electrophysiological responses from a type B gustatory sensillum on the pedipalps male of O. rufisternum to different stimuli



a)0.01 M KCl; b)0.01 M NaCl; c)0.01 M Proline; d)0.01 M Phenyl alanine; e)0.01M Hydroxy proline; f)0.01 M Glycine; g)0.01 M Tryptophan; h)0.01 M Inositol; i)0.01 M Fructose

DISCUSSION

The present study demonstrates that the pedipalps of *O. rufisternum* contain 8 morphologically distinct sensillum types. Comparing the cuticular specializations with sensilla described for other spider species[4] and insects[15-17] these sensilla are capable of responding to various stimuli viz., gustatory, olfactory and mechanoreception. Their distribution patterns of individual sensillar types provides evidence for the importance of gustation and olfaction by sensilla on the pedipalps and forelegs as reported in behavioral studies in several species of spiders. Type A with a spur at the apical tip covering a pore and types B,C and D with a blunt tip and an apical pore possess typical characteristics of a gustatory sensillum while types F,G and H are olfactory. TEM studies reveal a double lumen and the presence of 2-3 neurons innervating similar blunt tipped hairs in *Araneus diadematus* which further branch into 16-20 branches[2]. While such a description closely resembles typical gustatory sensilla, two of its features appear strikingly different. Such blunt tipped sensilla were observed to lack a mechanosensory dendrite, typically found in insect gustatory sensilla[2]. Besides, branching of dendrites is commonly observed in olfactory sensilla where the cuticular surface has numerous pores. In a recent study on parasitic Hymenoptera, Isidoro, et. al.,[18-19] document evidence to ascribe a gustatory function to the multiporous potted sensilla which are characterized by dendritic branching. TEM investigations reveal several unique features with the pores in the apical region being covered by movable structures and innervation by a large number of neurons in the range of 200-220. It is difficult to draw firm conclusions on the possible functional role of type E and transmission electron microscope studies are required to further elucidate their role. It has been suggested that olfactory neurons have a range of sensitivities to different compounds and that large numbers may facilitate discrimination at a distance. It seems reasonable to speculate that at close range, the olfactory sensilla on the pedipalps are able to detect low weight molecular components of surface waxes of the prey. In addition, palpal drumming may serve to increase ventilation around the pedipalps which may enable successive reception of stimuli at different points in space ensuring a high resolution between the incoming stimuli. Several behavioral studies have emphasized the perception of chemical stimuli in spiders, particularly the males in recognition of sex pheromone[20-22] and it seems probable that palpal drumming probably exposes the gustatory sensilla to tactile and chemical stimuli. Also, several behavioral studies suggest that surface compounds are able to inhibit aggressive behavior between conspecifics and prevent cannibalism [19-20]. These substances act as releaser pheromones and are identified by spiders after contact with another spider. The gustatory sensilla identified in the present study are likely to be involved in the detection of these substances. Tip recording from various sensilla confirmed the gustatory role of some of these sensilla. Responses obtained were multicellular, *ie.*, several neurons respond to individual stimuli and that some of these neurons were sensitive to salts, sugars and amino acids. While chemosensilla in spiders share similarities with insect sensilla, a notable difference in gustatory sensilla of spiders is their innervations. Insect contact chemosensilla are innervated by 4 chemosensitive neurons in addition to a mechanosensitive neuron, for eg., the lateral and medial sensillum styloconicum on the maxillary galea of caterpillars; in contrast, gustatory sensilla in spiders are innervated by as many as 19 chemosensitive neurons in addition to 2 mechanosensitive neurons[3-4]. Multicellular activity of individual sensilla in *O. rufisternum* is similar to that described in insects but it has a greater number of active units. This limited the sorting of spikes into individual classes. Thus, the responses reported here suggest the activation of groups of neurons from which the individual sensitivity spectra remains unknown.The study demonstrates that the pedipalp of *O. rufisternum* contains 8 morphologically distinct sensillum types; these sensilla are capable of responding to various stimuli viz, gustatory, olfactory and mechanoreception. The chemosensitive sensilla occur on the distal leg and palp segments and are most concentrated on the tarsi of the first legs, especially on the ventral side and the steep insertion angle which is favourable location for contacting substrate, prey or sexual partners. The gustatory sensilla on the pedipals and forelegs were sensitive to a variety of chemicals testing viz., salts such as Nacl, Kcl; Aminoacids – Phenylalanine, hydroxyl proline, tryptophan, proline and glycine, sugar – fructose and to sugar alcohol, inositol. The gustatory sensilla in spiders are innervated by as many as 19 chemosensitive neurons, in addition to 2 mechanosensitive neurons. The response reported in the study suggest that the stimulus activated more than one sensory neuron and included 2-4 classes of spikes as judged by their amplitude and shape. The activation of groups of neurons from which the individual sensitivity spectra remains unknown.

CONFLICT OF INTEREST

Conflict of interest none.

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