Neural processing of chemosensory information from the locust legs

Dissertation

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1.0 Introduction

In insects chemosensory perception of the external environment is through specific chemoreceptors on their cuticular surface. Chemoreception plays an important role in mediating a diverse range of behaviours, including avoidance (White and Chapman, 1990), detection and selection of food (Dethier, 1976). The chemical senses may be divided into taste, as detection of aqueous chemicals, and smell, as airborne molecules. Alternative terms are contact (taste, gustatory) and distant (smell, olfactory) chemoreception (Gullan and Cranston, 1995).

Most insect contact chemoreceptors and many olfactory sensilla contain more than one sensory neuron (Zacharuk, 1980). For aquatic insects, all chemicals sensed are in aqueous solution, and strictly all chemoreception should be termed taste. However, if an aquatic insect has a chemoreceptor that is structurally and functionally equivalent to one in a terrestrial insect that is olfactory, then the aquatic insect is said to smell the chemical (Gullan and Cranston, 1995). Chemosensors trap chemical molecules, which are transferred to dendrites of chemosensory neurones for recognition, where they specifically depolarize a membrane and elicit a nerve impulse. Effective trapping involves adequate localization of the chemoreceptors. Thus many contact (taste) chemoreceptors occur on the mouthparts, such as the labella of higher Diptera (*Phormia regina*) where salt and sugar receptors occur, as described in the classic work of Dethier, (1976).

The contact chemoreceptors of maxillary palps of *Locusta migratoria* (L.) play an important part in food selection when the insect has not been deprived of food for a long period (Blaney and Chapman, 1970; Blaney, 1974; White and Chapman, 1990). On the ovipositor (Kalogianni, 1995,1996), contact chemoreceptors assist with identification of suitable oviposition sites (Ma and Schoonhoven, 1973). The antennae, which often

point forward to encounter sensory stimuli first and are endowed with many distance chemoreceptors, some contact chemoreceptors and many mechanoreceptors. The legs, particularly the tarsi that are in contact with the substrate (Gaaboub, 1990), also carry many chemoreceptors (Gaaboub and Hustert, 1998). In butterflies *Pieris brassica* stimulation of the tarsi by sugar solutions evokes an automatic extension of the proboscis (Ma and Schoonhoven, 1973.).

Tarsal contact chemoreceptors of fore and middle legs can help in the search for food. In blowflies, a complex sequence of stereotyped feeding behaviours is induced when a tarsal chemoreceptor is stimulated with sucrose. The proboscis starts to extend and, following sucrose stimulation of the chemoreceptors on the labellum, further proboscis extension occurs and the labellar lobes open. With more sugar stimulus, the source is sucked until stimulation of the mouthparts ceases. When this happens, a quite predictable pattern of search for further food follows (Dethier, 1976).

In phytophagous insects in general and in Orthoptera in particular (White and Chapman, 1990; Szentesi and Bernays, 1984) observed that stimulation of the tarsi of *Schistocerca gregaria* by nicotine hydro*gen* tartrate elicited a behavioural response. Chapman *et al.* (1987) suggested that detection of a host-specific chemical by tarsal chemoreceptors may be important in host-plant recognition by the monophagous grasshopper *Bootettix argentatus.* There have been very few structural studies of tarsal chemoreceptors in Orthoptera (Kendall, 1970; Henning, 1974; White and Chapman, 1990).

Almost all-previous behavioural and electrophysiological work on chemoreception in grasshoppers has concentrated on feeding behaviour and the responses of mouthpart sensilla (Haskell and Schoonhoven, 1969; Blaney, 1974, 1975, 1980; Winstanley and Blaney, 1978), yet here too,

many questions on the mechanism of discrimination by chemoreceptors remain unanswered (Chapman, 1988). Early work (Haskell and Schoonhoven, 1969) suggested that, as in Dipteran and Lepidopteran chemoreceptors (Hanson, 1987; Schoonhoven, 1987), each neurone within a sensillum responded to a different range of compounds. The most extensive study of grasshopper chemoreceptors to date (Blaney, 1974. 1975. 1980; Winstanley and Blaney,1978), however, found that each neurone in the mouthpart sensilla responded to many different compounds, and moreover, that several neurones within any one sensillum responded in a similar manner to any chemical stimulus.

The number of afferents innervating an individual sensillum varies according to its location and the insect species: 6 for receptors on locust tarsi (Kendall 1970), 6-9 on locust maxillary palps (Blaney and Chapman, 1969), 4-12 for cricket palps (Klein 1981) and 5 for gustatory receptors in the flies *Phormia* and *Drosophila* (Dethier, 1976; Murphey *et al.* 1989). This suggests that a fundamentally different discrimination mechanism is operating in grasshopper chemoreceptor systems, which deserves further study.

Insect chemoreceptors are sensilla with one or more pores (holes). Two classes of sensilla can be defined based an their ultrastructure:
Uniporous, senses of taste (gustation) with one pore, and multiporous senses of smell (olfaction) with several to many pores and the apex of the sensilla (Gaaboub, 1990; Boeckh, 1980; Kaissling, 1971). Uniporous sensilla range in appearance from hairs to pegs plates or simply pores in a cuticular depression, but all have relatively thick walls and a simple permeable pore, which may be apical or central. The hair or peg contains a

chamber, which is in basal contact with a dendritic chamber lying beneath the cuticle. The outer chamber is often seen to contain (and extrude) a

viscous liquid, presumed to assist in the entrapment of chemicals and in their transfer to the dendrites (Chapman, 1982). Basiconic sensilla are short peg-like structures which act as multimodal receptors by responding to mechanical stimuli and to contact with chemicals through their terminal pore which connects to a fluid-filled lumen containing the sensory dendrites (Dethier and Bowdan, 1989; Newland, 1998). It is assumed that these uniporous chemoreceptors predominantly detect chemicals by contact, although there is evidence for some olfactory function (Gaaboub and Hustert, 1998).

The tarsus of *S. gregaria* or *L. migratoria* is divided into three segments and an arolium set between a pair of claws. The first segment bears three pairs of pulvilli in the fore and middle legs, and one pair and two single pulvilli in the hind legs. Segment two bears a pair of pulvilli, segment three one long pulvillus and the terminal arolium bears a similar pad on the undersurface. The outer layers of the arolium differ from those of the pulvilli in possibly lacking an epicuticle (Kendall, 1970; White and Chapman, 1990). The claws and dorsal surfaces of the tarsus bear trichoid sensilla, basiconic sensilla and campanifonm sensilla. The ventral surface of the tarsal pulvilli is covered with characteristic sensilla, which come into contact with the substrate during locomotion. These are the so- called pulvillar basiconic sensilla, which are provided with a small cone-shaped hair (length 5-10 µm; socket diameter 10 µm; diameter of the hair 4-6 µm). Generally, each sensillum basiconicum is innervated by 5 sensory neurones present below the hair base and surrounded by the enveloping cells one of which is a mechanosensory neuron to responding to mechanical stimuli while the other are chemosensory (White and Chapman, 1990).

Basiconic sensilla are widely distributed over the body, including the legs, but are particularly concentrated on the antennae and on the tips of the mouthparts. They are peg-like structures with a shaft that is typically much

shorter than that of the trichoid sensilla and which has a pore at its tip. The afferent neurones from tarsal receptors project only in one ganglion. The central projection of tarsal chemosensitive receptors always project ventrally (Gaaboub and Hustert, 2000; Newland *et al.* 2000), and the pulvillar canal sensilla, with an external appearance of simple holes within the cuticle (diameter 4-5 µm) (Mücke, 1991). Trichoid sensilla are present on the underside of the arolium. They are about 25 um long and arranged in two rows running proxi-mal-distal with two separate sensilla nearer the base. On average there are about ten sensilla per arolium (White and Chapman, 1990).

The trichoid sensilla act as mechanoreceptors signalling tactile stimulation, although on other parts of the body, such as the cerci and head and neck, they also respond to air currents. Each hair is separately innervated by a single sensory neuron with its cell body just below the socket in which the hair shaft is articulated. The dendrite of this sensory neuron extends into the shaft and transducer movements into sequences of spikes that are conducted along the axon to the central nervous system. The thickness of the shaft varies for hairs in different regions of the body so that at one extreme the hairs are called filiform and at the other, bristle hair.

Although the insect's tarsus plays an important role in contact chemoreception between the insect and its host plant, little attention has been directed the features of its sensilla. Chemoreceptors on the tarsus of the migratory locust could be involved in the recognition of host plants or sites for oviposition. When locusts walk, land or manipulate their food the tarsal pulvilli (pads) make most of the contact with the substrate. The basiconic chemoreceptive sensilla of the tarsal pulvilli should record the chemical composition of the surface but it is not known what the adequate stimuli are and which regular behavioural responses occur.

For the tibia of insects four types of chemosensory neurones for water, sugar, salt and alkaloids (White and Chapman, 1990) have been identified. A study of the sensory and motor responses to different chemical stimuli as to their attractant or repellent effects arising from a specific pulvillus of the locust. (Gaaboub and Hustert, 1998).

This study focussed on the processing of different chemical stimuli by the local circuits that control leg movement in the prothoracic and mesothoracic ganglion of *L. migratoria* and *S. gregaria*. Behavioural as well as physiological responses were compared. The behavioural responses of freely moving locusts to aqueous solutions of different chemicals over a large range of concentrations applied to the pro and meso leg were determined. Next, the physiological responses of the gustatory basiconic sensilla, leg motor neurones and spiking local interneurones, which are an important component of the local circuits generating leg movements, were determined.

Locusts exhibit an increasing possibility of moving the leg away from an applied droplet as the concentration of a given chemical increases. This applies not only to chemicals that are known to be noxious or phagodeterrent such as nicotine, but also to nutrient chemicals such as sucrose or amino-acids. Also critical is the concentration to which the behavioural response becomes more frequent than with applying just water. Intracellular recordings of motor neurones have demonstrated that they receive concentration and chemical dependent graded inputs from the gustatory basiconic sensilla on the legs.

2.0 Material and Methods

2.1 The experimental animals.

Adult *Locusta migratoria* and *Schistocerca gregaria* (Forskal) from the laboratory colony maintained at the Zoological Institute of the University of Göttingen were reared under crowded conditions and fed on wheat and oat seedlings at 25^{o}C reared under a 12h:12h light : dark regime. Adult locusts of either sex were used in all experiments. Ages from 5 to approximately 8 days post-moult, before the onset of breeding. All locusts were examined prior to use to ensure that all the limbs were intact and undamaged. They were kept isolated without food overnight prior to the experiments. Insects were cooled to 4^{o}C 10 minutes prior the experiment.

The tarsus is supplied by four tibial nerves (Mücke, 1991). N5B2a3 is purely a motor nerve and innervates levator, depressor, and distal retractor muscles. N5B2-a, -a2, and -b are pure sensory nerves. N5B2b supplies sense organs on the posterior half of the distal tibia and tarsal segments I-III; N5B2a2, supplies the anterior half of the distal tibia; and N5B2a, supplies the anterior half of ta I-III and the entire pretarsus (Fig. 1).

2.2 Scanning electron microscopy

To identify the external chemoreceptive and mechanoreceptive sensilla, tarsi were fixed in chloroform then either critical point dried following dehydration in ethanol, or air-dried. After drying they were coated either with gold-palladium or carbon followed by gold-palladium and examined and photographed with a scanning electron microscope (SEM).
Apparatus model LEO – UIF (Leo 438VP).

2. 3 Anatomy

2.3.1 Staining by cobalt chloride

Backfills of the motor nerve (levator and depressor tarsi N5B2a3) and sensory nerve N5B2-a, -a2, and -b N5B2b supplies sense organs on the posterior half of the distal tibia and tarsal segments I-III; N5B2a2, of the

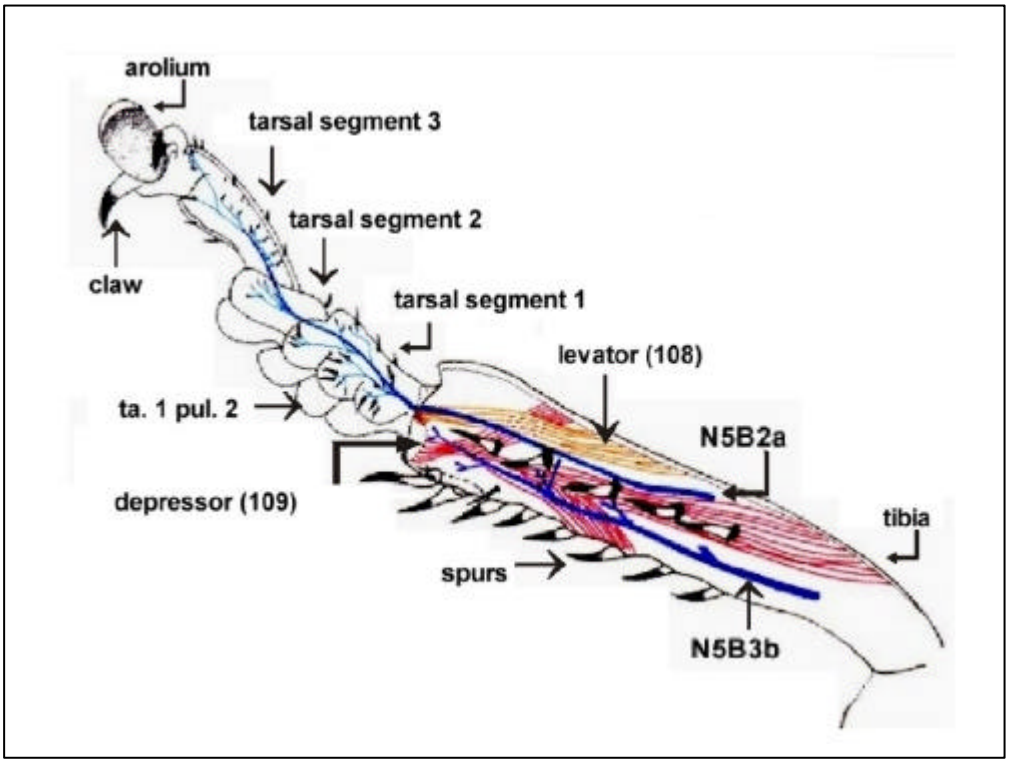


Fig. (1) Overview of the mesothoracic tibio-tarsal region with tarsal muscles and sensory supply of the posterior tarsal segment inset: expended view of pulvillar sensilla and peripheral backfill of the sensory nerve N5B2a and motor nerve N5B3b with cobalt choloride in the mesothoracic ganglion.

tibia; and N5B2a, the anterior half of ta I-III and the entire pretarsus were prepared by surrounding an individual cut nerve end with vaseline and distilled water in a well 5 for minutes before cobaltous chloride, at a concentration of 6% was replaced the water and this was sealed against desiccation with more vaseline.

The whole locust was placed in a moist chamber and incubated for 5-7 days at 4^{o}C to allow the cobalt to diffuse throughout the afferent neuron. Subsequently, animals were dissected from the ventral surface, the mesothoracic ganglia removed, and cobalt was precipitated the into the black sulphide (by some droplet at ammonium sulphide $(NH_4)_2S$ 10 minutes according to) (Pitman *et al.*,'72). The leg was washed 2 times for 5 minutes in ringer solution, then fixed in 5% formaldehyde (buffered to pH 7.2) and silver intensified according to (Bacon and Altman, '77).

The preparations were dehydrated through an acending alcohol series and cleared in methyl salicylate. Stained afferents were drawn from wholemounts by using a camera Lucida attachment on a Zeiss standard microscope and photographed with a Leitz Aristoplan microscope (Fig. 1). In some experiments the mesothoracic legs were also removed from the animal and its nerve stumps were stained centrifugally, in a similar manner to confirm the identity of the stained receptor.

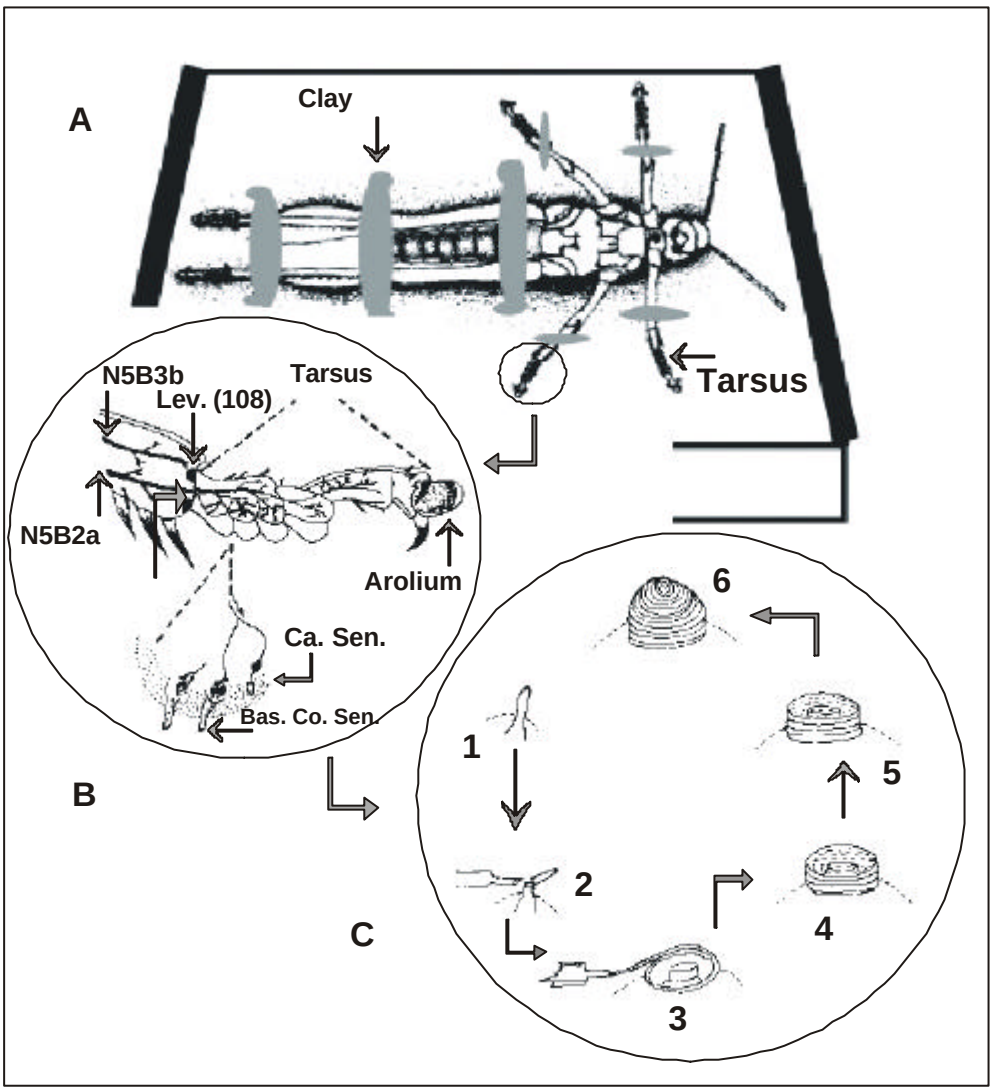


Fig. (2) Anatomy and staining method of basiconic sensilla (A) view of the mounted locust before saining. (B) Overview of the mesothoracic tibio-tarsal region with tarsal muscles and sensory supply of the posterior tarsal segment inset: expended view of pulvillar sensilla and peripheral backfill of the sensory nerve N5B2a with neurobiotin in the mesothoracic ganglion. Arrows indicate the four cell bodies of the pulvillar basiconic sensillum, arrow shows to the spindle-shaped cell body of one pulvillar canal sensillum. (C) Overview of the methods to backfilling one pulvillar basiconic sensillum (1) basiconic sensillum, (2) cutting by broken microglass electrode, (3) vasline ring, (4) vasline pool with DW. (5) Replace DW with neurobiotin, (6) sealed with vasline.

2.3.2 Staining by neurobiotin

The central projection of both contact chemoreceptor and mechanoreceptor neurons of the tarsal pulvilli were visualized with neurobiotin (Vector Laboratories Inc.) in backfills (modified from: Bayer and Wilcheck 1980, Consoulas *et al.* 1996) from single receptors in the periphery (Fig. 2). The chemosensitive and the mechanosensitive canal sensilla from the pulvilli were stained by surrounding the receptor with a well of wax or vaseline. In this well a droplet of distilled water was placed and the sensillum was shaved of with a fine piece of razor blade or perforated with a broken glass microelectrode, exposing the sensory dendrites.

The distilled water was replaced with a droplet of 3% aqueous neurobiotin solution. The animals were kept in a moist chamber at 4^{o}C for 72-120 hours or in room temperature for 48-72 hours. After that, the thoracic ganglia were dissected out in insect saline, fixed in 4% paraformaldhyde for 5 hours, and then dehydrated and cleared in xylene. The preparations were then rehydrated with a descending alcohol series. The labelled ganglia were incubated for 1 hour at 37^{o}C in a solution of 1mg collagenase, 1mg hyaluronidase in 1 ml 0.1 M phosphate buffer, then rinsed in 0.1M phosphate buffer with two changes of 15 minutes followed by three changes of 15 minutes with 0.5% TritonX-100 (Sigma Chemical Co) added.

The preparations were then incubated in ABC complex (ABC-kit PK400 standard Vector Laboratories, Burlingure) in buffer for 5-12 hours at room temperature, then rinsed for 15 minutes with two changes, followed by pure 0.1 M phosphate buffer for 15 minutes. For the DAB reaction, the preparations were incubated for 5-15 minutes in a solution of 30 mg 3,3-diaminobenzidine tetrahydrochloride (Sigma Chemical Co.) and 45µl hydrogen peroxide (30%) in 100 ml 0.1 M phosphate buffer.

The reaction was stopped by two changes of 0.1 M phosphate buffer for 5 minutes. The preparations were dehydrated through a ascending alcohol series and cleared in methyl salicylate. The results were drawn by using a camera lucida attachment on a Zeiss standard microscope and were photographed with a Leitz Aristoplan microscope.

The major tracts and commissures of the central nervous system were studied in the neurobiotin-stained wholemounts which were embedded in soft Durcupan (Fluka-Chemie) and serially sectioned at 20-30 µm. The cytoarchitecture in relation to the afferent projections was visualized with a phase and interference contrast microscope (Leitz Aristoplan). Tracts and commissures in the neuropile were identified according to what is known of the thoracic ganglia of locusts from (Pflüger *et al.* 1988) and (Kalogianni, 1995). Peripheral nerves were named as by (Mücke, 1991)

2.4 Stimulation and sensory physiology

Responses from individual sensilla (basiconic sensilla) to chemical stimuli on the ventral side of the tarsus were recorded using the tip recording technique (Hodgson *et al.* 1955). The potentials were amplified and filtered using AC amplifiers. A blunt glass microelectrode filled with different solutions was placed over the shaft of the sensillum. Electrodes containing salt (NaCl 0.01 to 3 M), sugar [glucose 0.1M to1M], acid [citric acid 0.01M to 1M], alkaloid (nicotine hydrogen tartrate 0.01 M) and quinine hydrochloride diluted 0.01 M in 0.01 M NaCl and Ringer solution were used to stimulate the *chemosensory* afferents. Controlled movements of this electrode were used to deflect the sensillum so as to elicit spikes in the mechanosensory afferents. The same electrode was therefore used simultaneously to evoke and record the spikes of the afferents. The displacement of a sensillum did not deform its short and stout shaft.

2.5 Responses of motor neurones to chemical stimulation of the pulvilli:

The activity of motor neurones innervating the muscles depressor and levator tarsi in a meso leg was recorded in three ways.

2.5.1 Electromyography (EMG) with implanted wires. EMG activity was recorded from depressor and levator tarsi utilizing 2 or 3 pairs of extracellular electrodes (30 µm steel wire insulated but for the tip) inserted through the cuticle and fixed with wax.

The animals were restrained ventral side uppermost, and, unless otherwise indicated, the legs were restrained at the coxal and femoral segments, the tibiae and tarsi alone were free to move (Siegler and Burrows, 1986; Laurent and Hustert, 1988). The activity of motor neurones innervating muscles in a mesothoracic leg was recorded. Observations were made on the animals during stimulation by different chemical concentration of salt (NaCl 0.01 to 3 M), sugar (glucose 0.1M to1M), acid (citric acid 0.01M to 1M) alkaloid (0.01 M nicotine hydrogen tartrate (NHT) diluted in 0.01 M NaCl and Ringer solution (Sigma Chemical Co.). The responses of the motor neurones were recorded by an AC-tape recorder (Tascam).

2.5.2 Electromyography with surface suction electrodes. The animals were mounted in plasticine, ventral side up with all legs immobilised by staples in plasticine. Extracellular recordings were made with suction electrodes from the surface of the depressor and levator muscles.

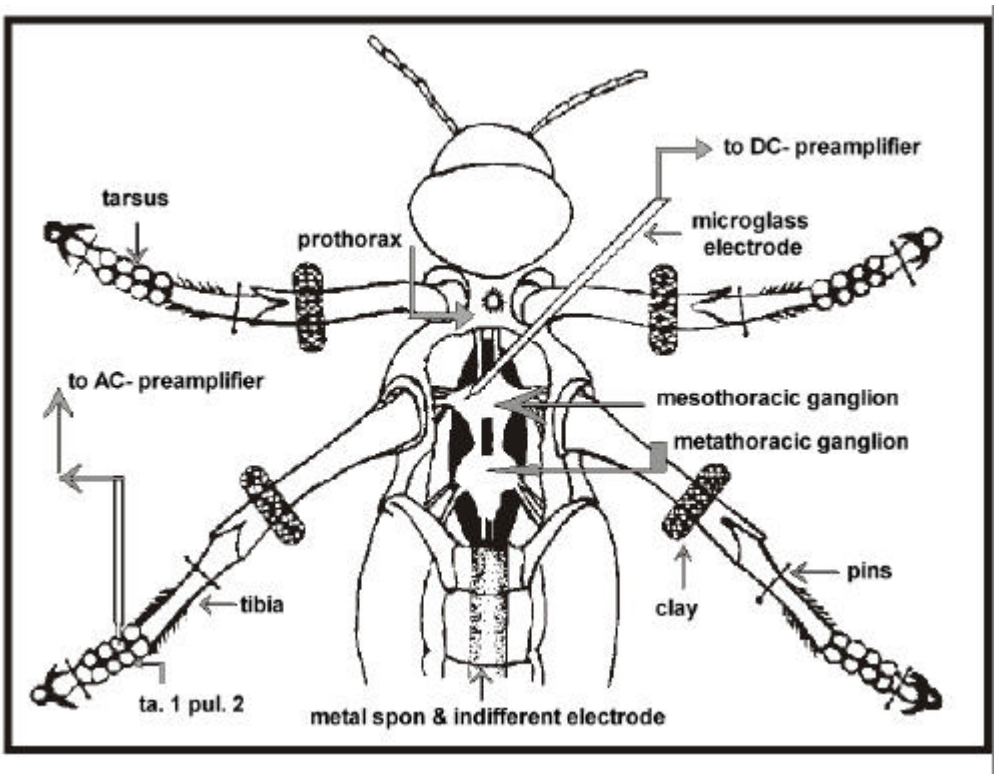
2.5.3 Motor nerve recording. The animals were mounted in Plasticine, ventral side up, with all legs immobilised by hooks in plasticine. Extracellular recordings were recorded with suction electrodes from some motor nerves to (depressor and levator) the nerve terminating on a muscle without

applying mechanical tension via the nerve. Recording from motor nerves was also done with hook electrodes there isolated from the saline with petroleum jelly. Individual exteroceptors were stimulated on the ventral side of the tarsus and were recorded using the tip recording technique (Hodgson, et al. 1955).

2.6 Intracellular recording.

The locust was restrained ventral side up in plasticine with all legs immobilised by hooks in plasticine, the thoracic ganglia were then exposed and a stainless steel plate was placed beneath them to provide stability (Robertson and Pearson 1982)(Gee and Robertson 1994) (Fig. 3). The body cavity was filled with saline at room temperature in (147 mM NaCI, 10 mM KCI, 4 mM $CaC1_2$, 3 mM NaOH, 10 mM HEPES buffer). Nerves 3 and 4 of both meso- and metathoracic ganglia were cut to increase stability of the preparation. In some preparations, all thoracic nerves except nerve 5 were cut or crushed and the abdominal connectives were crushed to reduce background synaptic activity.

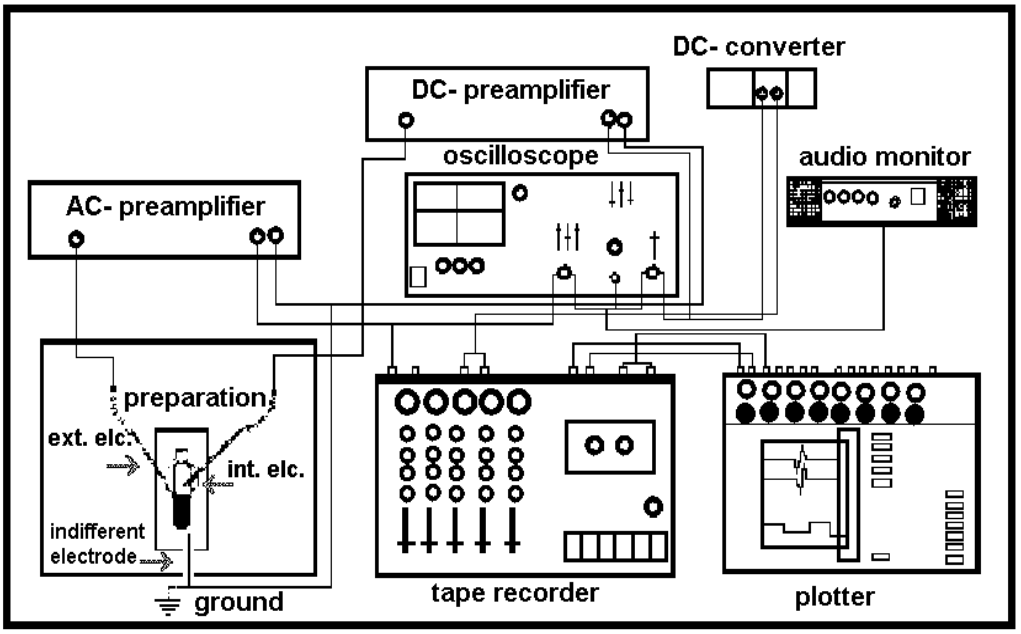
To facilitate electrode penetration the ganglionic sheath was treated with a 0.1% solution (w/v) of protease (Sigma type XIV) for 1-2 min before recording. The thorax was continuously perfused with locust saline at 20-22^{o} throughout an experiment. Microelectrodes were filled with 2 M potassium chloride and had DC resistance in saline of *about* 60-200 MΩ (Fig. 4). Intracellular recordings were made from the somata of spiking local interneurones or from the somata of the mesothoracic tarsal motor neurones (depressor and levator tarsi). Interneurones of this population were identified by their responses to stimulation of basiconic sensilla on the tarsus (pulvilli) that define their receptive fields.



(Fig. 3) Ventral view of the thorax, preparation for intracellular recording and staining by Lucifer Yellow.

2.6.1 Intracellular staining

Recordings were made with glass microelectrodes filled at the tip with Lucifer Yellow CH (4% in distilled H_20) used to stain all spiking inter-neurones motor neurone. The shafts were filled with 1 M lithium chloride (electrode resistance ~80-200 MΩ). In some cases it was necessary to inject small amounts of hyperpolarizing current to stop the cell from spiking and enable us to measure the EPSPs. After recording EPSPs, hyperpolarizing current was used to fill the impaled cell with Lucifer Yellow. In many instances, prior to removing the microelectrode, the preparation was examined under epifluorescence illumination in order to determine the recording site in the interneuron. The ganglia were fixed in 4% paraformaldehyde for 1 h, dehydrated in an ethanol series and cleared in



(Fig. 4) Diagram of the recording arrangements.

methyl salicylate. Ganglia were examined under a compound fluorescence microscope from the dorsal aspect and a drawing of the ganglion‘s outline and the filled intemeuron was made with the aid of a camera Lucida. Some interneurones were photographed. Preparations, from which the postsynaptic cell was not identifiable, due to insufficient filling with Lucifer Yellow, were not included in data.

The names of muscles and sclerites, nerves, and sense organs were used as by (Snodgrass, 1935; Hustert, 1978; Bräunig, et al. 1981). The gross internal architecture of the CNS was visualized under a phase contrast microscope. Tracts and commissures in the neuropile were defined according to (Pflüger, *et al.* 1988).

2.7 Behavioural experiments

A total number of 120 locusts species *S. geraria* divided into two groups were treated with different aqueous solutions of varying concentrations. The group of 60 locusts was arranged in 5 repetitions each comprising 12 locusts. Each individual within a repetition in the first group was treated with the following concentration of NaCl (10mM, 25mM, 50mM, 75mM and 100mM). The same was applied to the second group using sucrose in the following concentration (10mM, 100mM, 250mM, 500mM, 1000mM and 2000 mM). The distilled water control was performed primarily to measure the effectiveness of mechanical stimulation alone in eliciting a response. There was an interval of at least 20 min between subsequent presentations of solutions, and the animals were placed in individual containers between tests. During each test, the locust was removed from its container, and an opaque hood fashioned prepared from a heat-shrink insulation was placed over its head, covering the eyes and chemosensory receptors on the mouthparts and antennae (Rogers and Newland, 2000). The locust was placed on a test arena consisting of a rigid 1 mm mesh nylon sheet raised 25 mm above the work surface.

The solutions were applied as droplets using a Pasteur pipette held 10 -15 mm above the right hind tarsus or above the right pro tarsus. The nylon mesh allowed applied droplets to run easily around the entire surface of the tarsus but prevented them from falling straight through. More importantly, it allowed the locust a firm hold on the substratum, ensuring that any movement was a positive reaction, not merely the result of the animal losing its grip and sliding away from the point of contact.

The droplets had a mean volume of 0.04±0.008 ml (mean ± S.E.M., N=40). The mechanical component of the stimulus presented was, therefore, always similar. Droplets were only applied when the locust had

come completely to rest on the mesh and the hind leg was at an angle of approximately ± 30^0 from vertical. All experiments were performed at 23 - 25 ^{0}C.All tests were filmed using a video camera (Panasonic WVBP500) mounted on a tripod with a 50 mm lens at 25 frames s^{-1} and recorded on a Panasonic NV-HD680 video recorder for 10 s following the application of the droplets. A date/time marker (Video timer type: For A) was mixed with the video signal, allowing easier analyses of the responses of the animals. After each test, the tarsus was rinsed with distilled water, and the locust was returned to its container.

Each experiment was repeated five times with new locusts for each repetition, so that there were 60 tests with any given solution (Rogers and Newland, 2000). All the tests were recorded for 10 s following application of the droplet. As the locusts were free to move at any time, there is potentially some difficulty in separating movements due to the application of the stimulus from spontaneous movements. In the following work, only movements that occurred within 1 second (s) of the droplet being applied are included. Using this upper latency limit of 1 s excludes all movements of the fore and hind leg that were preceded by movements of other limbs and were therefore clearly part of a non-local sequence of motion.

The latency to first response followed an approximately exponential function, with 62.8 % of all the recorded movements of the fore and hind leg (in response to all the test chemicals) occurring within a latency of 1 s. The proportion of locusts responding at longer latencies declined rapidly over the remaining 9 s recording period. The natural logarithm of concentration was used in all statistical analyses to render a more linear dose-response relationship. The frequencies and durations of response were analysed using analysis of variance (ANOVA).

3.0 Result

3.1 Features and function of the basiconic sensilla.

The fine structure and distribution of various types of tarsus sensilla in the desert locust, were investigated by scanning electron microscopy. The tarsus of locust is divided into three segments and an arolium set between a pair of claws. The first segment bears three pairs of pulvilli in the fore and middle legs, and one pair and two single pulvilli in the hind legs. Segment two bears a pair of pulvilli, segment three one long pulvillus and the terminal arolium bears a similar pad on the undersurface. The claws and dorsal surfaces of the tarsus bear trichoid sensilla, basiconic sensilla, canal sensilla and campaniform sensilla (Fig. 5 and 6). The ventral surface of the tarsal pulvilli is covered with characteristic sensilla, which come into contact with the substrate during locomotion.

The basiconic sensilla is the most abundant type, especially on the pulvillus. The sensillum length ranges between (5-10 µm), the basal diameter is about (4 µm), the sensillum lacks a basal socket. Each basiconic sensillum is innervated by 5 sensory neurons present below the sensillum base and surrounded by the enveloping cells.

The canal sensilla found on the pulvillus have an outer opening about (5 µm) in diameter. The canal sensilla are innervated by a single large sensory neuron, similar to campaniform sensilla which are mechanoreceptors.
The mechanosensory trichoid sensilla which are (20-40 µm) long, are more slender and have a smaller basal diameter (about 3 µm) than the basiconic sensilla.

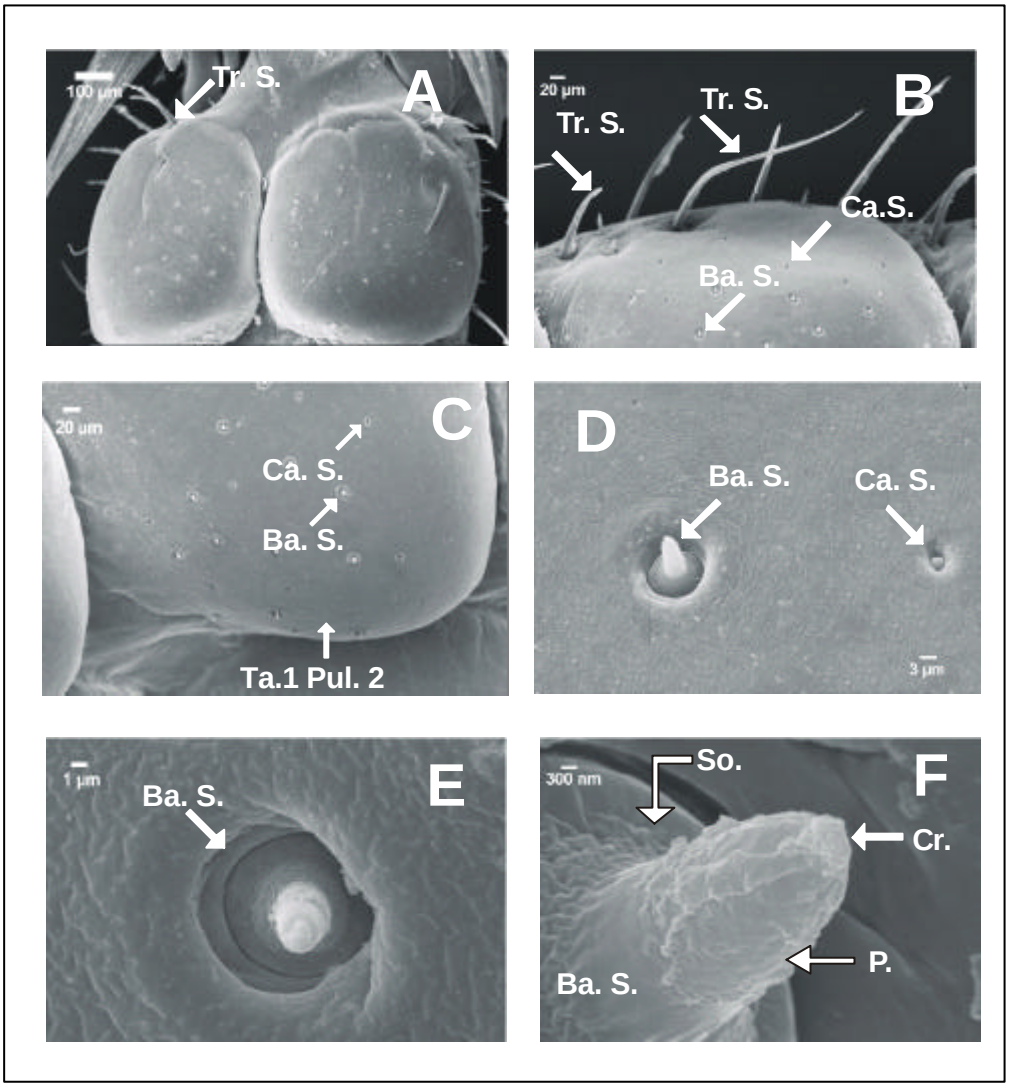


Fig. (5) Scanning electron micrograph showing the ultrastructure of the ventral exteroceptors on the tarsus. (A) Anteroventral view of the first pair of tarsus 1 pulvilli 1 arrow shows lateral trichoid sensilla (Tr.s.). (B) Lateral part of the pulvillus 2, the surface is smooth and has 3 types of receptors, pulvillar canal sensilla (ca.s.), pulvillar basiconic sensilla (Ba.s.) and trichoid sensilla (Tr.s.). (C) and (D) The smooth surface carries two types of receptors; pulvillar canal sensilla (Ca.s.) and pulvillar basiconic sensilla (Ba.s.). (E) Individual basiconic sensillum, of small size, altered shape and lacking pores. (F) Higher magnification of one pulvillar basiconic sensillum, with its socket (So.), smooth peg (P), and crest (Cr.).

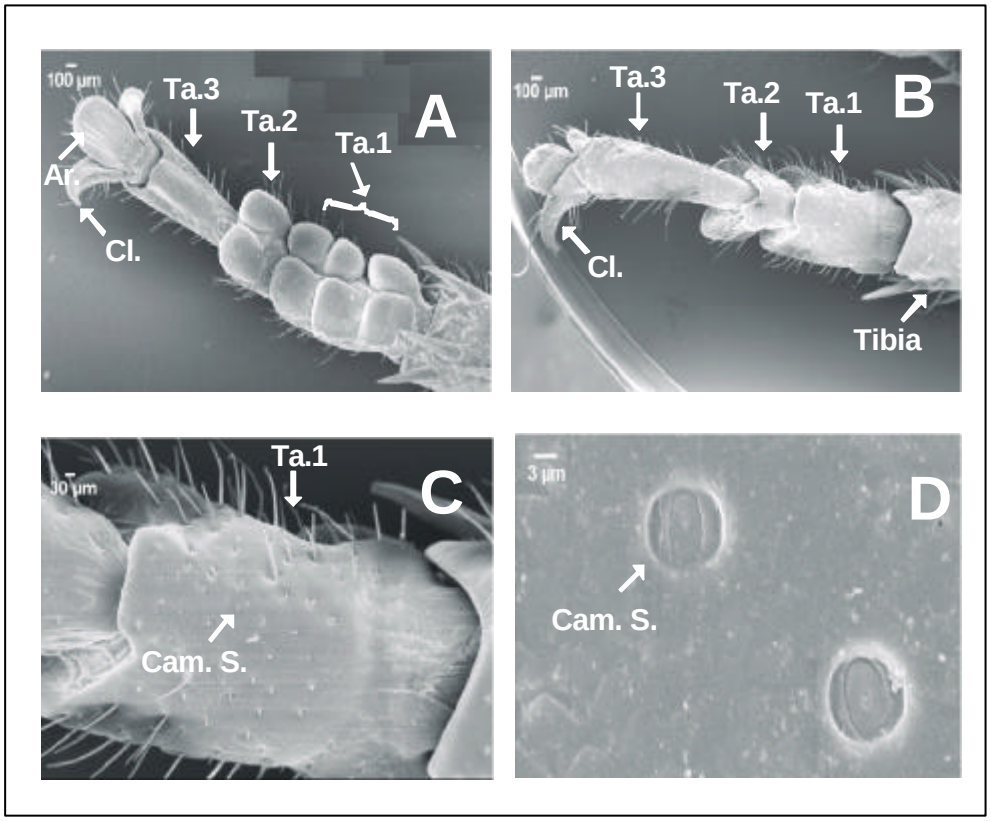


Fig. (6) (A) ventral view of the mesothoracic leg. (B) dorsal view of the mesothoracic leg. (C) Dorsal view of the tarsus 1, arrow shows campaniform sensilla (Ca. s.), scale bar 30 µm. (D) Higher magnification of campaniform sensilla (scale bar 3 µm).

The trichoid sensilla are most abundant on the dorsal side and are also present on the underside of the arolium. Each hair is separately innervated by a single sensory neuron with its cell body just below the socket in which the hair shaft is articulated. The thickness of the shaft varies for hairs in different regions of the body so that at one extreme the hairs are called filiform and at the other, bristle hair. The trichoid sensilla are found in larger number on the lateral and dorsal side.

Only two mechanoreceptors (campniform sensilla) are present on the dorsal side of the first segment of the tarsus, which is innervated by a single sensory neuron (Fig. 6).

The number of basiconic sensilla and canal sensilla is higher in the pulvillus of the foreleg than in the pulvillus in the middle leg. The density of basiconic sensilla in both fore and middle pulvillus is higher in the middle of the pulvillus (Fig. 7). But, the canal sensilla occur more dense in the lateral side. This means that the chemoreceptive sensitivity is stronger in the middle of the pulvillus and the mechanorecptive sensitivity is stronger on the lateral side. On the dorsal side of the tarsus there are mechanoreceptors and chemoreceptors, the mechanoreceptors are longer than the chemoreceptors.

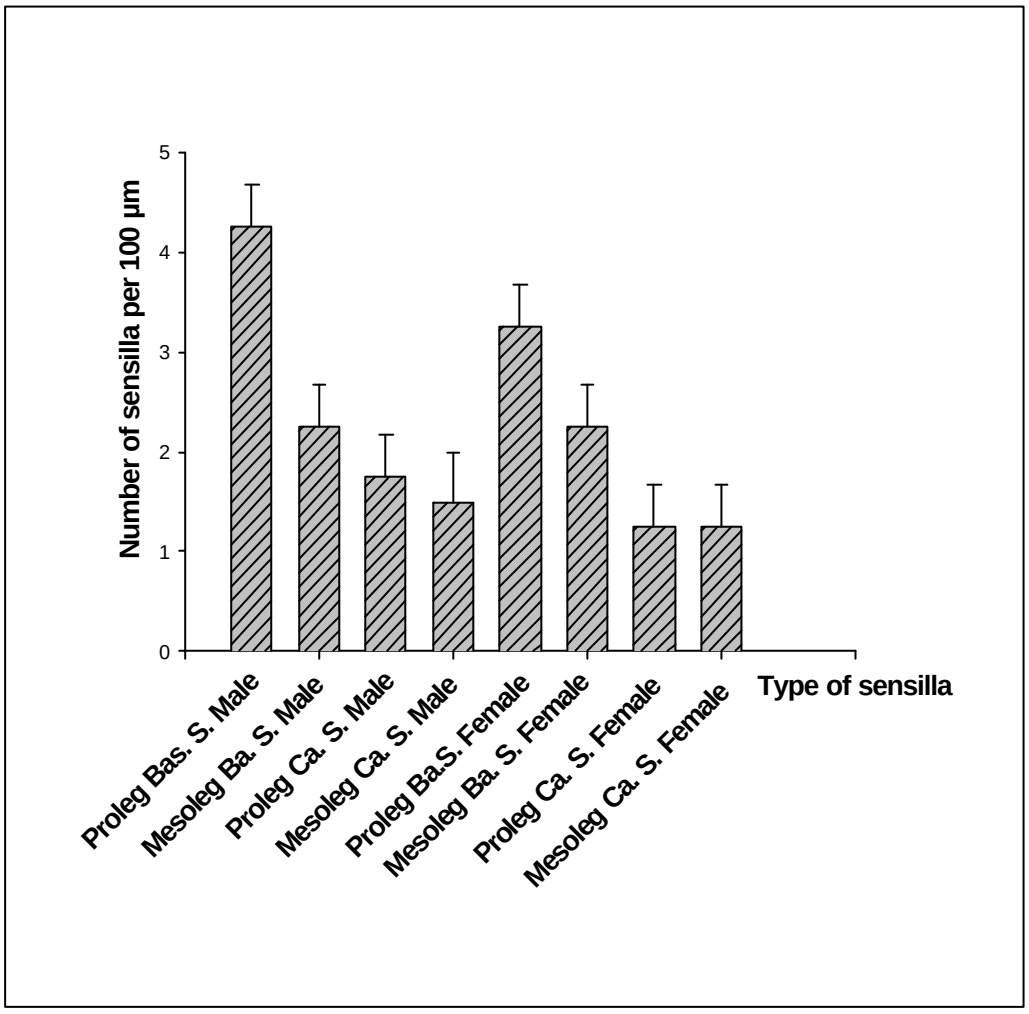


Fig. (7) Comparison of the mean number of pulvillar canal sensilla and pulvillar basiconic sensilla in the tarsus 1 pulvillus 2 in pro and mesothoracic legs.

3.2 Electrophysiological recordings

Electrophysiological recordings were carried out to study the afferent responses to different concentrations of NaCl, sucrose, glucose, citric acid, NHT, Quinine, soladinine and saline (Clements and May) on the electrical activity of pulvillar basiconic sensilla. The investigation showed that the pullvillar basiconic sensilla were sensitive to all mentioned stimuli.

The results indicated that both the frequency and the amplitude of afferents from pulvillar basiconic sensilla differed according to the type of chemical and its concentration (Fig. 8).

High concentrations of NaCl the stimulation were more effective than at low concentrations. The responses of a typical sensillum to different concentrations of NaCl are shown in (Fig. 8). Most traces contain at least two and possibly three neurons firing. The distributions of spike amplitudes for the same recording also suggest the presence of two or three neurons firing per trace. Increases in salt concentration increase the amplitude of the response, but does not increase the number of neurones which fire. Deliberate movement of a sensillum by the recording electrode produced activity in a mechanosensory neurone. The mechanosensory neurone spikes with a large amplitude.

The responses of sensilla to sucrose, glucose or quinine showed a significantly greater number of action potentials in response to sugars than to NaCl used her as electrolyte alone. Investigations for all chemicals tested are shown in (Fig.8).

For NaCl and glucose as stimulants the number of neurones which fired appears to be similar for both stimuli. Increases in the total spike count in

response to sugars or alkaloids were due to increased firing rates of several rather than a single neurone.

Two different response types occurred. In most cases the chemical sensitive neurone began to fire immediately upon stimulation, followed by a period of decreasing frequency as adaptation occurred. Some neurones, however, showed an initial latency of around 100ms, followed by a period of increasing frequency. Both types were due to the activity of a single neurone in each sensillum, and in both cases, after a suitable recovery time (10 min), it was possible to record further responses compare: (White and Chapman, 1990).

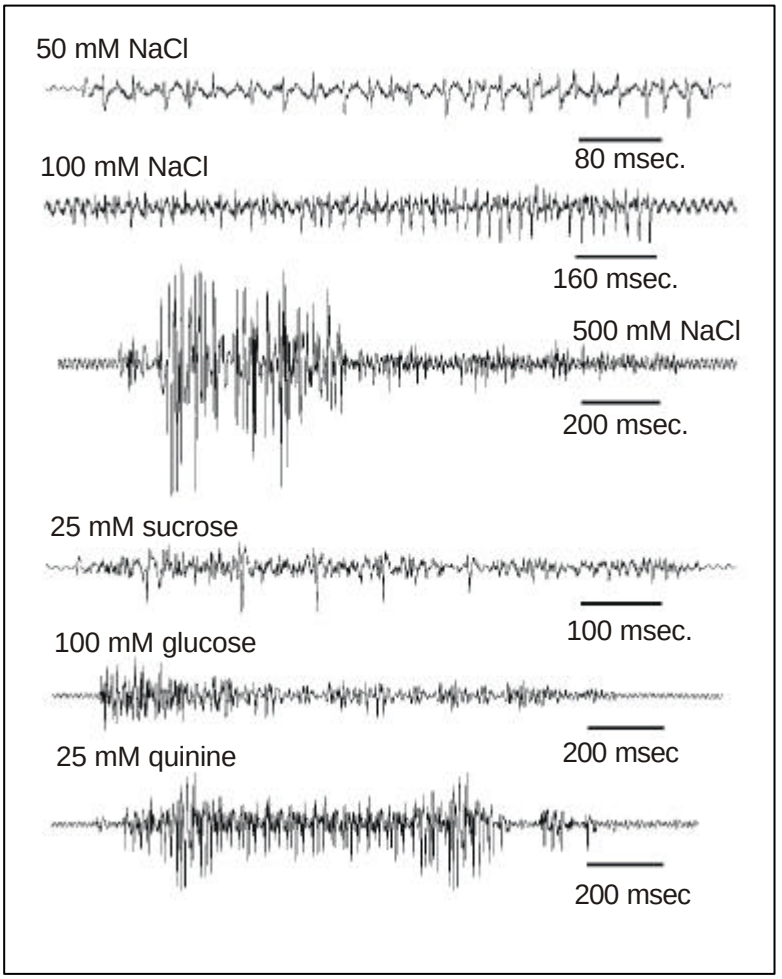


Fig. (8) Recording from a pulvillar basiconic sensillum to different concentrations of NaCl, sucrose, glucose and quinine.

3.3 Chemoreceptor projections:

Afferent chemoreceptor projections from single basiconic sensilla on a pulvillus were traced using the backfilling techniques to see whether chemoreceptor afferents from the pulvilli of the mesothoracic legs converge or segregate in chemospecific glomerular compartments of the central nervous system (CNS) like antennal smell receptors, or distribute according to topological rules similar to other contact chemoreceptors of less specialized regions on the legs (Newland et al 2000), or extend to other ganglia as most chemosensory afferents from abdominal segments do (Tousson and Hustert 2000).

For practical reasons, we primarily stained afferents from the second posterior pulvillus of the first tarsal segment (Ta1/pul.2) (Fig. 9 C) which during a step cycle of a middle leg on horizontal surfaces often makes the initial contact with the substrate. Comparisons were made to afferent projections from the second anterior pulvillus (Ta1/pul.2)on the same leg tarsomere, which records the takeoff of the leg ending the stance phase.

Homologous afferents of the prothoracic pulvillus were also compared since the foreleg has different stepping trajectories and holds food (wheat leaves e.g.). A third comparison was made with tactile hairs on the dorsal tarsus.

Peripheral staining:

Cobalt backfilling of the basiconic sensilla on the tarsal pulvilli confirmed the "locust rule" of a typical supply with one mechanosensory neuron terminating at the base and four chemosensory neurons terminating near the pore of the hair tip (Fig. 9).

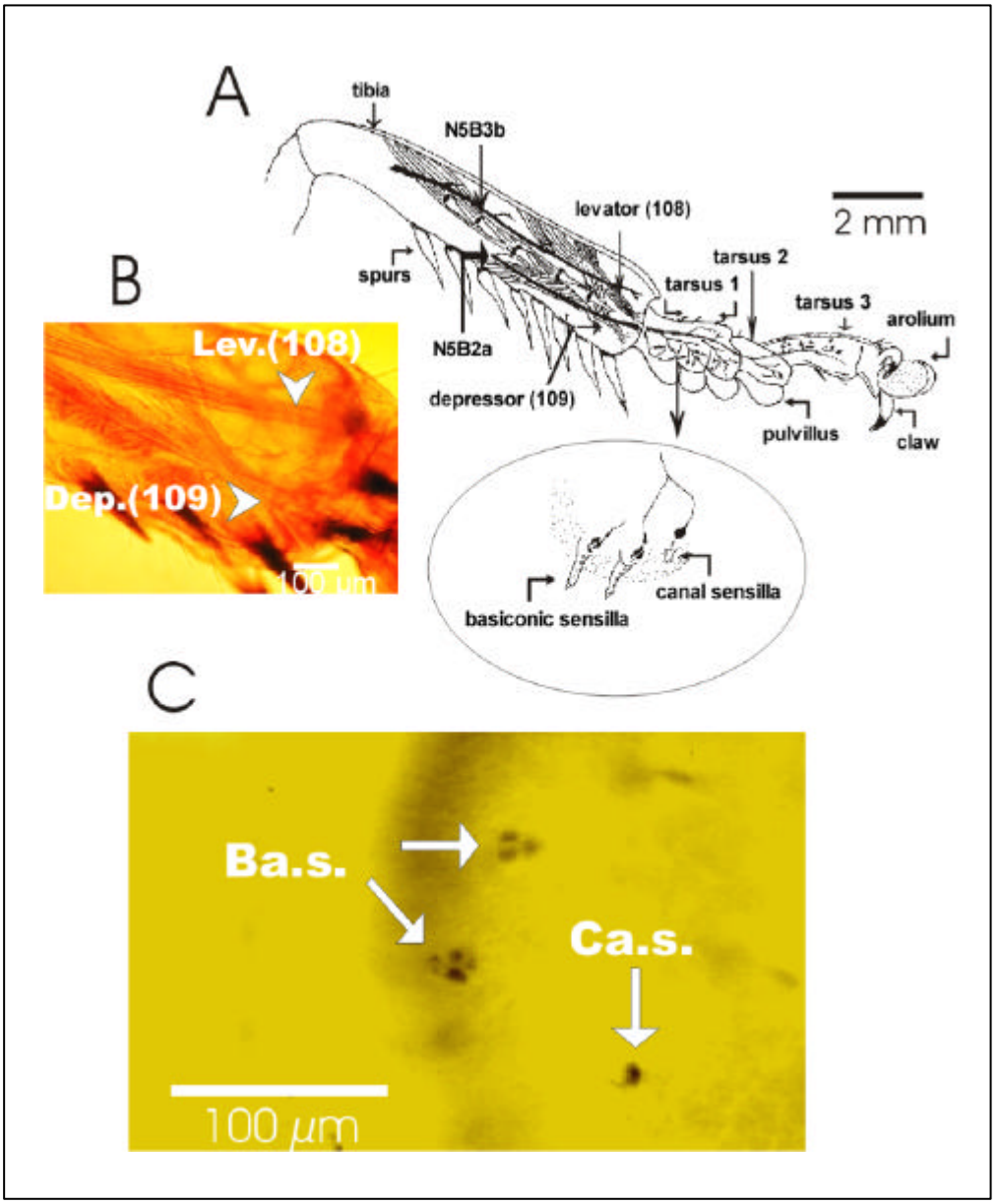


Fig. (9) (A) Overview of the mesothoracic tibio-tarsal region with the tarsal muscles and the sensory supply of the posterior tarsal segment. Inset : expanded view of a pulvillar sensillum. (B) Photographs of levator tarsi (108) and depressor tarsi (109). (C) Photographs of a peripheral backfill of the sensory nerve N5B2a with cobalt chloride in the mesothoracic ganglion. Left arrows indicate the four cell bodies of Ta1/pul.2 the pulvillar basiconic sensillum. The right arrow shows the large spindle-shaped cell body of one pulvillar canal sensillum.

The ovoid (spindle)-shaped cell bodies are about (4-5 μm) in diameter whereas the single mechanoreceptor neuron at the base of a canal sensillum is larger (6-8 μm) and terminates at the cuticular bottom of the canal that extends from the pulvillar surface.
The basiconic sensilla on the tarsus are supplied by groups of five deeply staining neurones that lie beneath each basiconic sensillum. Proximal to the somata each sensory neurone extends its axon, which joins with those from the other cells in a group to form a small nerve that finally enters one of the larger nerves leading to the mesothoracic ganglia.

3.3.1 Projections of sensory neurons innervating basiconic sensilla on pulvillus (tarsus):

Typical for all projections is that all five afferent axons from a pulvillar basiconic sensillum enter the neuropile in a close bundle *via* the root of the main leg nerve (nerve 5). The axons proceed medially at a ventro-median level. Branching occurs in a wide area of the ventral and lateral association center (IVAC and pLAC, Pflüger et al. 1988).

In the ipsilateral hemiganglion, afferent terminations extend in the neuropile from the level of the most lateral tracts to a line that would connect between the lateral halves of the anterior and posterior connective. The peripheral location of a basiconic sensillum more posterior or more anterior on the pulvillus seems to determine the rostro-caudal target area of projections in the neuropile: projections from a lateral (posterior) basiconic sensillum on a posterior pulvillus terminate more caudally in the neuropile, while afferents from a medial location (anterior on the same pulvillus) terminate more rostrally (Fig. 10).

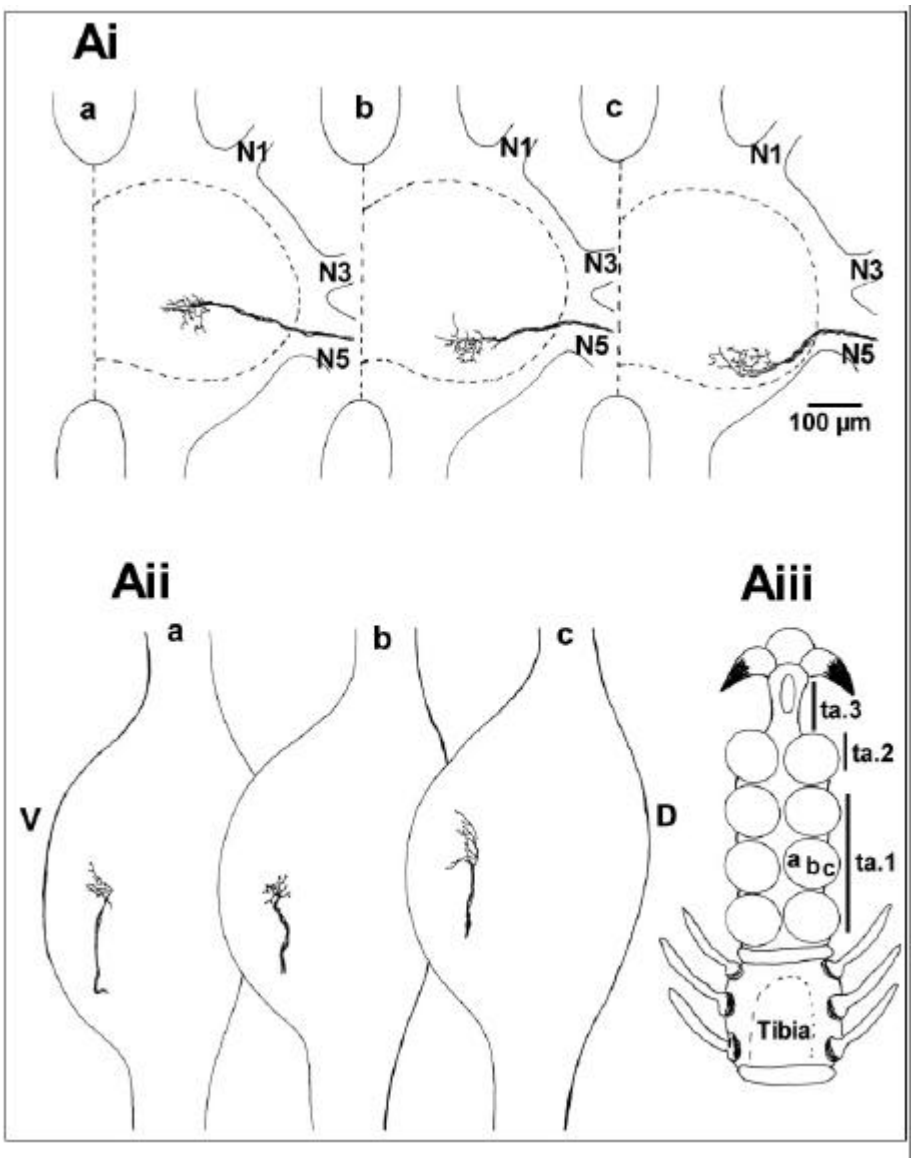


Fig. (10) Comparison of central projections from basiconic sensilla located on different sites on a tarsal pulvillus. (Ai) Central projections of pulvillar basiconic sensilla (a,b&c) on the posterior pulvillus of ta1 (see Aiii) branching in the ventral neuropile of mesothoracic ganglion. (Aii) Lateral view of the central projections of the pulvillar basiconic sensilla from the sites (a,b&c, seeAiii). (Aiii) The position (a,b,c) of the stained pulvillar basiconic sensilla on the ventral pulvilli of the first tarsomere are marked on the drawings. Pulvillar basiconic sensillum project always ventrally with 5 axons.

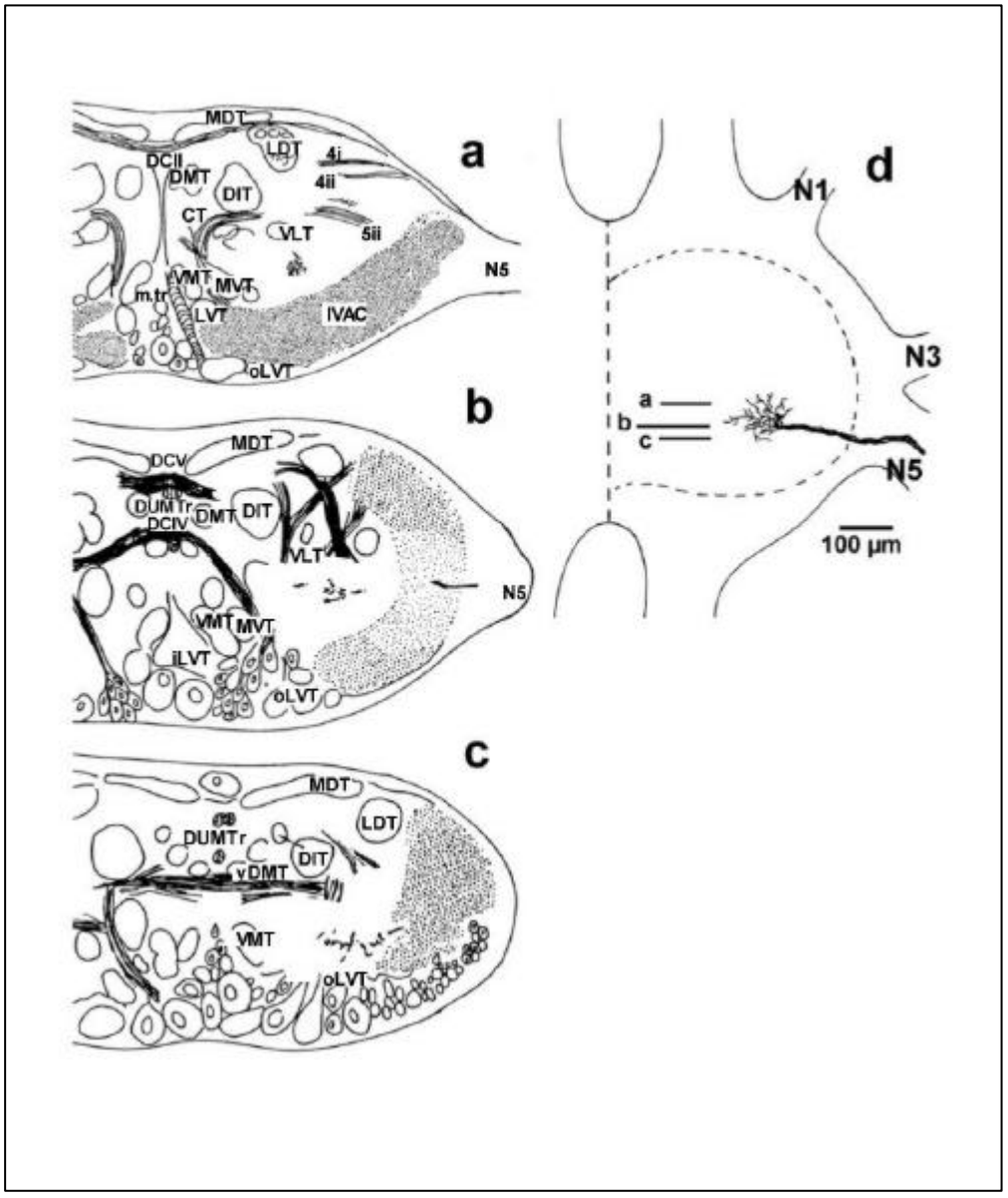


Fig. (11) Transverse 16 µm sections (a-c) through a mesothoracic ganglion at the levels shown in the wholemount of the central projection (d) of a pulvillar basiconic sensillum stained with neurobiotin (Tracts and nerve roots named according to Pflüger, et al. 1988).

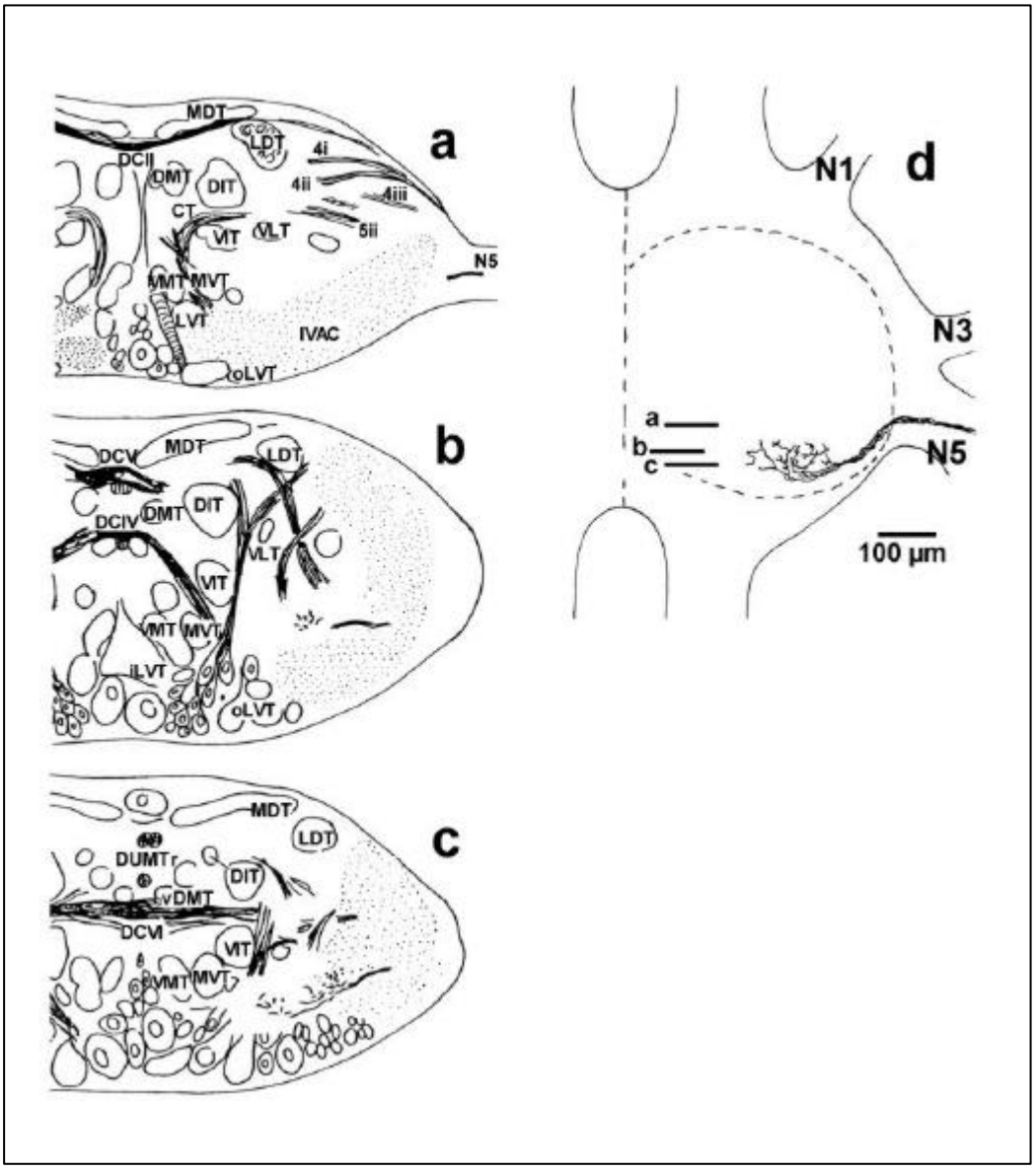


Fig. (12) Transverse 16 µm sections (a-c) through a mesothoracic ganglion at the levels shown in the wholemount of the central projection (d) of a pulvillar basiconic sensillum stained with neurobiotin (Tracts after Pflüger, et al. 1988).

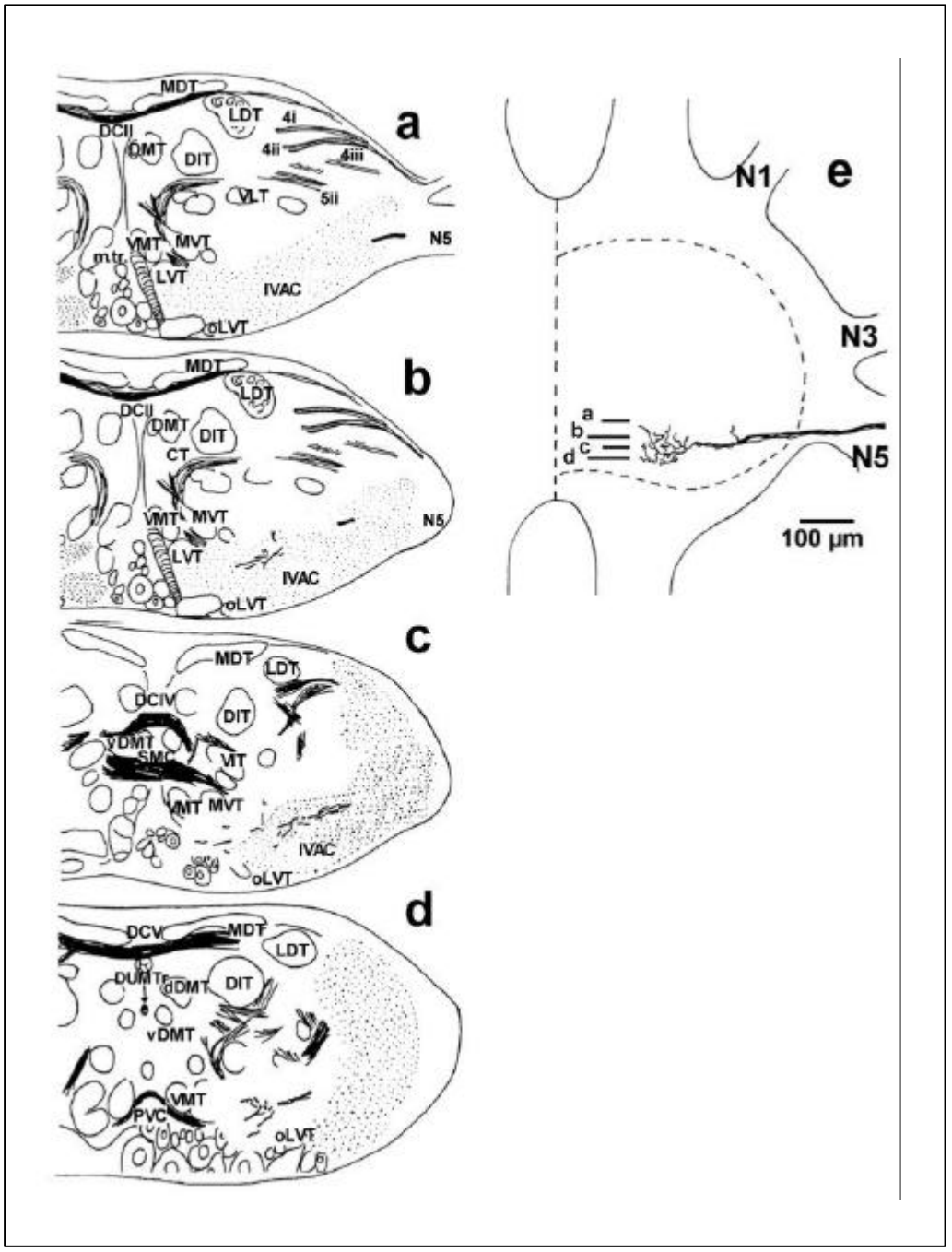


Fig. (13) Transverse 16 µm sections (a-d) through a mesothoracic ganglion at the levels shown in the wholemount of the central projection (e) of a pulvillar basiconic sensillum stained with neurobiotin.

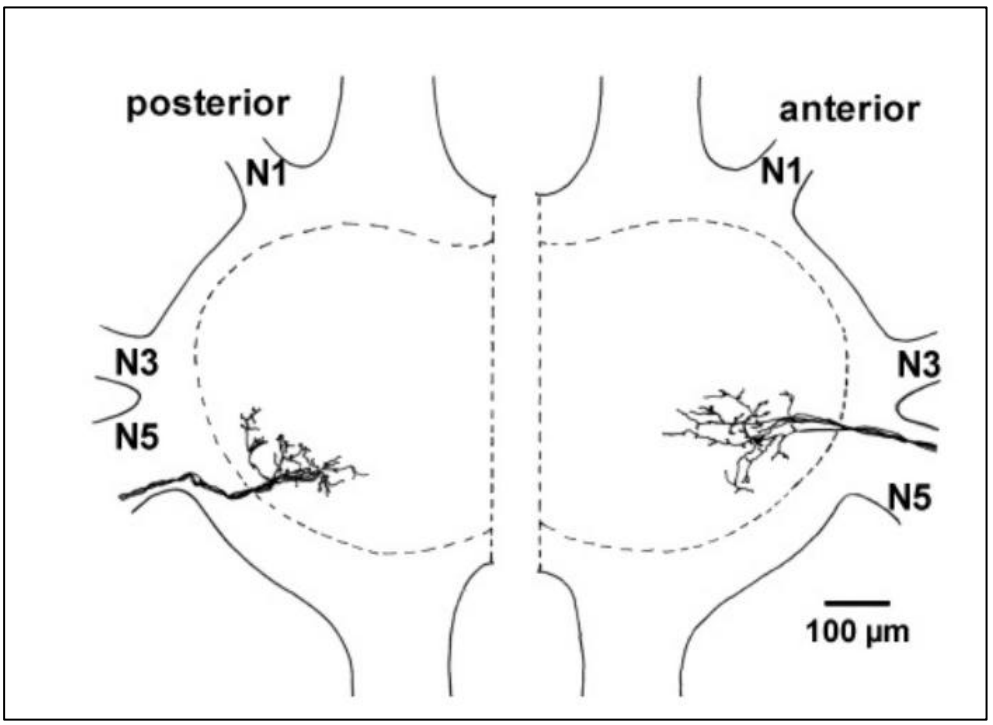


Fig. (14) Camera lucida drawings of the morphology of the central projections in the mesothoracic ganglion from individual pulvillar basiconic sensillum located on the anterior and posterior pulvillus Ta1 Pu2.

Afferents from a middle location on the pulviilus terminate in between (Fig. 10). In spite of these basic differences there can be considerable area for overlap between the most radial afferent branches of basiconic sensilla spaced apart on the tarsus.

All basiconic afferents from the posterior pulvillus project medio-ventrally in the IVAC and pLAC of the neuropile (Fig. 11-13). The comparison with afferents from the second anterior pulvillus of the first tarsomere shows a continuing the trend: they project more anteriorly than those of the posterior pulvillus projections (Fig. 14).

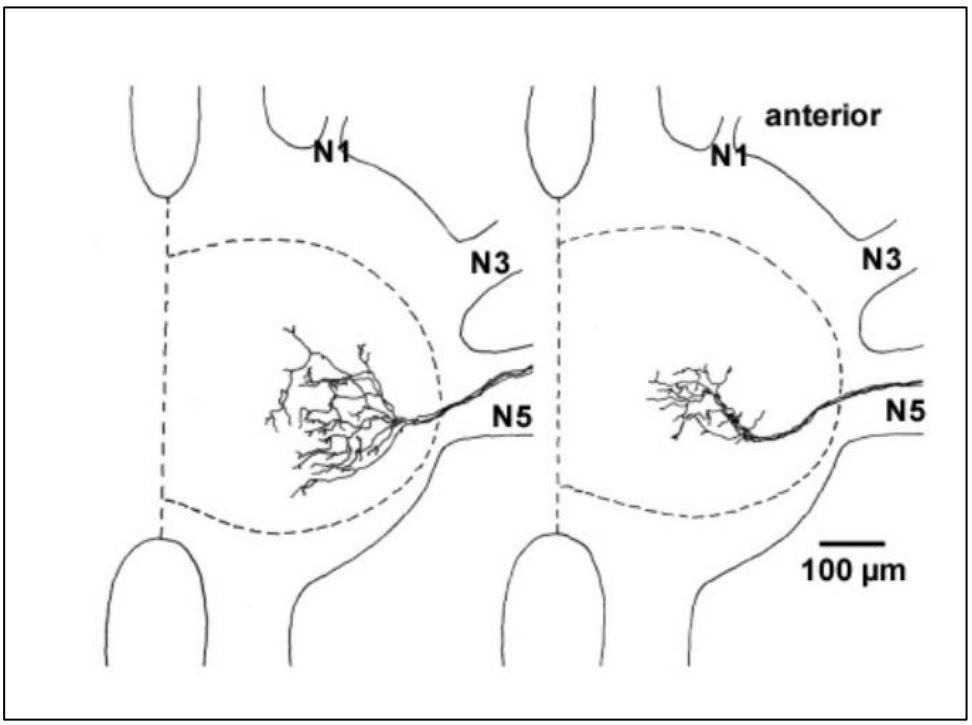


Fig. (15) Camera lucida drawings of the morphology of the central projections in the prothoracic ganglion from individual pulvillar basiconic sensillum located on the anterior pulvillus Ta1 Pu2.

Basiconic sensilla projections from the homologous pads on the prothoracic leg often show more extensive branching (Fig. 15), but basically follow the same topographic relations: location on the ventral tarsus surface corresponds to rostro -caudal central projections (Fig. 16,17).

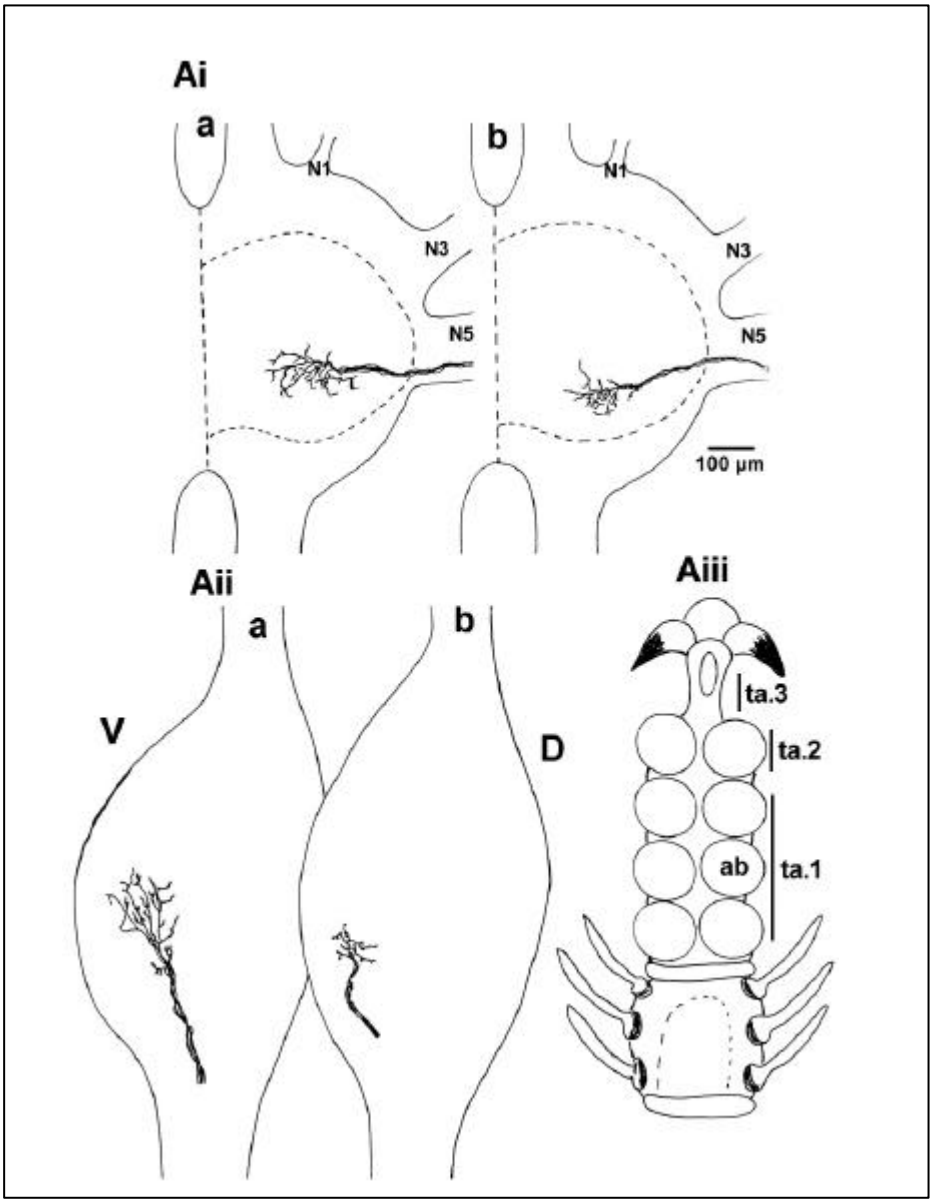


Fig. (16) Comparison of the morphology of the central projections in the prothoracic ganglion from individual pulvillar basiconic sensillum located on the posterior pulvillus Ta1 Pu2. (Ai) Central projections of pulvillar basiconic sensilla (a&b) on the posterior (ta.1 pul.2) (see Aiii) branching in the ventral neuropile of mesothoracic ganglion. (Aii) Lateral view of the central projections of the pulvillar basiconic sensilla from the sites (a&b) (see Aiii). (Aiii) The position of the pulvillar basiconic sensilla on the ventral pulvilli of the first tarsomere (ta.1 pul.2) are marked on the drawings. Pulvillar basiconic sensilla project always ventrally with 5 axons.

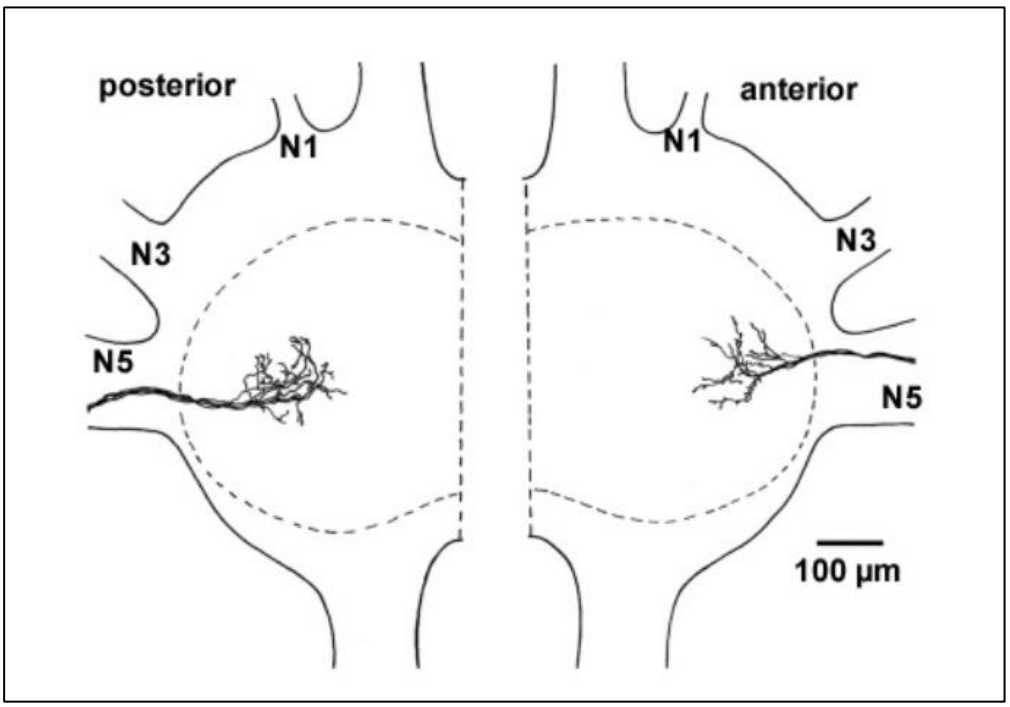


Fig. (17) Camera lucida drawings of the morphology of the central projections in the prothoracic ganglion from individual pulvillar basiconic sensilla located on the anterior and posterior pulvillus Ta1 Pu2.

3.3.2 Afferent projections from dorsal basiconic sensilla on the femur, tibia and tarsus:

The greatest spatial separation between central projections of sensory neurons from tactile hairs was for neurons from hairs on the distal three leg segments (the femur, tibia and tarsus) (Fig.18A). We therefore chose basiconic sensilla on the dorsal surface of these three distal leg segments to compare their central projections with those from tactile hairs along the proximo-distal axis of the leg.

Figure18 illustrates pulvillar contact chemoreceptors representative sensory projections from basiconic sensilla from each of these three regions of the leg. Four sensory neurons from a basiconic sensillum on the proximal dorsal femur entered the ganglion via nerve *5* and ran anteriorly and centrally terminating in an area of ventral neuropil midway between the anterior and posterior borders of neuropil, just lateral to a line drawn between the medial edges of the connectives (Fig. 18A).
Axons from a basiconic sensillum on the proximal dorsal tibia travelled towards the middle of the ganglion before turning slightly posteriorly and giving rise to numerous small branches in an area lateral to the central projections from the femoral basiconic sensillum (Fig. 18B). The central projections of sensory neurons from a basiconic sensillum on the dorsal tarsus projected more laterally still (Fig. 18C).

Thus, the positions of basiconic sensilla on the proximo-distal leg axis are represented by the positions of the arborizations of their sensory neurons along a medio-lateral axis in the ganglion (Fig. 18E), an organisation similar to that of the sensory neurons from tactile hairs. This mapping can be clearly seen in a preparation where sensory neurons from basiconic sensilla on both the femur and the tarsus were stained in the same preparation (Fig. 18D).

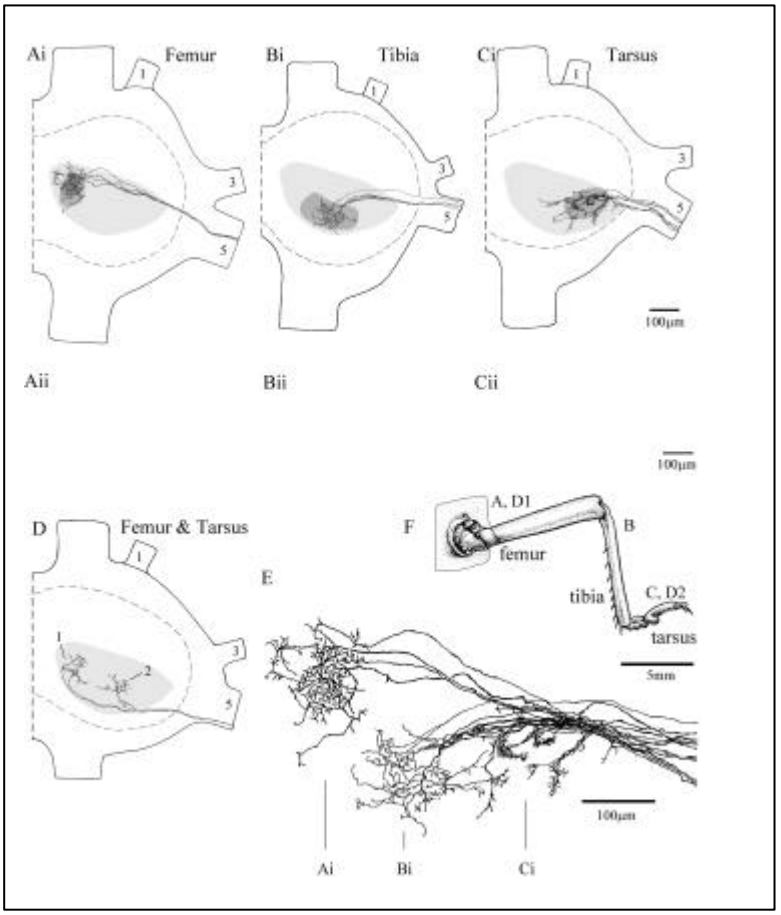


Fig. (18) Mapping of the central projections of sensory neurons from basiconic sensilla along the proximo-distal leg axis. **Ai, ii.** Two examples of the central projections from basiconic sensilla located on the proximo-dorsal femur. **Bi, ii.** Projections of sensory neurons from basiconic sensilla situated on the proximo-dorsal tibia. **Ci, ii.** Central projections from basiconic sensilla located on the dorsal tarsus. On the drawn ganglia the light stippling represents the area occupied by the sensory neurons from all the basiconic sensilla. The darker stippling indicates the projection areas of tactile hair afferents from similar proximo-distal locations of the middle leg taken from Figure 2. Note the close correlation between the branching areas of sensory neurons from both classes of receptor. **D.** Staining a basiconic sensillum on the femur and another on the tarsus in one animal shows a clear separation in projection areas within the ventral neuropil. **E.** Superimposing the drawings of sensory neurons from different areas on the leg (**F**) shows the central projection from basiconic sensilla map according to the spatial position of their corresponding receptor on the proximo-distal axis of the leg.

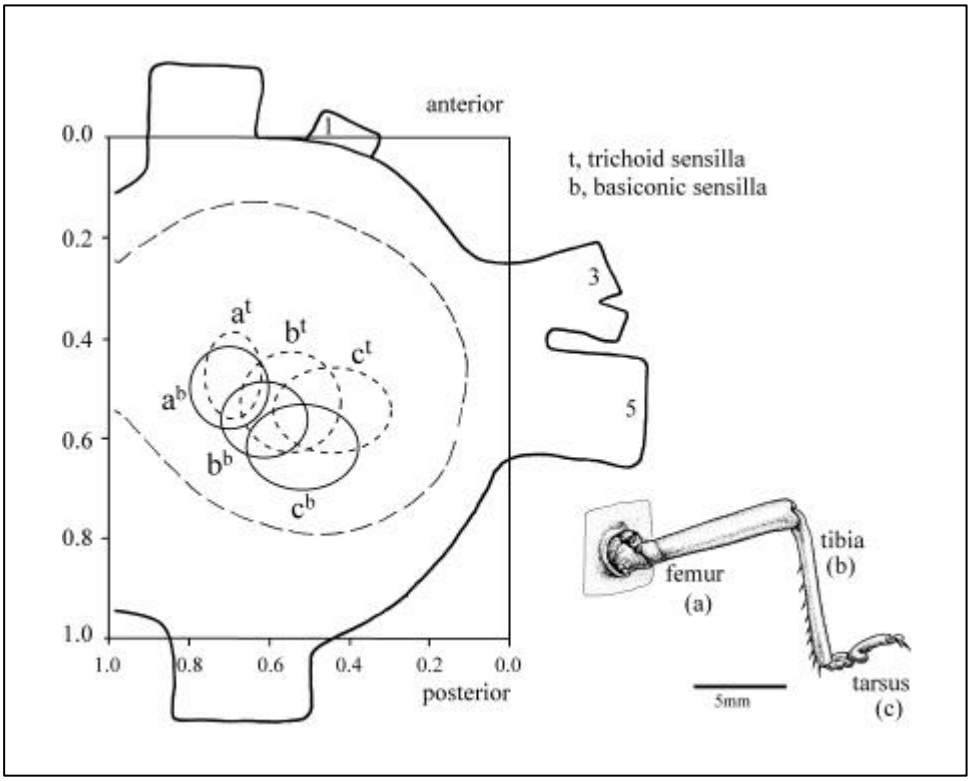


Fig. (19) Average positions of the most anterior, posterior, medial and lateral extents of the arborizations from basiconic (solid lines) and trichoid sensilla (dotted lines) from the femur (a), tibia (b) and tarsus (c) as shown by ellipses connecting each of the four points. Each position was calculated as the ratio of the distance from the anterior or lateral edges of the ganglion (0 on the axes of the Figure) to the extremities of the projections relative to the total length or maximum width of the hemi-ganglion. Average positions were calculated from 46 femoral, 34 tibial and 10 tarsal basiconic sensilla and 10 femoral, 9 tibial and 9 tarsal trichoid sensilla. t, trichoid sensilla; b, basiconic sensilla.

Two clear projection sites were evident, one that overlaps with the area where femoral basiconic sensory neurons project (Fig. 18A), and another that overlaps with the area to which tarsal basiconic sensory neurons project (Fig. 18C).

3.3.2.1 Organisation of sensory afferents from basiconic sensilla

The total area occupied by the arborizations of all the sensory neurons from individual basiconic sensilla was similar to the area occupied by those of the single neurons from tactile hairs (Fig.19). Moreover, the total arborization area of the projections from individual basiconic sensilla was not significantly correlated with the number of axons staining in nerve 5 (area calculated by multiplying arborization length by width ratios as described above, Spearman's coefficient =0.198, P >0.05, n =74).

The projection patterns of 19 basiconic sensilla on the dorsal tibia were analysed in detail to determine if there were any clear differences in the projection areas of the different neurons that might indicate separate destinations for neurons with different modalities or sensitivities (Fig.20). The numbers of sensory neurons within the basiconic sensilla on the leg has not been systematically investigated, although five neurons, one of which is mechanosensory has been reported for some leg sensilla in locusts (Chapman 1982). Conversely, numbers of sensory neurons within palp-dome gustatory sensilla are known to be variable (Blaney *et al.* 1971). Therefore, the variability in the number of axons staining in the mesothoracic ganglion may reflect genuine differences in the sensory neuron complement of basiconic sensilla as well as experimental artefacts. Over 83% of successful stains from basiconic sensilla on the tibia consisted of 3 or more axons entering the ganglion with 44% of stains consisting of 5 or 6 neurons, the maximum number stained. As with the sensory neurons from tactile hairs, axons from basiconic sensilla entering the ganglion took a number of routes to their destination. In 7 of the 19 analysed projections all the stained axons travelled in a narrow bundle and took a path anterior and medial of nerve 5 before curving back and beginning arborize (Fig. 20A i-iii, D). In other projections, the axons travelled in a more widely spaced diffuse bundle across the ganglion (Fig. 20B i - iii), with some axons taking

anterior paths and others travelling more directly to their destination. In a further 5 preparations the axon paths through the ganglion were widely divergent with at least one axon travelling around the posterior edge of the neuropil before turning towards the anterior and branching (Fig. 20C i-iii, E). There were no instances of stains consisting of more than one axon that exclusively took this posterior route. The variety of paths would seem to indicate genuine differences between sensilla rather a variety of partial stains as there was no difference in the mean number of axons in stains where the neurons ran directly to their destination compared to stains where axons took both anterior and posterior paths (Mann-Whitney test, Z=-0.97, P=0.945, n=29, range in axon numbers 1-6 in both types). Further to this, there was no indication of a consistent numerical differentiation between the number of neurons taking anterior and posterior paths that might suggest a modality linked difference in route (Fig. 20C). Neither was there any clear indication of the consistent presence of axons with different diameters that could correlate with the presence of a single mechanosensory and several chemosensory neurons, such as have been reported for the sensory projections from bimodal gustatory sensilla of Diptera (Edgecomb and Murdoch, 1992).

The arborizations of stains from basiconic sensilla on the tibia were further examined to determine whether there were any readily apparent spatial subdivisions between the branching patterns of different neurons within the 'tibial region' that could be related to differences in modality or chemical sensitivity. A common arborization pattern, particularly associated with stains in which the axons travelled closely together was for the neurons to arborize extensively in two separate regions linked by a narrow connection (Fig. 20A i). Although there was one instance in which the distalmost arbor was clearly composed of branches from a single neuron (Fig. 20 A iii), in all other stains both regions consisted of branches from two or more neurons

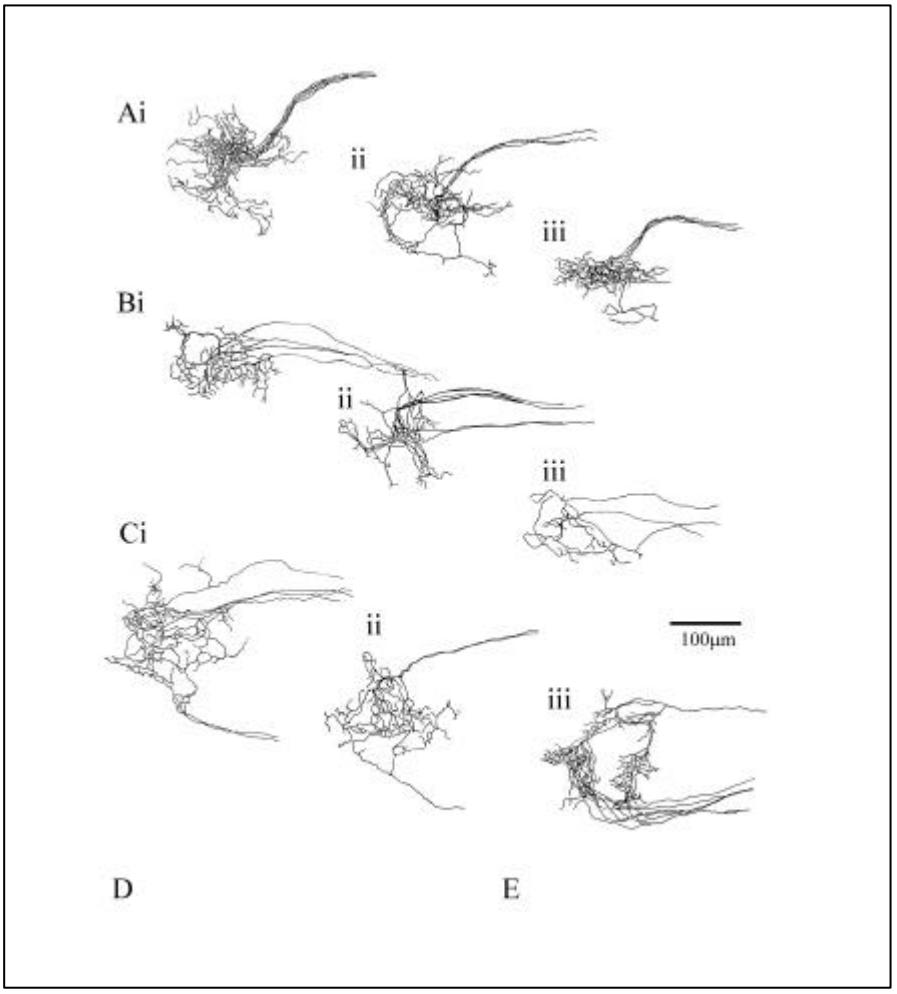


Fig. (20) Axonal projections of basiconic sensilla. Sensory afferents from basiconic sensilla travel across the ganglion and arborize in a number of different ways, but there is no consistent observable spatial separation of neurons into different regions consistent with differences in modality. Projections from single basiconic sensilla on the dorsal tibia. **Ai, ii, iii.** Three examples of projections in which the axons run in a narrow bundle anterior of nerve 5 before turning towards the posterior and arborizing in two distinct zones connected by a narrow waist. **Bi, ii, iii.** Three examples of sensory projections in which the axons travelled in a diffuse bundle across the ventral neuropil before arborizing in a variety of forms. **Ci, ii, iii.** Three examples of sensory projections from single sensilla in which some axons ran around the posterior edge of the neuropil and others travelled more directly to their arborization region. For each drawing anterior is to the top and the midline of the ganglion is to the left. The photographs show two further examples of sensory projections in which (**D**) the neurones travel together in a narrow bundle or (**E**) take divergent routes to their arborization region.

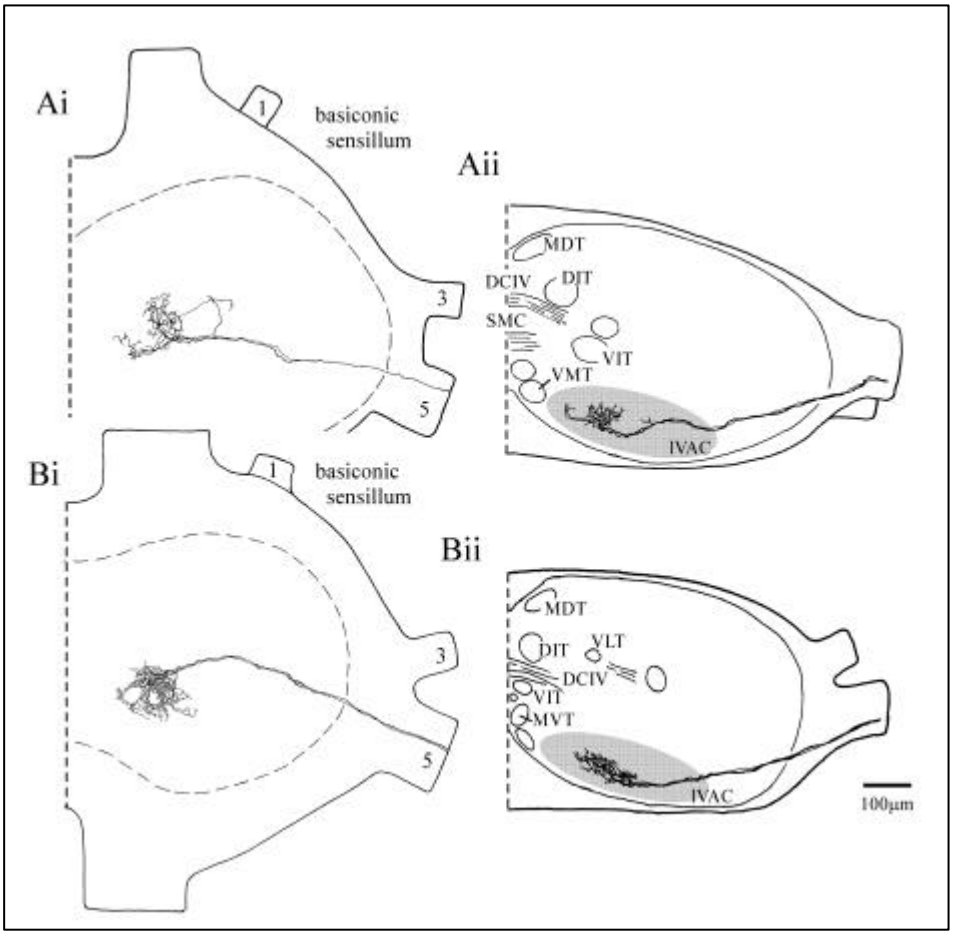


Fig. (21) Sensory afferents from the basiconic sensilla project to the same part of the ventral association centre. (A) Sensory neurons from a basiconic sensillum on the dorsal tibia, shown in wholemount (i) and in section (ii). (B) Sensory afferents from a basiconic sensillum on the dorsal femur, shown in wholemount (i) and in section (ii). There was no apparent dorso-ventral separation of basiconic sensilla afferents consistent with any putative differences in modality. The lateral ventral association centre (lVAC) is shown in grey.

(Fig. 20 A i and ii, D). In preparations where axons approached their arborization area from different directions, some of the neurons commonly bifurcated and travelled some distance further before giving rise to their main mass of branching, resulting in some cases in spatially separate branching regions (Fig. 20C ii-iii).

Each region, however, received branches from more than one neuron; there were no instances of exclusive regions composed of arbors from single neurons only. Therefore although there are a number of different branching patterns, which may give rise to spatially separate areas of arborization, there is no observable evidence that these zones are exclusively comprised of branches from individual neurons. Consequently there is no evidence to support a modality or sensitivity dependent spatial separation of neurons across the ganglion.

Several ganglia containing stains of basiconic sensilla afferents were drawn in thick transverse sections to determine whether there could be any dorso-ventral partitioning of sensory afferents consistent with modality.

Two features of the central projections of basiconic sensilia sensory neurons were apparent in the transverse sections (Fig. 21 B-C ii). First, the arborizations of all the sensory neurons occur within the same region of the IVAC as that occupied by tactile hairs afferents from similar locations on the leg. Second, the branches of all the sensory neurons were intermeshed. There was no clearly observable separation between different neurons consistent with the existence of spatially separate neuropil regions for processing the different modalities. All basiconic sensillum afferents branched within a restricted dorso-ventral region within the IVAC regardless of the route the axons took across the ganglion to reach their arborization site.

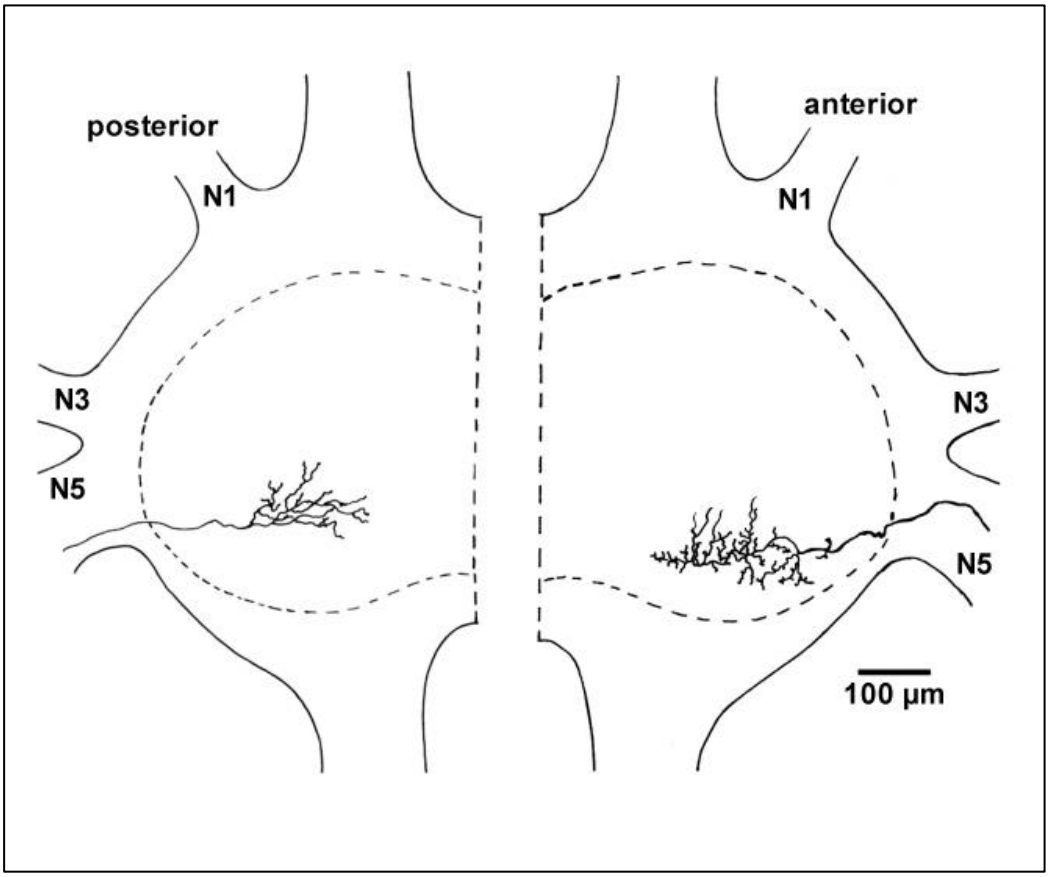


Fig. (22) Camera lucida drawings of the morphology of the central projections in the mesothoracic ganglion from individual pulvillar canal sensillum located on the anterior and posterior pulvillus Ta1 Pu2.

3.3.3 Canal sensilla:

For comparison with the adjacent basiconic contact chemoreceptor the projections of canal sensilla, the only other type of sensilla on the pulvilli were stained.

The single neuron at the base of each canal sensillum with an average diameter of 6-8 μm has an afferent axon of 1-2 μm and resembles campaniform sensilla. Central projections of tarsal campaniform sensilla rarely show the very lateral bifurcation of the incoming axon in the neuropile that is seen in most projections of campaniform sensilla from more proximal leg segments but they extend to the level of the lateral halves of the connectives (Laurent and Hustert 1988). They show narrow and slender projection areas. Staining of canal sensilla afferents from an anterior and a posterior pulvillus in the same segment shows a segregation of their projection areas in more anterior neuropile region for the anterior pulvillus and more posterior neuropile region for the posterior pulvillus in the mesothorax (Fig. 22).

This apparently does not hold for canal sensilla located centrally on a pulvillus in the prothorax, probably due to the fact that projections of prothoracic afferents tend to be more extensive in as compared to homologlus mesothoracic afferents (Fig. 23). The dorsoventral level of the projections in the neuropile (Fig. 24). For all canal sensillum afferents from the posterior pulvillus project medio–dorsally in the (pLAC) of the neuropile. In addition canal sensillum projections from the homologous pads on the prothoracic leg often show more extensive branching in comparison to those of the mesothoracic leg.

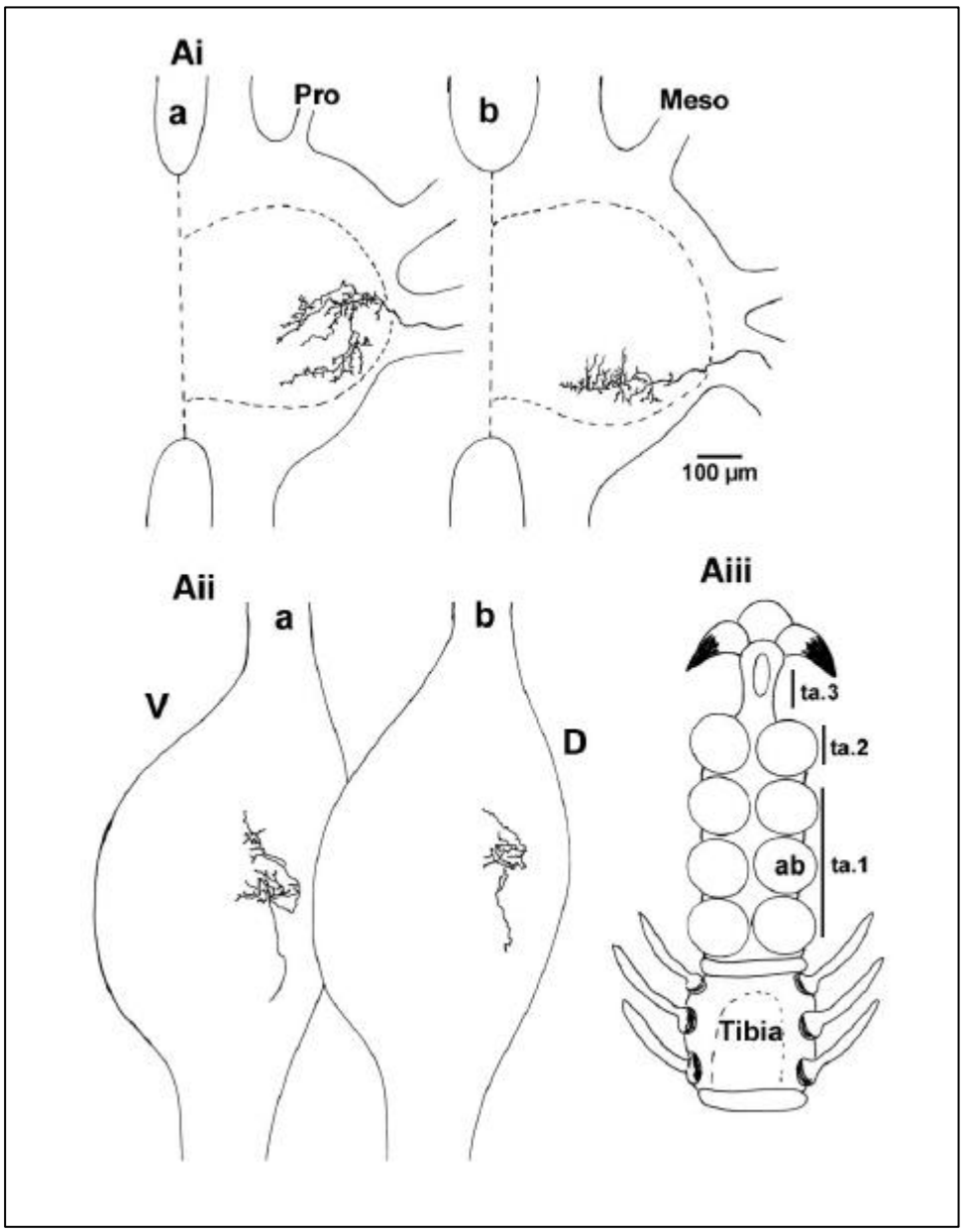


Fig. (23) Comparison of central projections from canal sensilla located on posterior sites of the tarsal pulvillus on pro- and mesothoracic legs. (Ai) central projections of pulvillar canal sensilla on prothoracic ganglion (a) & on mesothoracic ganglion (b) on the (ta.1 pul.2) (see Aiii). (Aii) lateral view of the central projections of the canal sensillum from the site a&b (see Aiii). (Aiii) the position of the pulvillar canal sensilla on the ventral pulvilli of the first tarsomere (ta.1 pul.2) are marked on the drawings.

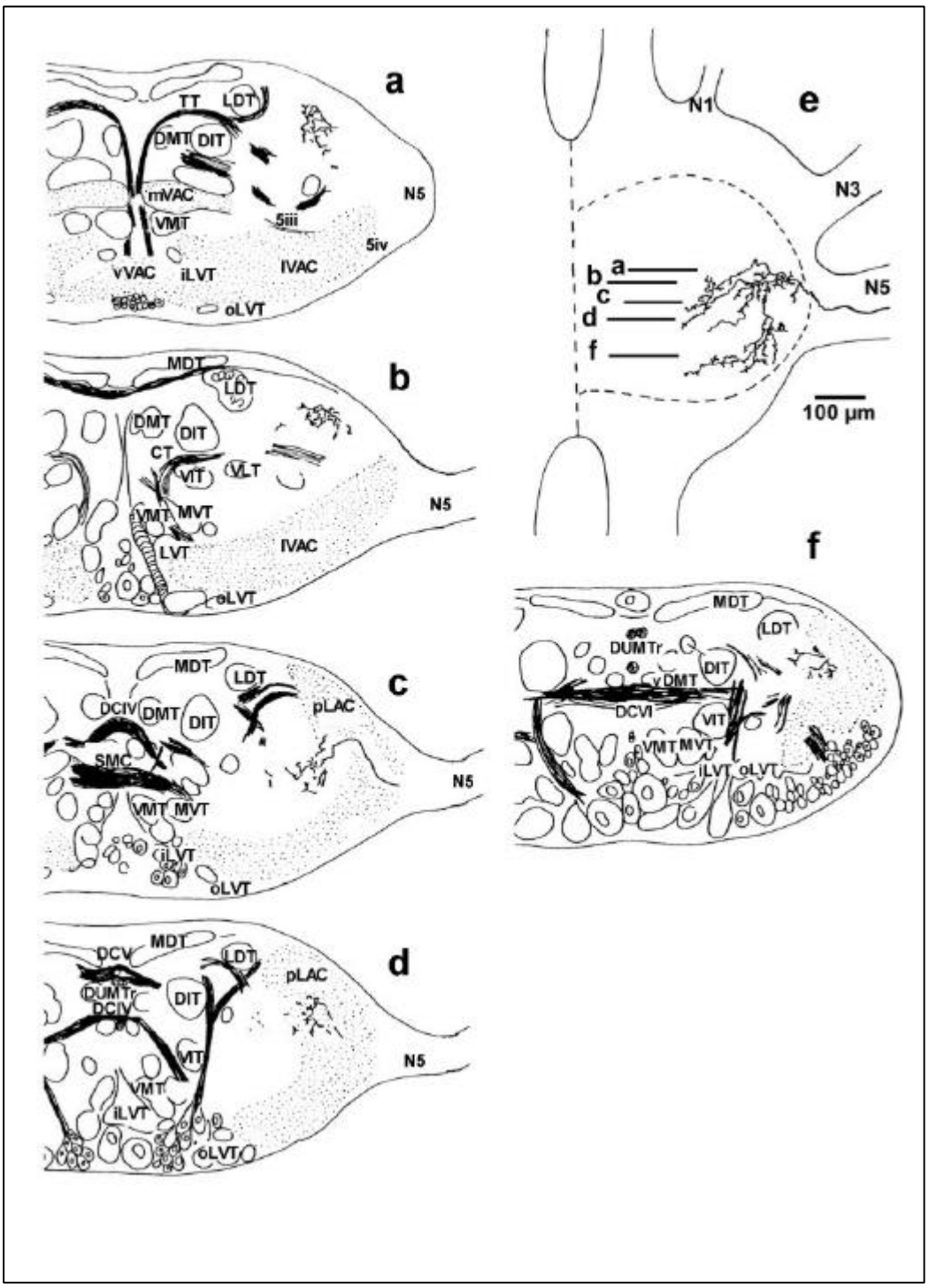


Fig. (24) Transverse 16 µm sections (a-d and f) through a mesothoracic ganglion at the levels shown in the wholemount of the central projection (e) of a pulvillar canal sensillum stained with neurobiotin.

3.3.4 Dorsal hairs

Backfills, using neurobiotin, resulted in the central projections from one sensory neuron only being stained using this method.

Axon entered the mesothoracic ganglion through nerve 5 and projected to an area just lateral to a line drawn between the lateral edges of the anterior and posterior connectives. Central projections of tarsal mechanoreceptors (trichoid sensilla) show narrow and slender projection areas.

Mechanosensory hair afferents from pro-and mesothoracic legs show a segregation of areas in more anterior neuropile region for the fore-legs in prothoracic ganglia, the projections of prothoracic afferents tend to be more extensive as compared to homologous mesothoracic afferents (Fig. 25). The projections in the neuropile for mechanoreceptor sensilla afferents from the prothorathic leg extend medio-dorsally in aLAC of the neuropile. (Fig. 26).

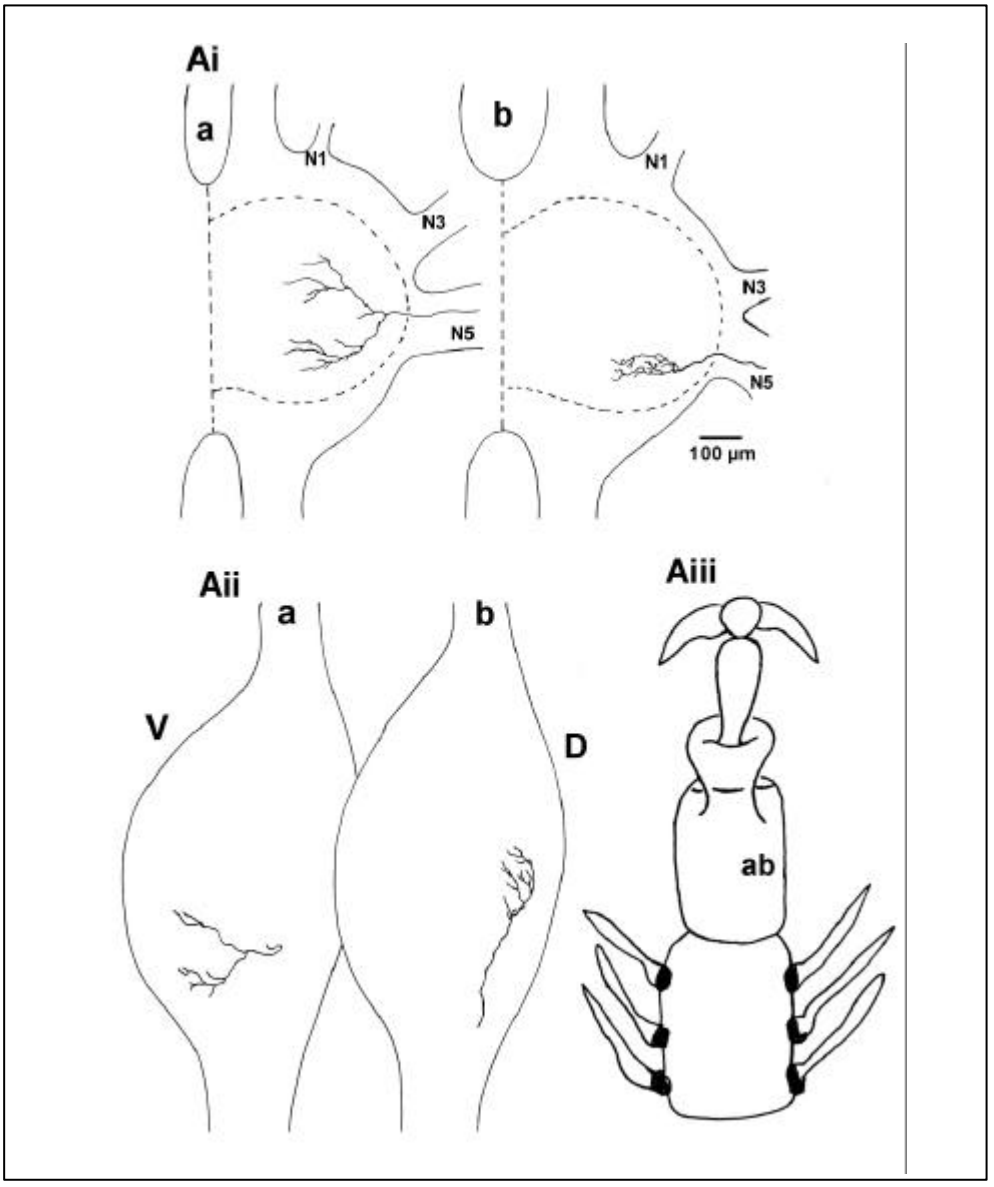


Fig. (25) Comparison of central projections from the trichoid sensilla located on posterior sites of the dorsal tarsus on pro-mesothoracic legs. (Ai) Central projections of the trichoid sensilla in the prothoracic ganglion (a) in the mesothoracic ganglion (b) of the first tarsomere (ta.1) (see Aiii). (Aii) Lateral view of the central projections of the trichoid sensilla from the site a&b (see Aiii). (Aiii) The position of the trichoid sensilla on the dorsal tarsus of the first tarsomere (ta.1) are marked on the drawings.

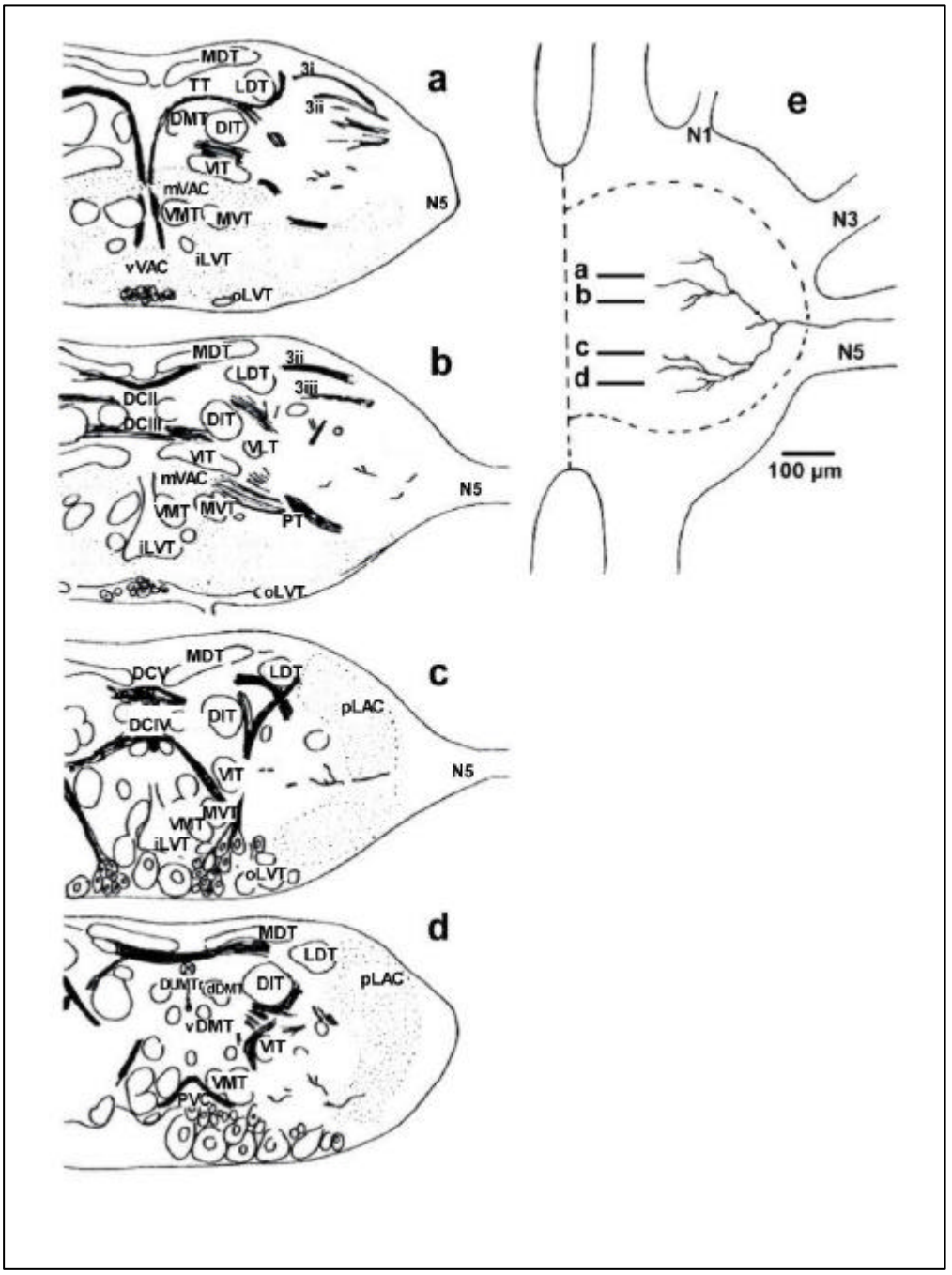


Fig. (26) Transverse 16 µm sections (a-d) through a prothoracic ganglion at the levels shown in the wholemount of the central projection (e) of a dorsal trichoid sensillum stained with neurobiotin.

3.4 Neuronal pathways producing the avoidance reflex

The information from the numerous contact chemoreceptors and mechanoreceptors on the body surface of an insect is usually processed by local and projection interneurons before it is passed to the motor neurons to affect a change in movement. This section was aimed to study the antagonistic effect on depressor and levator tarsi elicited by chemical stimuli on tarsal pulvilli. The objective here was thus, to study the role of the pulvillar basiconic sensilla in shaping the patterns of leg muscle (depressor & levator tarsi) activity of the locust during contact with specific chemical stimulant. Contact chemoreceptive signals from the sensory neurons to the leg motor neurons were studied using intracellular recording and evaluated the contribution of the receptive fields so defined by extracellular recording.

3.4.1 Motor responses to afferent input from the pulvillar basiconic sensilla:

Tarsal motor neurons were revealed in the mesothoracic ganglion by backfilling (neurobiocytin) from their terminal nerve branches near the individual muscle in the periphery. Backfilling of the branches of the nerve (N5B3b) that runs into the levator or depressor tarsi, revealed that the depressor tarsi is supplied by 5-7 motor neurons.

The levator tarsi is supplied by 1 motor neuron. All the depressor motor neurones have a similar shape, with individual neurones differing in the details of their branching, but not in a way that allowed unequivocal characterisation of individual neurones on anatomical criteria alone.

The pool of depressor tarsi motor neurones can be further subdivided into three groups (anterior, lateral and posterior) each containing 2-3 neurones although, in practice, variation in position precludes the use of this feature as a reliable indicator of identity.

Within the whole pool are slow motor neurones, which are often tonically active, fast units which are active more phasically, and intermediate units with mixed features. The somata of depressor tarsi in prothoracic ganglion lie just below the surface of the ganglia, in contrast to the somata of the depressor tarsi in mesothoracic ganglion that lies deeply below the peripheral surface (Fig.27). Transverse sections show that the branches of the mesothoracic depressor tarsi are restricted to the lateral part of the dorsal and intermediate neuropil (Fig. 28).

Pulvillar basiconica sensilla are sensitive to all used chemical substances at different degrees. The spiking responses increased with higher concentrations but with increasing bursting tendency (except after application of sugars) at higher concentration. The adaptation was rapid with lower concentrations applied to pulvillar basiconica sensilla.

When a new sensillum was stimulated with a low concentration a clear response was observed. However, when a sensillum was first examined after applying high concentrations, a following low concentration elicited less response. Motor responses to chemical stimuli were different in the stimulated and neighbouring legs. The antagonism between depressor and levator muscles by exctracellular recordings was tested with suction electrodes or metal hook electrodes.

3.4.2 Antagonism between depressor and levator tarsi (exctracellular recording)

During walking, the depressor tarsi is active during the stance phase and silent during the swing phase. The levator muscle is, by contrast, mainly active during the swing phase and inhibited at the beginning of the stance phase (Laurent and Hustert, 1988).

Stimulation of single pulvillar basiconica sensilla by sugars [(100-1000 mM glucose) (100-1000 mM sucrose)] can evoke a inhibition in the depressor tarsi motor neurons, while the levator muscle is inhibited with a delay (Fig. 29

i, ii). The levator muscle was exited more by the stimulation with glucose than with sucrose while the depressor was depolarized similarly by both sugars.

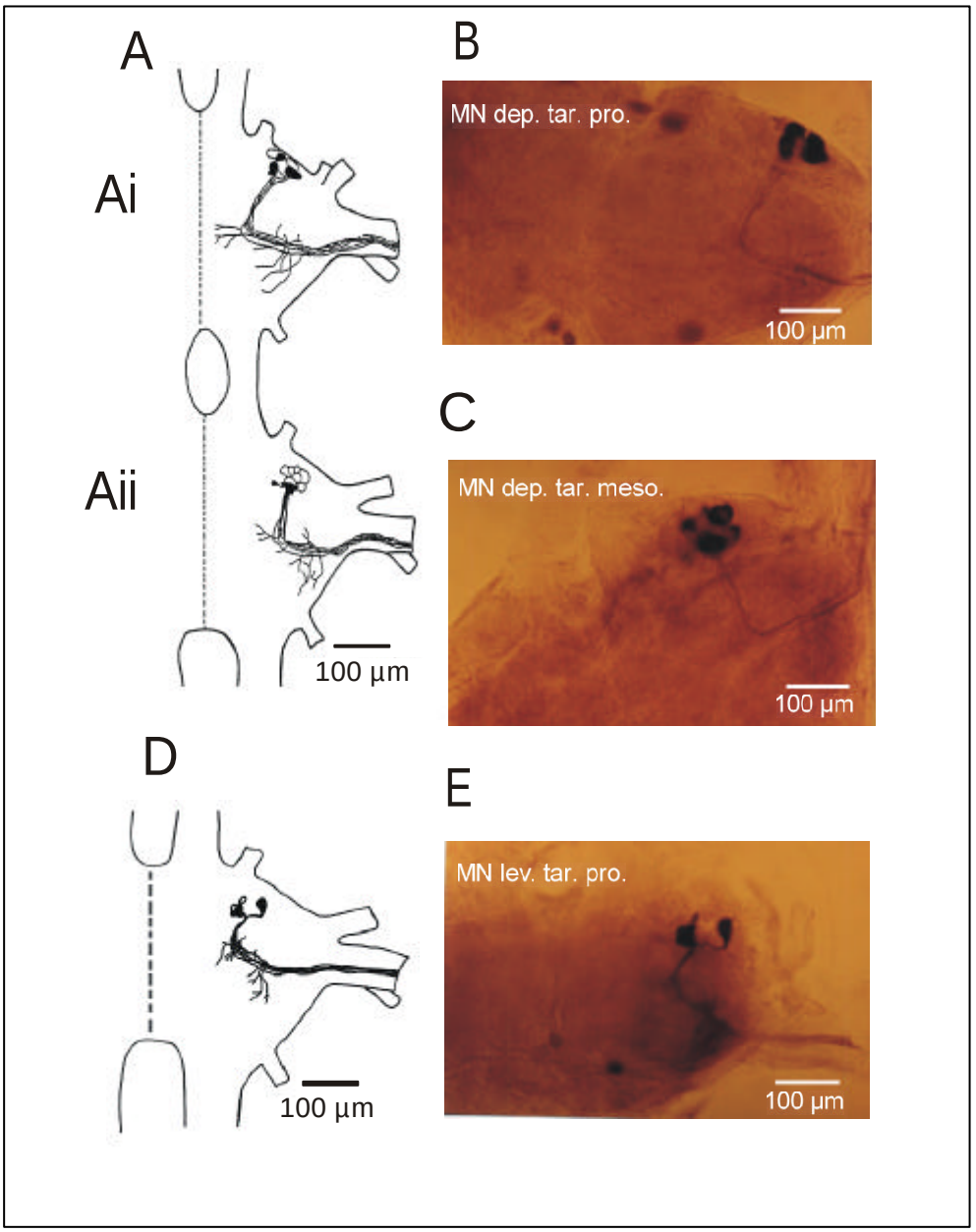


Fig. (27) Tarsal motor neurones stained with neurobiotin and viewed dorsally in whole mounts. (A) Backfill of nerve N5B3b revealing depressor tarsi motor neurones. (Ai) in the prothoracic ganglion , (Aii) in the mesothoracic ganglion. (B) Photographs of prothoracic depressor tarsi motoneurons backfills by neurobiotin from N5B3b (C) Photographs of the mesothoracic depressor tarsi motor neurones backfilled by neurobiotin from N5B3b. (D) Backfill of nerve N5B3b revealing levator tarsi motor neurones in the mesothoracic ganglion. (E) Photographs of mesothoracic levator tarsi motor neurones backfilled by neurobiotin in N5B3b.

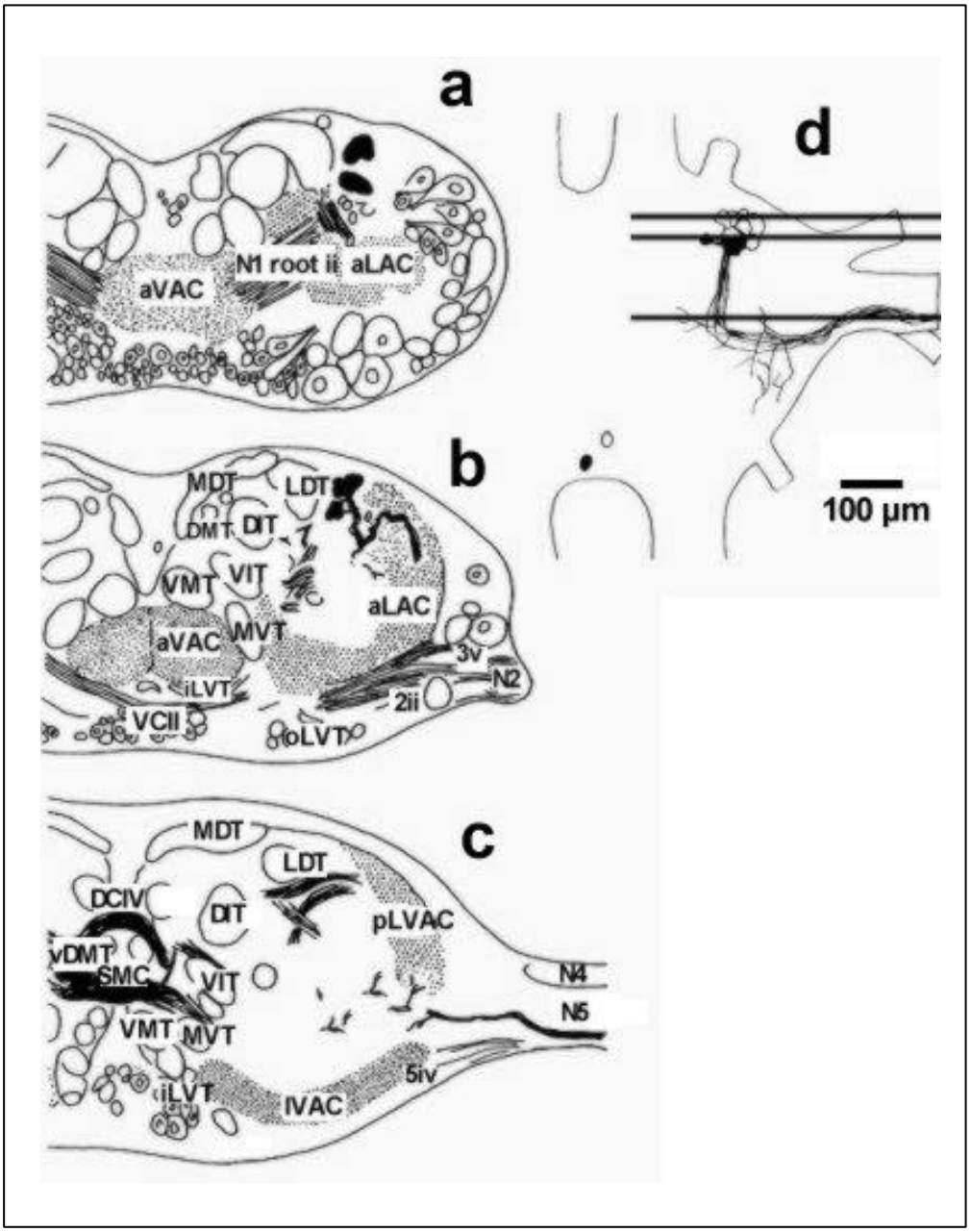


Fig. (28) Transverse 16 µm sections (a-c) through a mesothoracic ganglion at the levels shown in the wholemount of the central projection (d) of a depressor tarsi motor neurones stained with neurobiotin.

Bursting was observed in afferents from pulvillar basiconica sensilla afferents, whenever low concentrations of sugars were used.

Stimulation of pulvillar basiconica sensilla by (10mM NHT) first caused antagonistic discharges of depressor and levator tarsi motor neurons (Fig. 30). The levator motor neuron showed increased activity (depolarized stronger) directly after stimulation by (NHT)(phasic responses), followed by decreased muscular activity. In contrast, the depressor activity decreased directly after stimulation, followed by increased discharges, then followed by an adaptation.

Responses to citric acid varied in amplitude and duration of depolarization to motor neurons varied with different concentrations. At low concentrations (10-25 mM citric acid) (Fig. 31 i), the levator tarsi motor neuron was always excited after stimulation followed by adaptation (tonic responses). In contrast depressor tarsi motor neurons were inhibited by basiconic afferents later as seem (tonic responses).

At high stimulus concentrations (100-250 mM citric acid) (Fig. 31 ii), the depressor tarsi motor neurons were excited strongly through basiconic afferents. In contrast the levator tarsi motor neuron is inhibited first after stimulation, followed by increasing activity followed by excitation. The duration of excitation when using high concentration of citric acid was longer than the duration after application of low concentrations. In addition there are bursting responses in the depressor motor neuron (Fig. 31 ii).
Stimulation of pulvillar basiconica sensilla with different concentrations of NaCl resulted always in excitation of the levator motor neuron directly after stimulation (phasic response), in contrast, the depressor motor neurones were inhibited directly after stimulation.

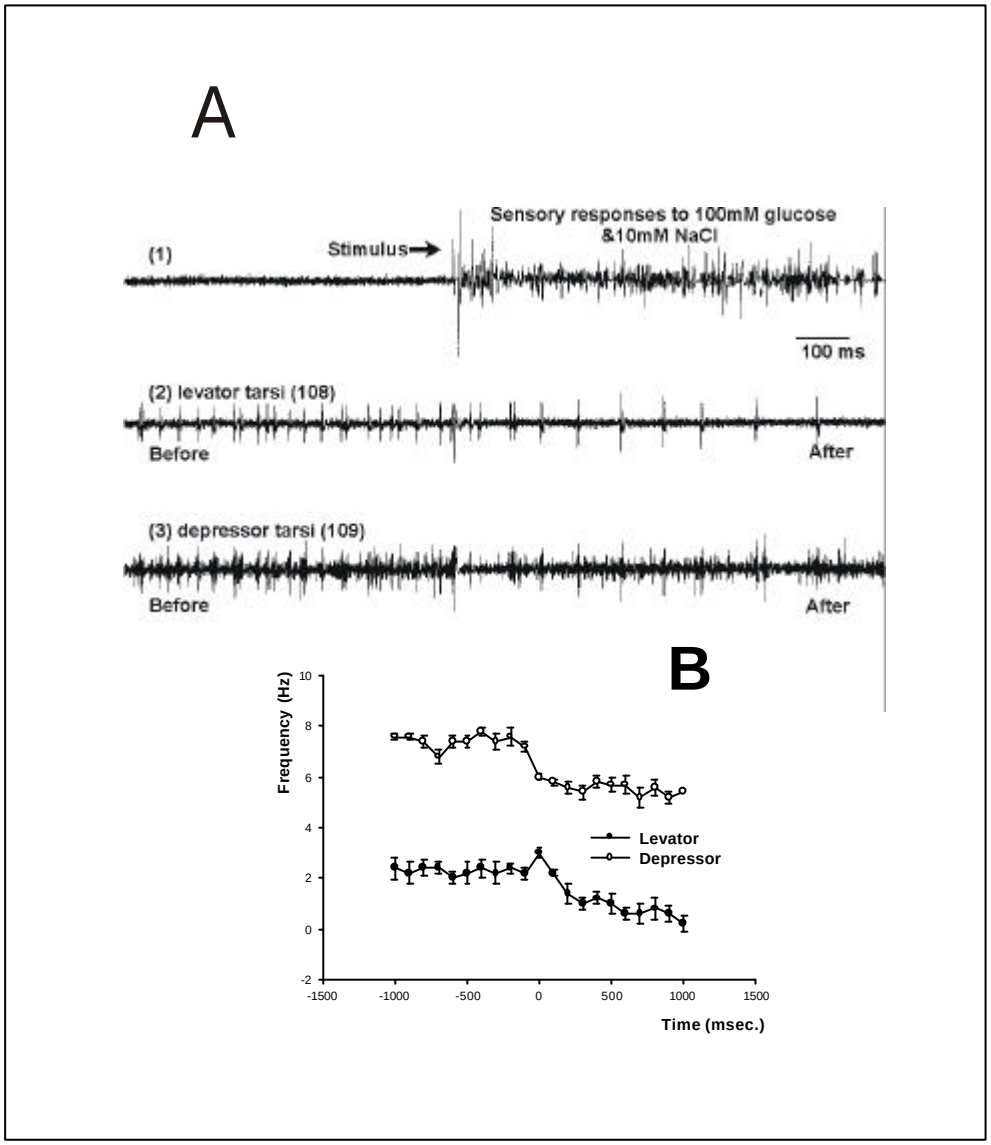


Fig. (29 i and ii) Responses from a single of pulvillar basiconic sensillum (trace 1) and resulting motor responses (traces 2 and 3). (A) Response to 100 mM glucose and 10 mM NaCl. (C) Response to 1 M glucose and 10 mM NaCl. (B) Response frequency of motor units from two antagonistic tarsal muscles (depressor and levator), the stimulus 100 mM glucose was applied at time zero. (D) Response frequency of motor units from two antagonistic tarsal muscles (depressor and levator), the stimulus 1M glucose was applied at time zero. Frequency measured as spikes per 100 msec. Each point represents the mean and ± SE (standard deviation) of 10 trials.

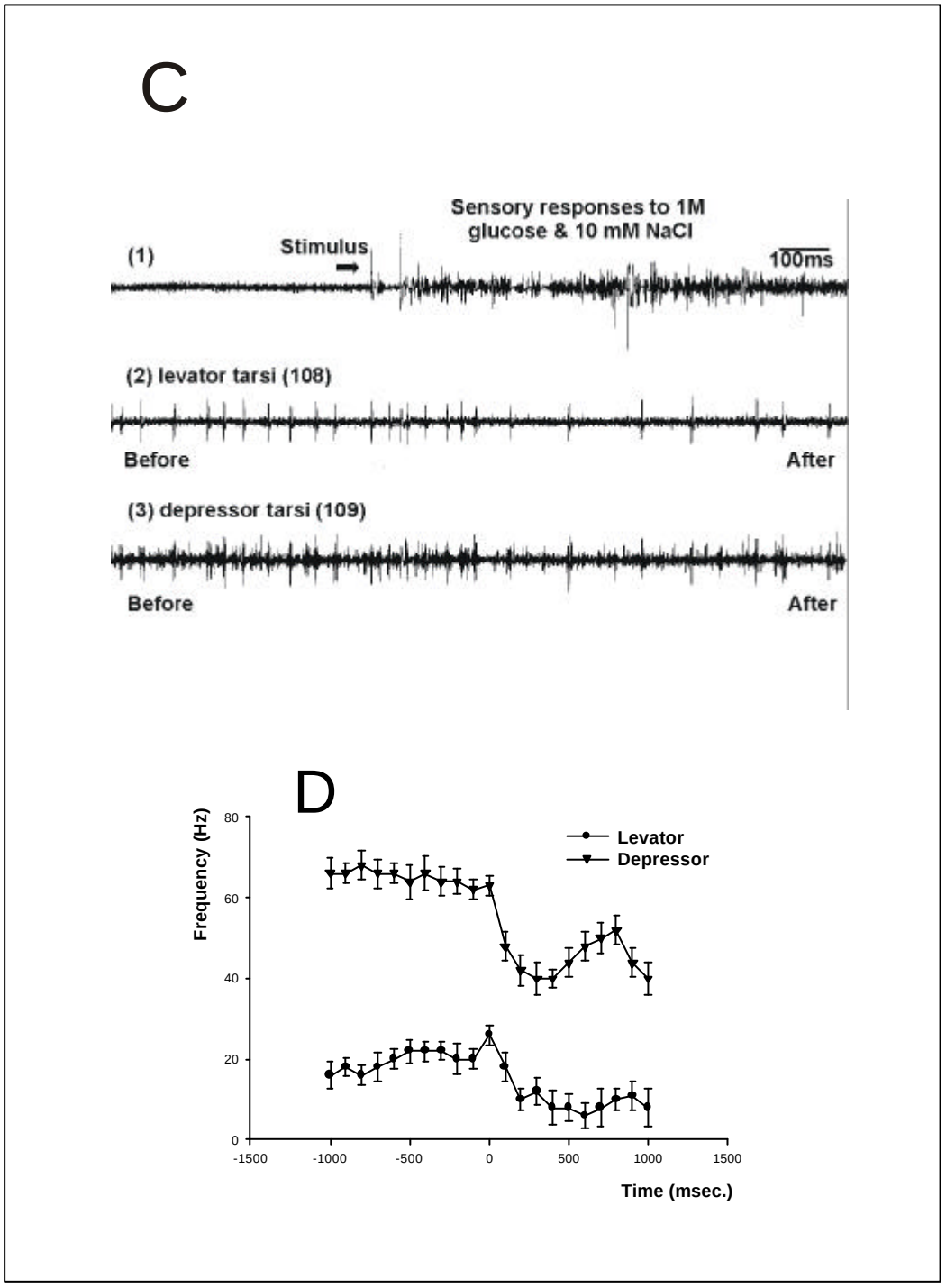

C
Sensory responses to 1M glucose & 10 mM NaCl
(1)
Stimulus
100ms
(2) levator tarsi (108)
Before
After
(3) depressor tarsi (109)
Before
After
D
Frequency (Hz)
80
60
40
20
0
Levator
Depressor
-1500
-1000
-500
0
500
1000
1500
Time (msec.)

Fig. (29 ii)

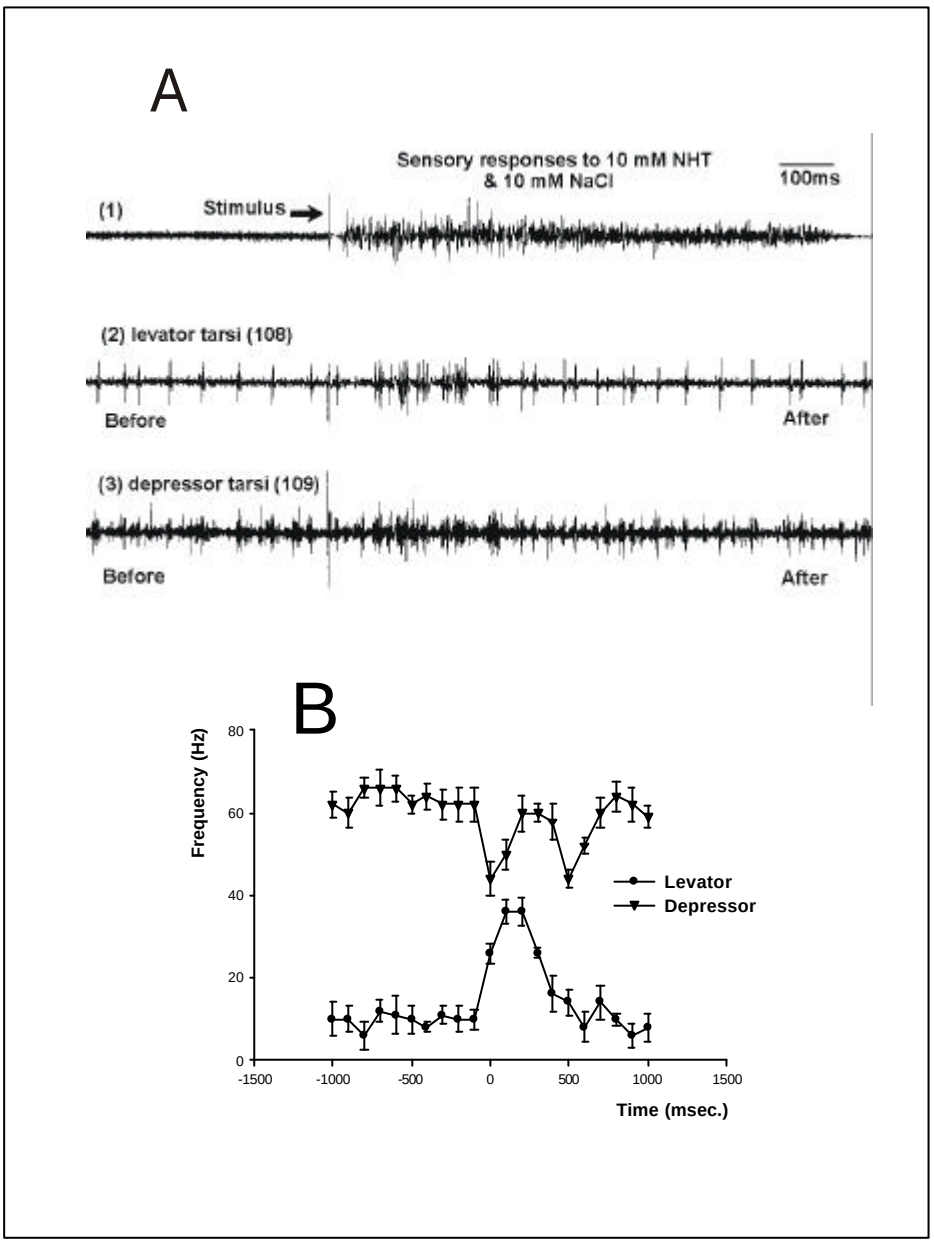


Fig. (30) Responses from a single of pulvillar basiconic sensillum (trace 1) and resulting motor responses (tarsal) (traces 2 and 3). (A) Response to 10 mM NHT and 10 mM NaCl. (B) Response frequency of motor units from two antagonistic tarsal muscles (depressor and levator), the stimulus 10 mM NHT was applied at time zero. Frequency measured as spikes per 100 msec. Each point represents the mean and ± SE (standard deviation) of 10 trials.

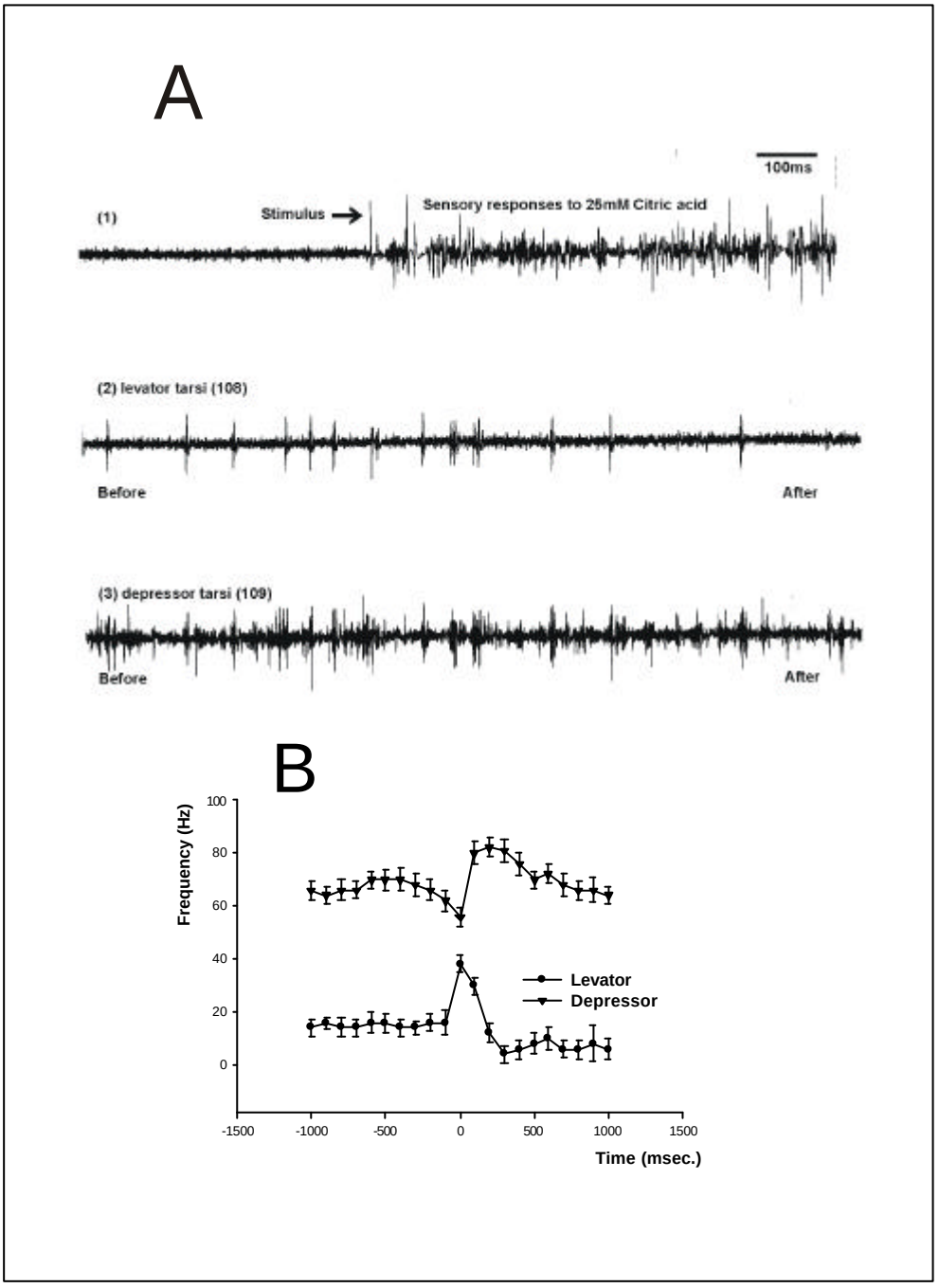


Fig. (31i) Responses from a single of pulvillar basiconic sensillum (trace 1) and resulting motor responses (tarsal) (traces 2 and 3). (A) Response to 25 mM citric acid and 10 mM NaCl. (C) Response to 250 mM citric acid and 10 mM NaCl. (B) Response frequency of motor units from two antagonistic tarsal muscles (depressor and levator), the stimulus 25 mM citric acid was applied at time zero. (D) Response frequency of motor units from two antagonistic tarsal muscles (depressor and levator), the stimulus 250 mM citric acid was applied at time zero. Frequency measured as spikes per 100 msec. Each point represents the mean and ± SE (standard deviation) of 10 trials.

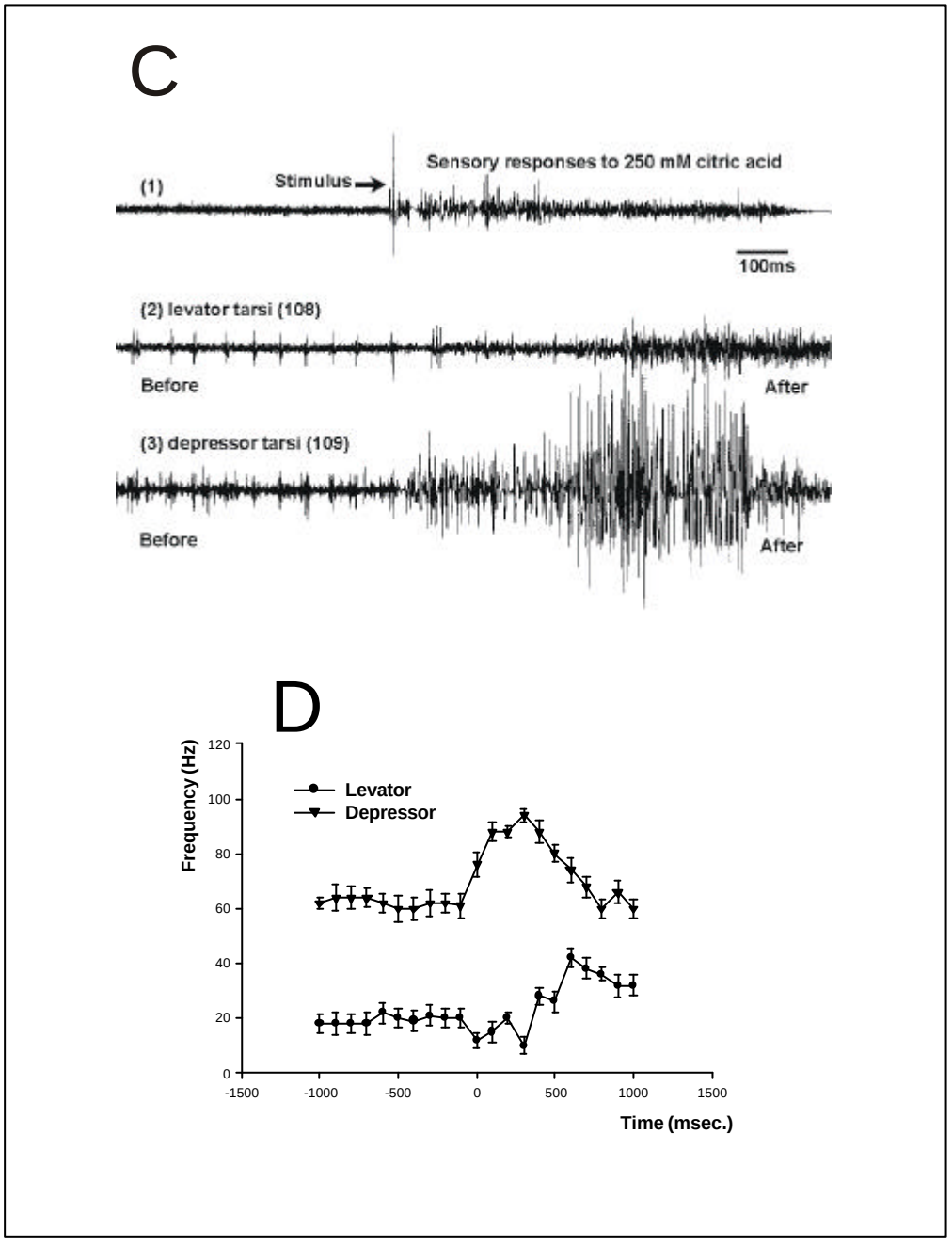

C
(1)
Stimulus
Sensory responses to 250 mM citric acid
100ms
(2) levator tarsi (108)
Before
After
(3) depressor tarsi (109)
Before
After
D
Frequency (Hz)
120
100
80
60
40
20
0
Levator
Depressor
-1500
-1000
-500
0
500
1000
1500
Time (msec.)

Fig. (31ii)

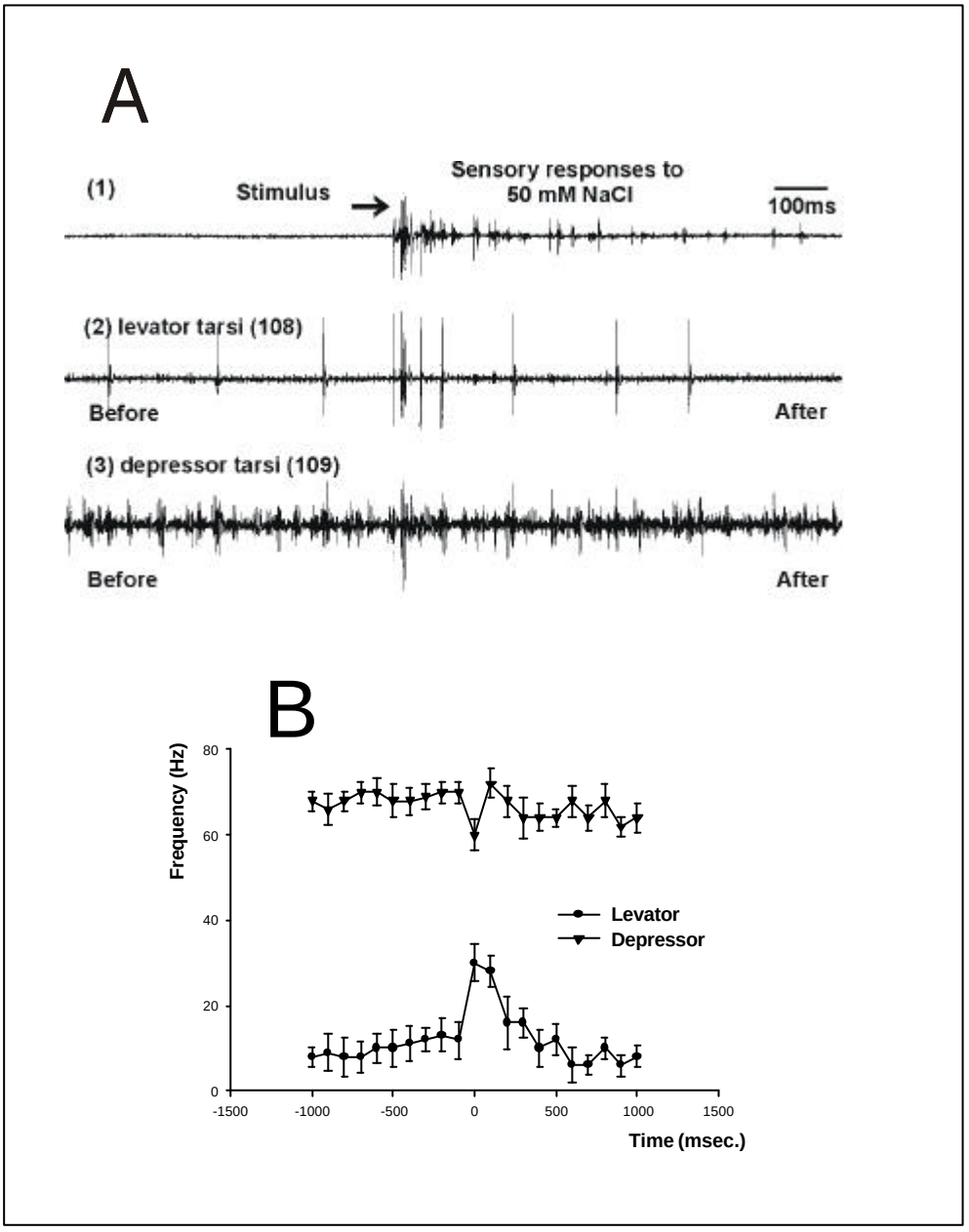


Fig. (32 i) Responses from a single of pulvillar basiconic sensillum (trace 1) and resulting motor responses (tarsal) (traces 2 and 3). (A) Response to 50 mM NaCl. (C) Response to 100 mM NaCl. (E) Response to 3 M NaCl. (B) Response frequency of motor units from two antagonistic tarsal muscles (depressor and levator), the stimulus 50 mM NaCl was applied at time zero. (D) Response frequency of motor units from two antagonistic tarsal muscles (depressor and levator), the stimulus 100 mM NaCl was applied at time zero. (F) Response frequency of motor units from two antagonistic tarsal muscles (depressor and levator), the stimulus 3 M NaCl was applied at time zero Frequency measured as spikes per 100 msec. Each point represents the mean and ± SE (standard deviation) of 10 trials.

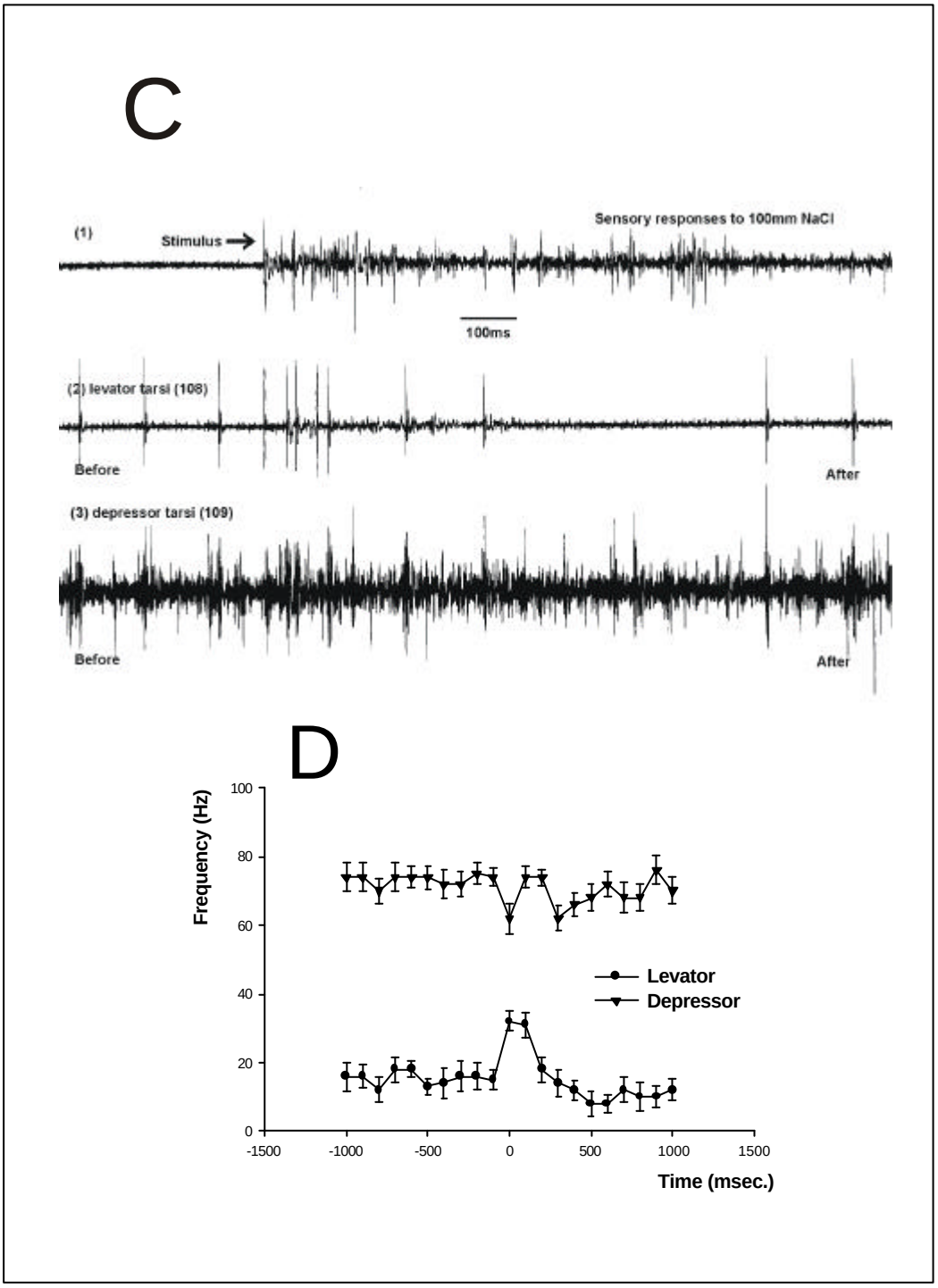

C
(1)
Stimulus
Sensory responses to 100mm NaCl
100ms
(2) levator tarsi (108)
Before
After
(3) depressor tarsi (109)
Before
After
D
Frequency (Hz)
100
80
60
40
20
0
-1500
-1000
-500
0
500
1000
1500
Time (msec.)
Levator
Depressor

Fig. (32ii)

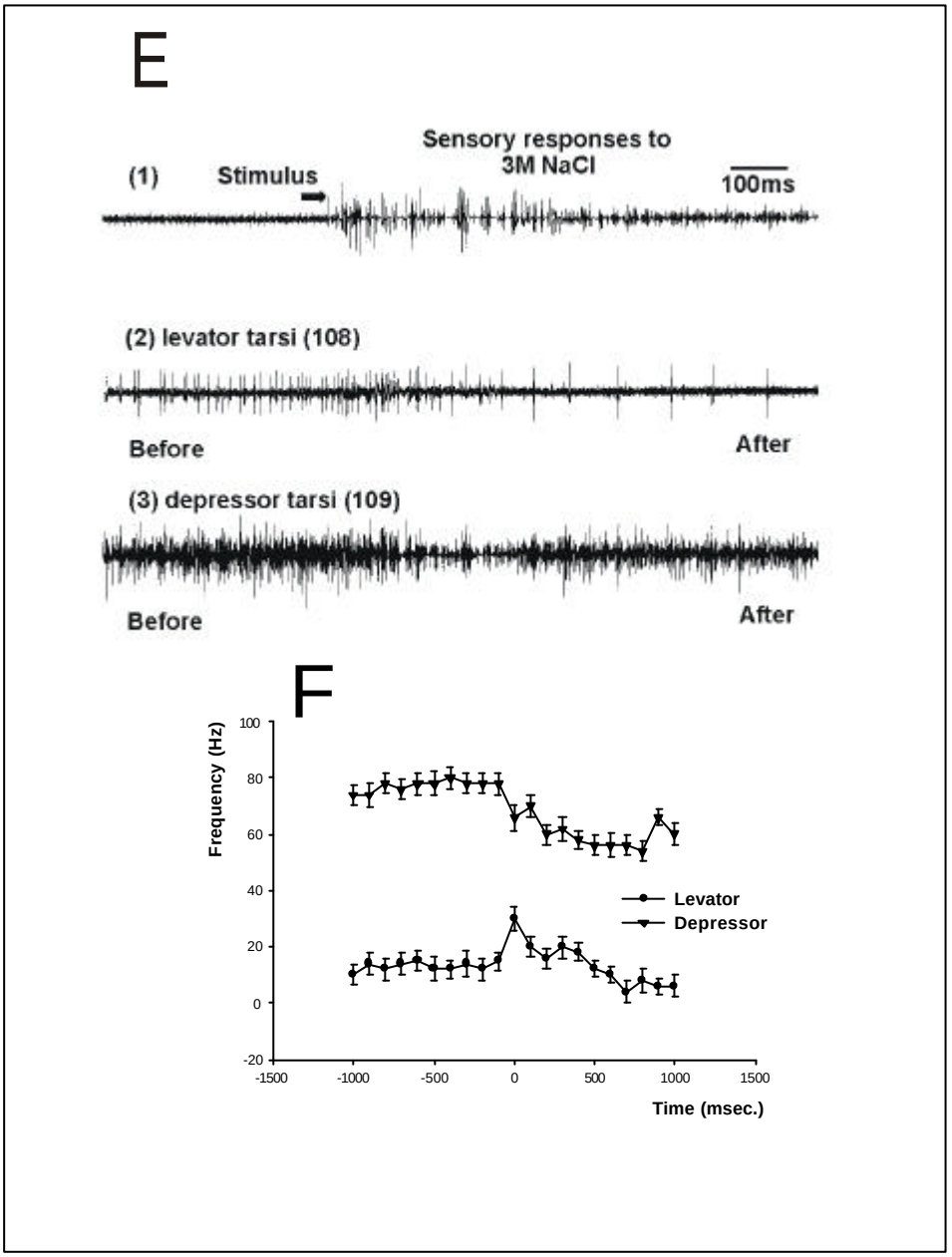

E
Sensory responses to
3M NaCl
(1)
Stimulus
100ms
(2) levator tarsi (108)
Before
After
(3) depressor tarsi (109)
Before
After
F
Frequency (Hz)
100
80
60
40
20
0
-20
-1500
-1000
-500
0
500
1000
1500
Time (msec.)
Levator
Depressor

Fig. (32 iii)

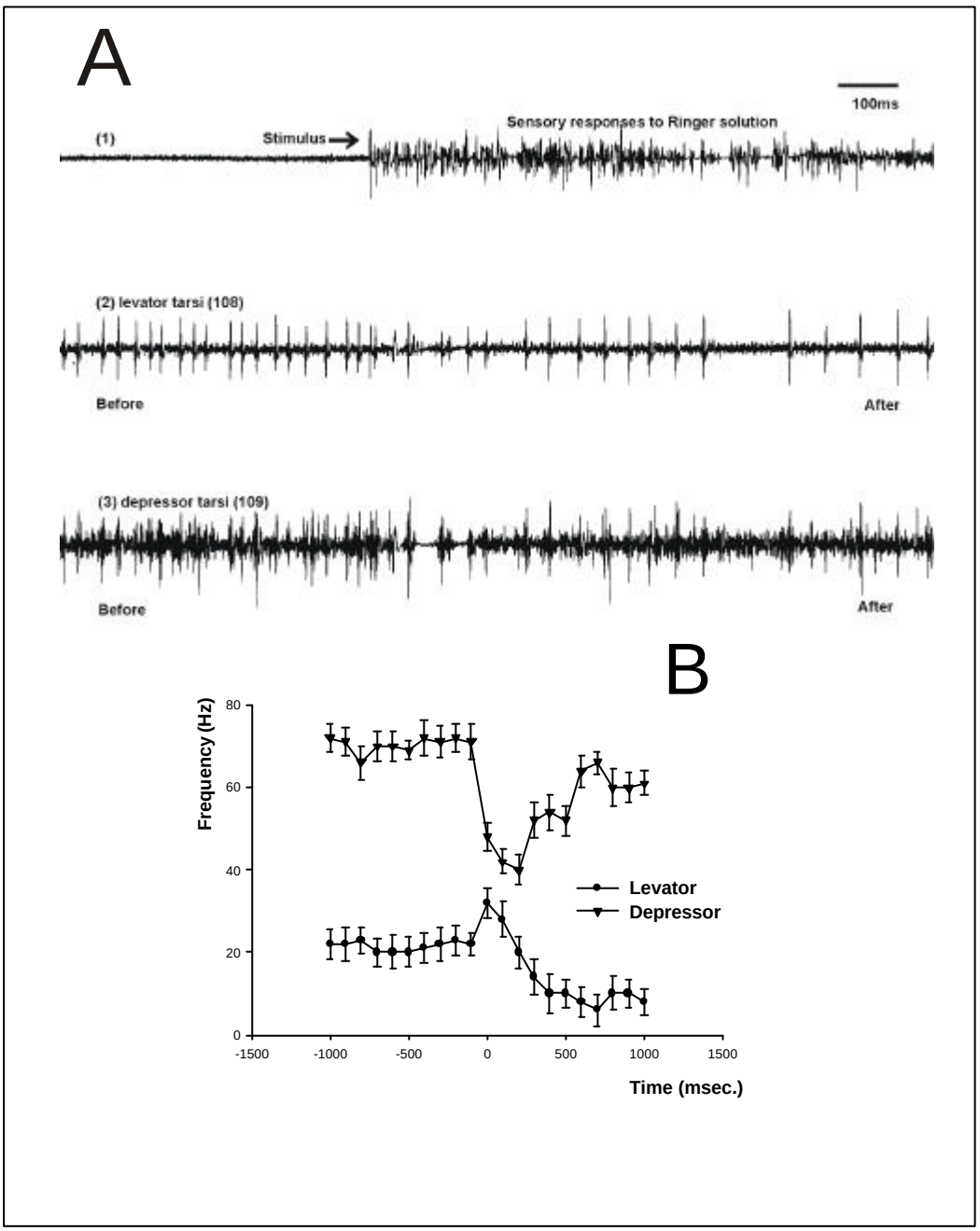


Fig. (33) Responses from a single of pulvillar basiconic sensillum (trace 1) and resulting motor responses (tarsal) (traces 2 and 3). (A) Response to clements and may solution. (B) Response frequency of motor units from two antagonistic tarsal muscles (depressor and levator), the stimulus saline was applied at time zero. Frequency measured as spikes per 100 msec. Each point represents the mean and ± SE (standard deviation) of 10 trials.

At low concentrations of NaCl (25, 50 and 100mM NaCl) the excitation of levator motor neuron or inhibition of a depressor motor neurones was followed by constant deplorizations in the activity of levator and depressor (Fig. 32 i, ii). At higher concentrations (1,5 M and 3 M NaCl) the excitation and the inhibition in the levator and depressor respectively, was followed regularly by a decrease in the activity of both motor neurons (Fig. 32 iii).

All depressor tarsi motor neurones were inhibited when applying saline, levator motor neurones was first excited followed by decreasing in activity of both levator and depressor motor neurons (Fig. 33).

3.5 Physiological properties of depressor and levator tarsi motor neurones:

Physiological mapping of the innervation was carried out by intracellular recordings from the somata of depressor or levator tarsi motor neurones in the mesothoracic ganglion. Motor neurones with their cell body in the mesothoracic ganglion were identified when they responded to chemosensory inputs from pulvillar basiconic sensilla of mesothoracic legs. Single pulvillar basiconic sensilla were stimulated with aqueous solutions of (NaCl, glucose, sucrose, citric acid quinine hydrochloride, solanidine and NHT). Motor neurones that responded to NaCl did not respond to glucose, solanidine, quinine and citric acid and vice versa, while glucose sensitive neurones often respond weakly to NaCl. Several of the recorded motor neurones were filled with Lucifer Yellow, and their morphology was reconstructed.

Stimulation of pulvillar basiconic sensilla with different concentrations of NaCl resulted always in excitation of one depressor tarsi motor neuron (MN.Dep.tar.1) (Fig. 34), in contrast there are no response to other chemicals. The activity of motor neurones correlated with the stimulation.

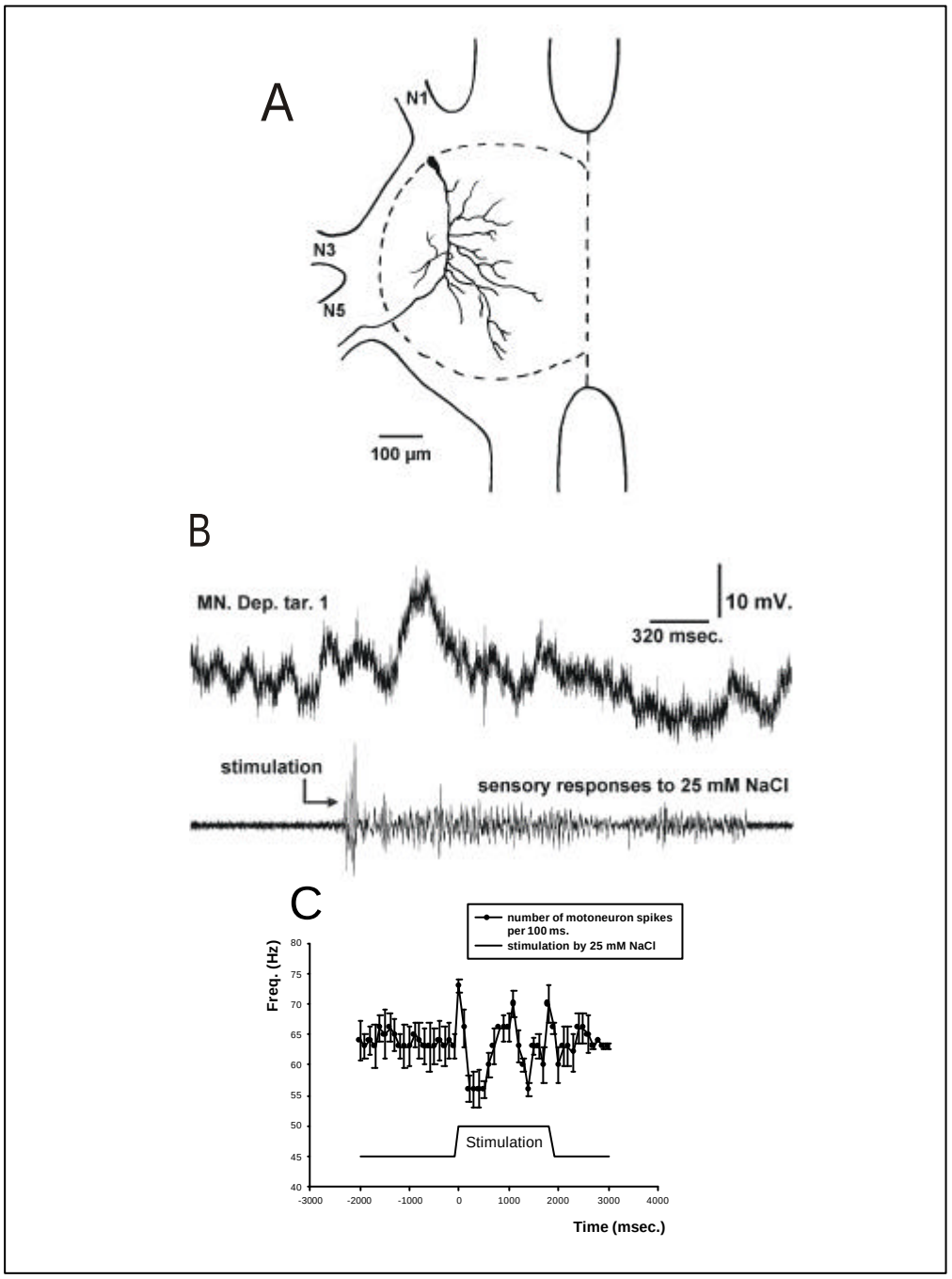


Fig. (34) Functional and morphological features of depressor tarsi motoneuron 1 (Dep. tar. Mot.1) in the mesothoracic ganglion, responsive to contact chemosensory input from one pulvillar basiconic sensillum. Responses to NaCl, sugars and citric acid (tested but not shown). (A) arborizations of (Dep. tar. Mot.1), (B) response to 25 mM NaCl, (C) peristimulus frequency changes of (dep. Tar. Mot.1) with 25 mM NaCl applied at time zero. Frequency measured as spikes per 100 msec. Each point represents the mean and $\pm$SE (standard deviation) each experiment with 10 trials.

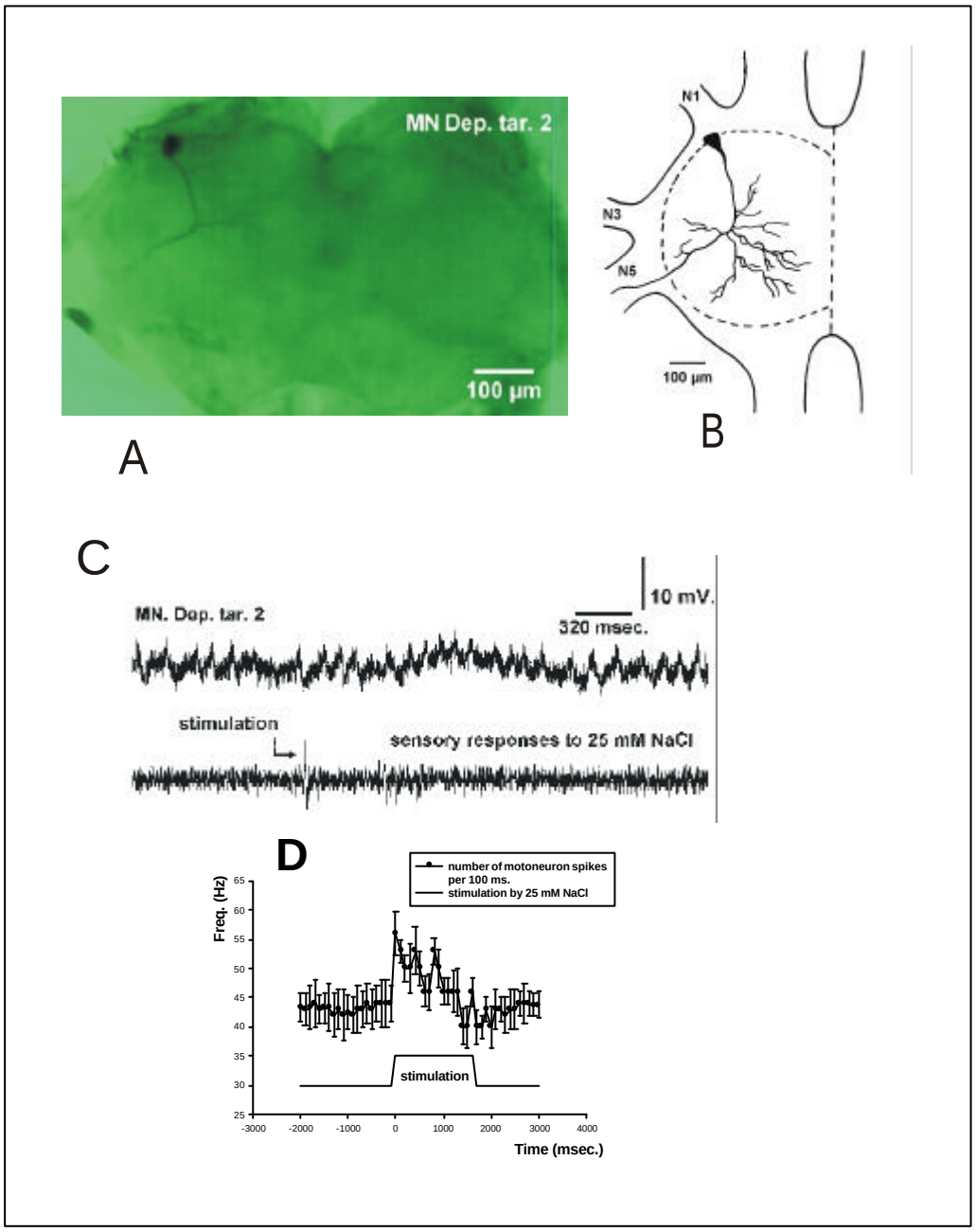


Fig. (35) Functional and morphological features of depressor tarsi motor neurone 2 (dep. Tar. Mot.2) in the mesothoracic ganglion, responsive to contact chemosensory input from one pulvillar basiconic sensillum. Responses to NaCl, sugars and citric acid (tested but not shown). (A) Photographs of Lucifer Yellow of (dep. Tar. Mot. 2), (B) arborizations of (Dep. tar. Mot.2), (C) response to 25 mM NaCl, (D) peristimulus frequency changes of (Dep. tar. Mot.2) with 25 mM NaCl applied at time zero. Frequency measured as spikes per 100 msec. Each point represents the mean and ±SE (standard deviation) each experiment with 10 trials.

The soma of a second motor neurone (MN.Dep.tar. 2) (Fig. 35) lies dorsally and the branches from the primary neurite extend in the ipsilateral half of the ganglion, as far medially as the median edges of the connectives. Stimulation by 25 mM NaCl can evoke depolarization in motor neurones; spiking discharge of the motor neurone varied with concentrations of NaCl, and responded weakly to citric acid and distilled water. In contrast there are no response with quinine or NHT.

In a third motor neurone (MN.Dep.tar.3) (Fig. 36) with its soma located dorso-laterally in the edge of the neuropil and branches from neurite extending in the lateral half of ganglion as far medially as the lateral edge of the connectives. This depressor motor neurone is excited by basiconic afferents stimulated with (25 mM NaCl or 25 mM Citric acid), the excitation in neurones correlated with the stimulation. Stimulation of pulvillar basiconic sensilla with 25 mM NaCl or glucose or sucrose resulted always in excitation of a fast depressor motor neurone (MN.Dep.tar.5) (Fig. 37 i, ii) correlated with the stimulation by sugars. The activity of neuron did not remain constant as the response to strong excitation by NaCl. It responds weakly to solanidine.

The morphologies of fast depressor tarsi and levator tarsi motor neurones are very similar. In both depressor and levator motor neurones the branches from the primary neurite extend in the lateral half of the ganglion, as far medially as the lateral edge of the connectives. Stimulation by 25 mM sucrose decreases the activity of the levator motor neurones (depression) (Fig. 38) followed by increasing in the activity.

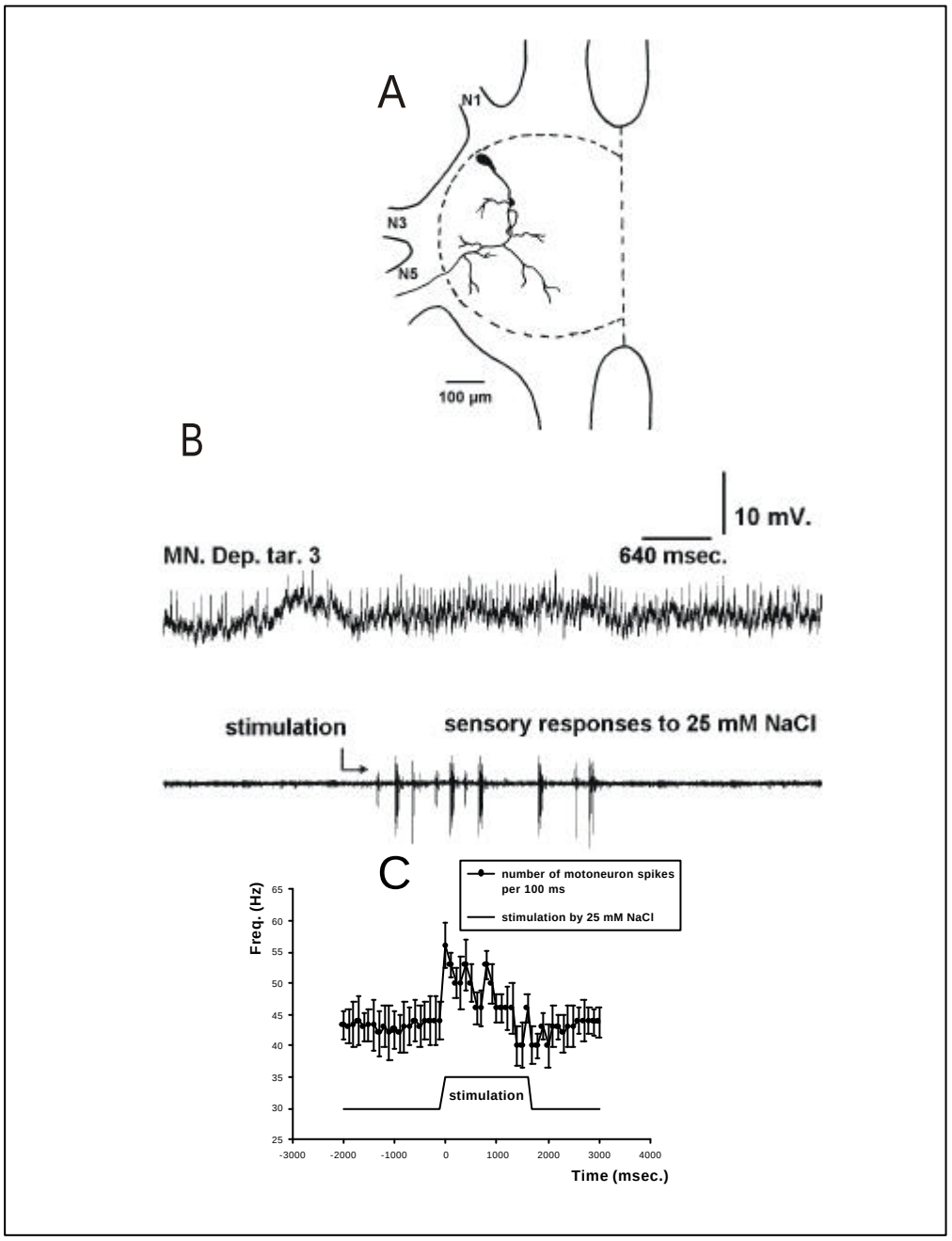


Fig. (36) Functional and morphological features of a depressor tarsi motor neurone (Dep. tar. Mot.3) in the mesothoracic ganglion, responsive to contact chemosensory input from one pulvillar basiconic sensillum. Responses to NaCl, sugars and citric acid (tested but not shown). (A) arborizations of (Dep. tar. Mot.3), (B) response to 25 mM NaCl, (C) peristimulus frequency changes of (Dep. tar. Mot.3) with 25 mM NaCl applied at time zero. Frequency measured as spikes per 100 msec. Each point represents the mean and $\pm$SE (standard deviation) each experiment with 10 trials.

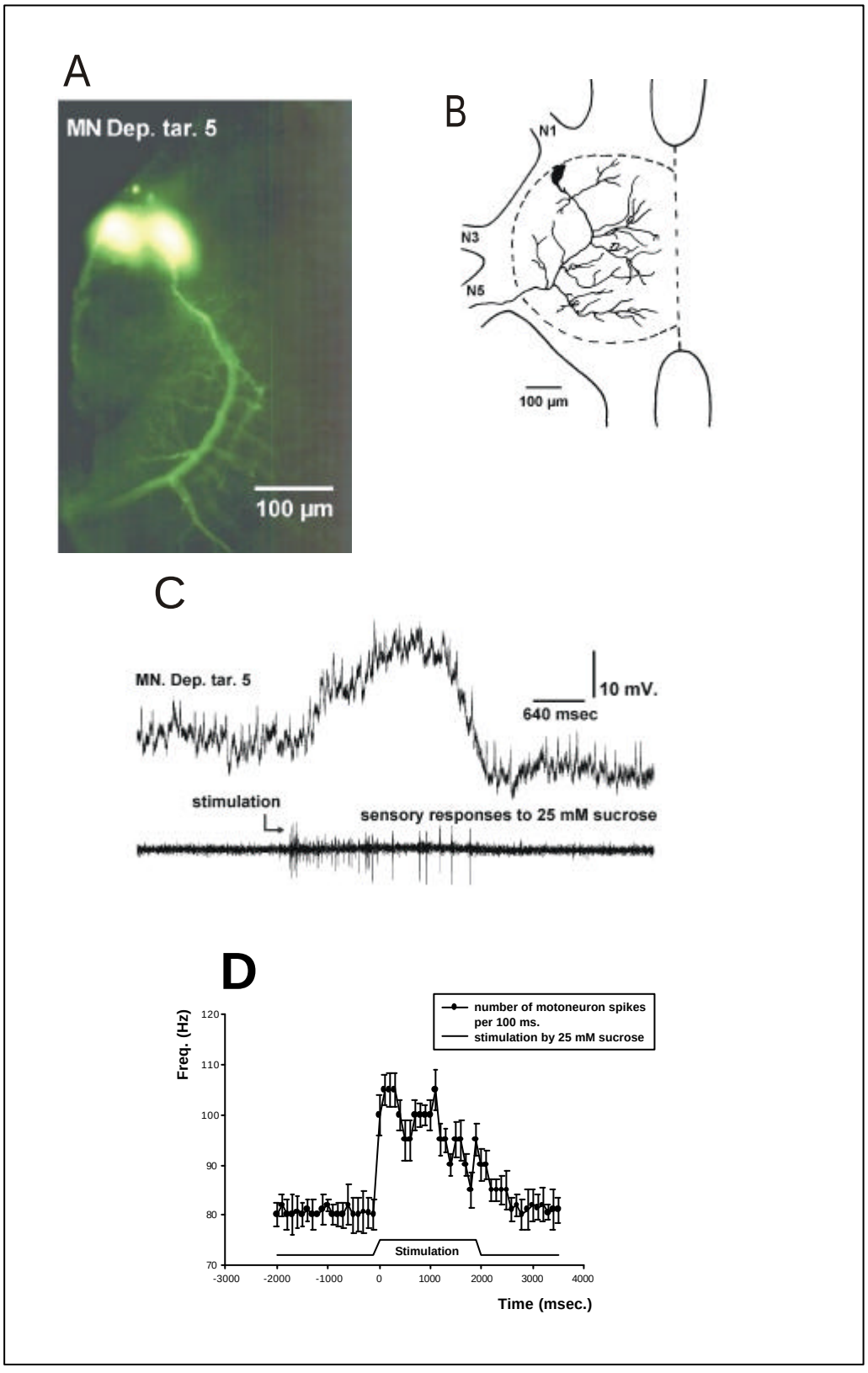


Fig. (37i) Legende next page.

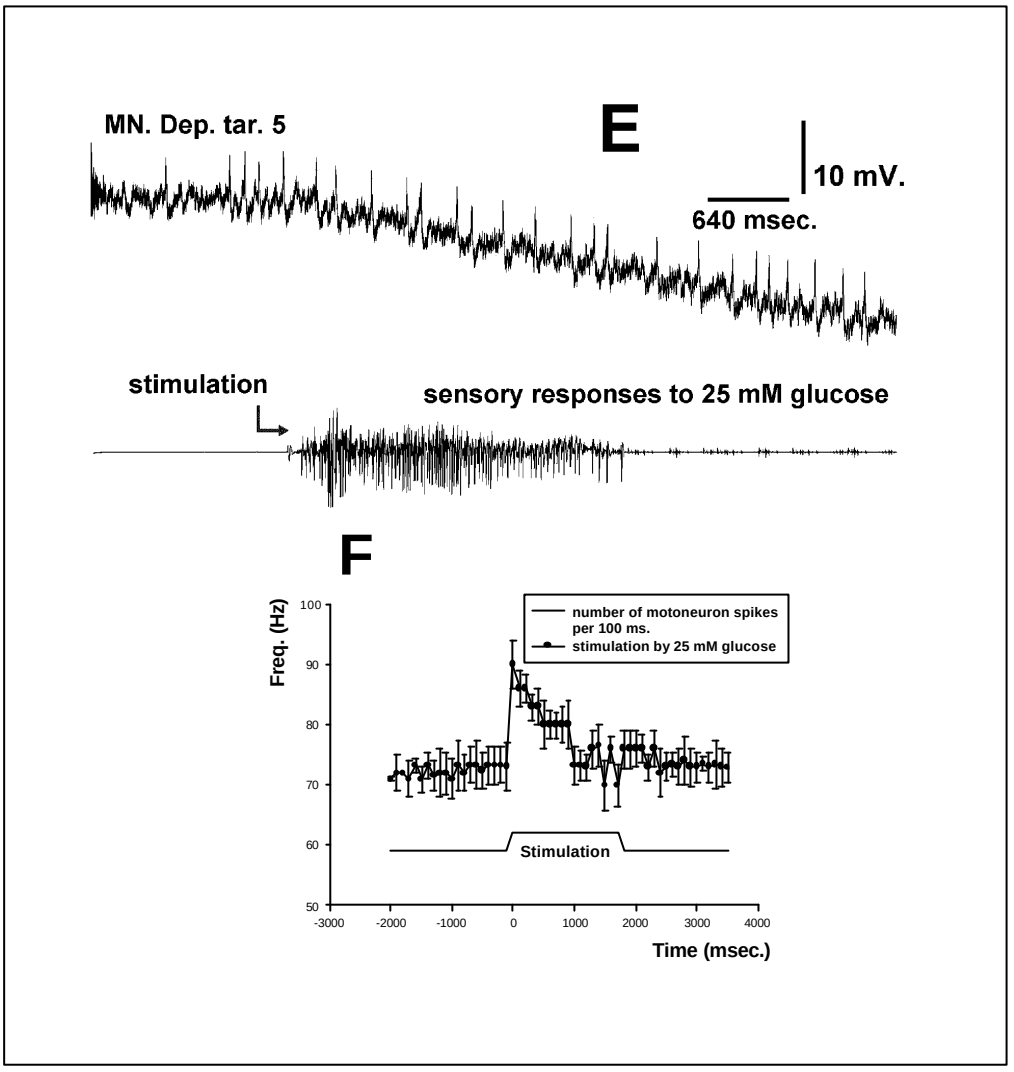


Fig. (37i & ii) Functional and morphological features of depressor tarsi motor neurone 5 (Dep. tar. Mot.5) in the mesothoracic ganglion, responsive to contact chemosensory input from one pulvillar basiconic sensillum. Responses to sugars and NaCl (C-G) no responses to quinine and solanidine (tested but not shown). (A) Photographs of Lucifer Yellow of (Dep. tar. Mot.5), (B) arborizations of (Dep. tar. Mot.5), (C) response to 25 mM sucrose, (E) responses to 25 mM glucose, (D) peristimulus frequency changes of (Dep. tar. Mot. 5) with 25 mM sucrose applied at time zero. (F) peristimulus frequency changes of (Dep. tar. Mot. 5) with 25 mM glucose applied at time zero. (G) peristimulus frequency changes of (Dep. tar. Mot. 5) with 25 mM NaCl applied at time zero. Frequency measured as spikes per 100 msec. Each point represents the mean and $\pm$SE (standard deviation) each experiment with 10 trials.

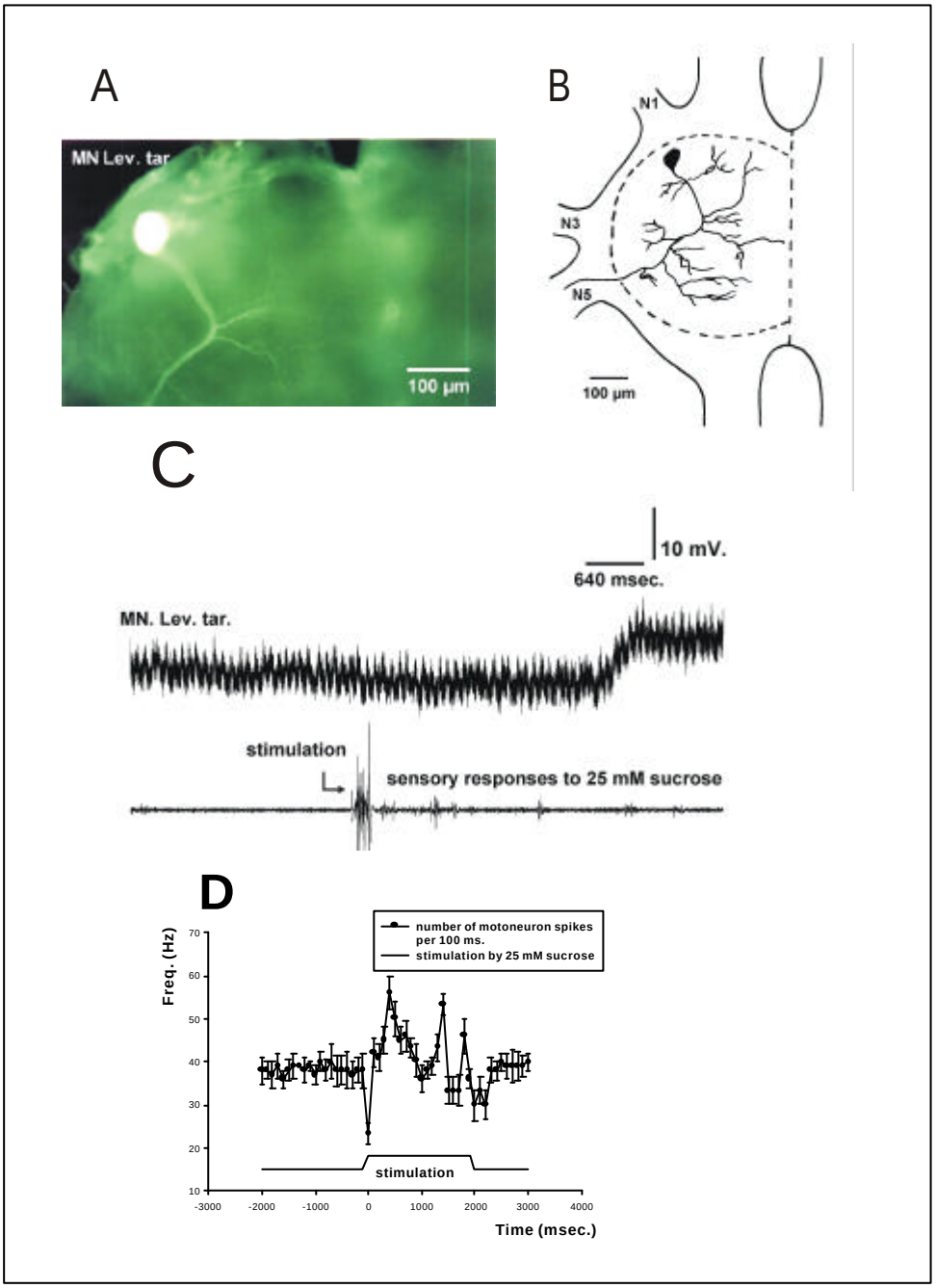


Fig. (38) Functional and morphological features of levator tarsi motoneuron 4 (Lev. tar. Mot. 4) in the mesothoracic ganglion, responsive to contact chemosensory input from one pulvillar basiconic sensillum. Responses to NaCl, sugars and citric acid (tested but not shown). (A) Photographs of Lucifer Yellow of (Lev. tar. Mot. 4), (B) arborizations of (lev. Tar. Mot.4), (C) response to 25 mM sucrose, (D) peristimulus frequency changes of (Lev. tar. Mot. 4) with 25 mM sucrose applied at time zero. Frequency measured as spikes per 100 msec. Each point represents the mean and $\pm$SE (standard deviation) each experiment with 10 trials.

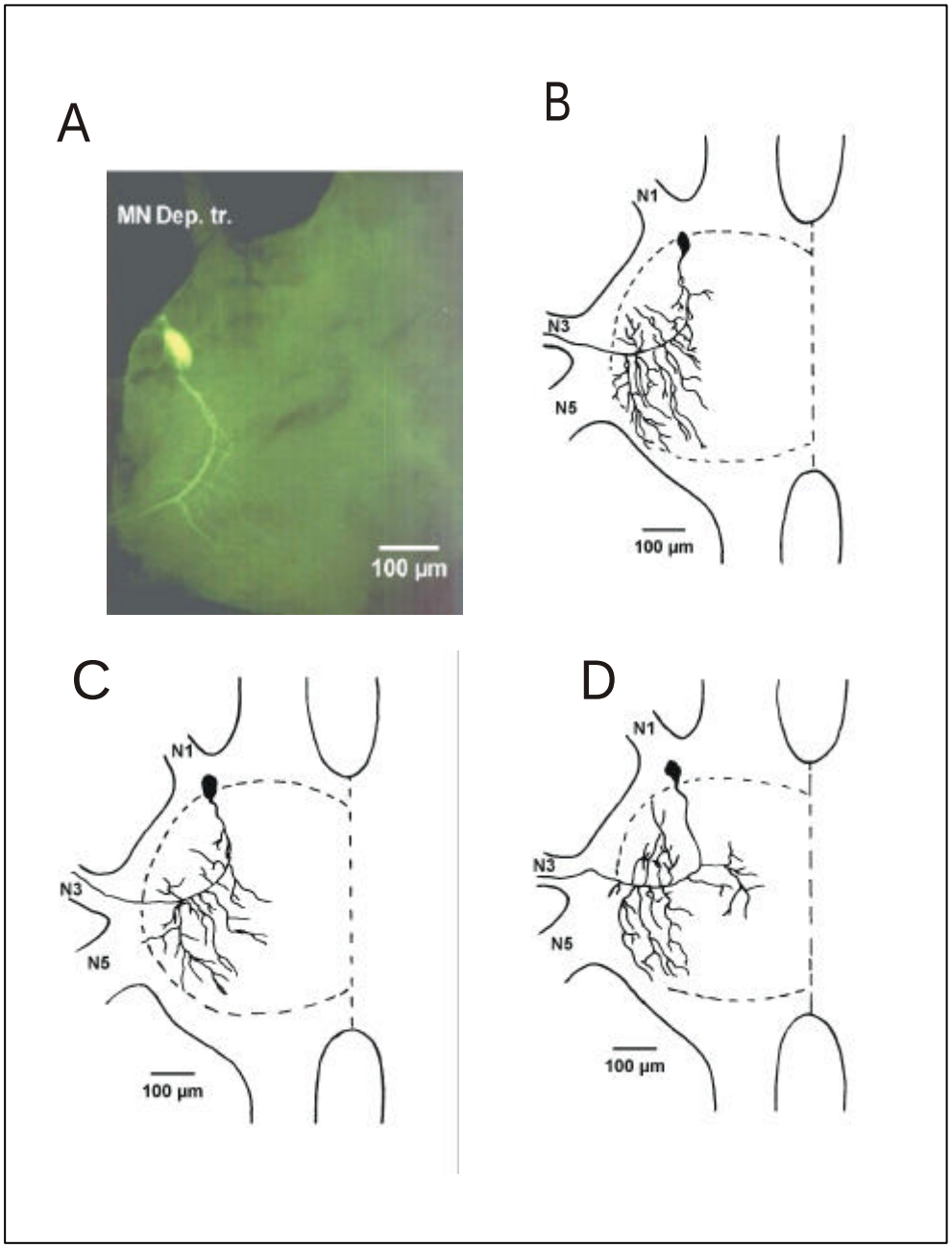


Fig. (39) Functional and morphological features of a depressor trochanter motoneuron (Dep. tr. Mot.) in the mesothoracic ganglion, not responsive to contact chemosensory input from one pulvillar basiconic sensillum. No responses to NaCl, sugars and citric acid (tested but not shown). (A) Photographs of Lucifer Yellow of (Dep. tr. Mot.), (B, C and D) arborizations of (Dep. tr. Mot.).

Stimulation of pulvillar basiconic sensilla by different concentrations of (NaCl, glucose, solanidine and NHT) caused no responses in one of the depressor tochachanter motor neurones (Fig. 39).

In contrast the stimulation by 25 mM NaCl resulted in excitation of a flight motor neurone followed by depression without stimulation (Fig. 40). The soma of the flight motor neuron lies dorsally near the lateral edge of neuropil, and ist axon extends contralterally through nerve 1 on the other side, the branches from the neurite extend in the middle line of the ganglion and medially in the lateral edge of midline from ganglion.

3.6 Physiological properties of interneurones:

The central arborizations of the afferents (sensory neurones from pulvillar basiconic sensilla), the local interneurones and intersegmental interneurones overlap in the ventral areas of neuropil regions of mesothoracic ganglion. The stimulation of pulvillar basiconic sensilla evokes excitatory postsynaptic potentials in the local interneurones. Each interneurone has a characteristic morphology defined by its array of branches in the regions of neuropil containing the projections of afferent that provide its inputs. Interneurones with inputs from contact chemoreceptor have branches in the most ventral regions of neuropil. The interneurones described were excited via pulvillar basiconic sensilla by aqueous solutions of solanidine, quinine hydrochloride, NHT, NaCl, glucose, sucrose and citric acid.

Stimulation of pulvillar basiconic sensilla by 25 mM solanidine or 25 mM citric acid resulted in excitation of local interneurones (1) (phasic response) (Fig. 41 i, ii), the duration of activity elicited by solanidine is longer and more tonic than the response to citric acid. The soma lies contralateral and the

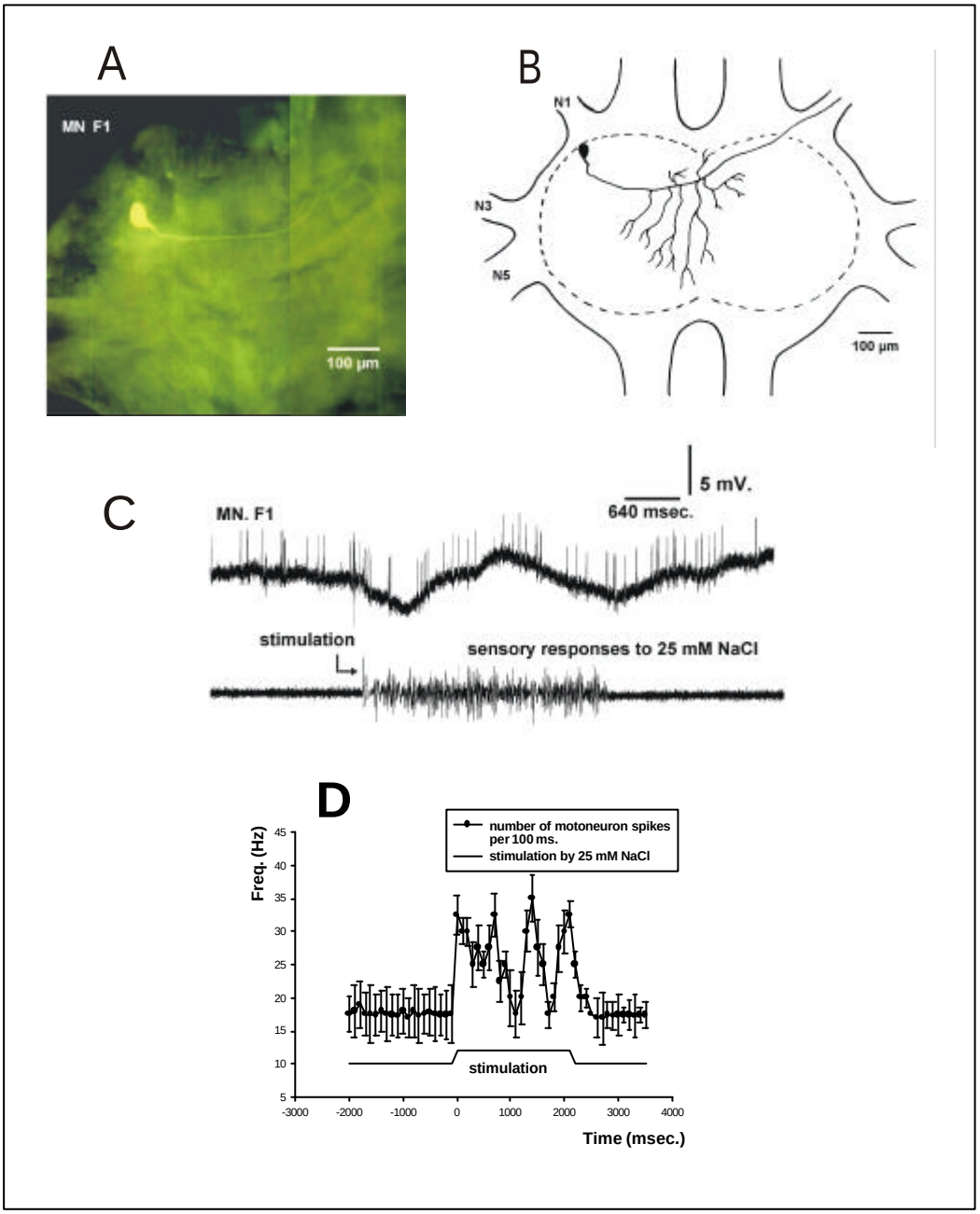


Fig. (40) Functional and morphological features of a flight motoneuron 1 (MN. F1) in the mesothoracic ganglion, responsive to contact chemosensory input from one pulvillar basiconic sensillum. Responses to NaCl, sugars and citric acid (tested but not shown). (A) Photographs of Lucifer Yellow of (MN. F1), (B) arborizations of (MN. F1), (C) response to 25 mM NaCl, (D) peristimulus frequency changes of (MN. F1) with 25 mM NaCl applied at time zero. Frequency measured as spikes per 100 msec. Each point represents the mean and ±SE (standard deviation) each experiment with 10 trials.

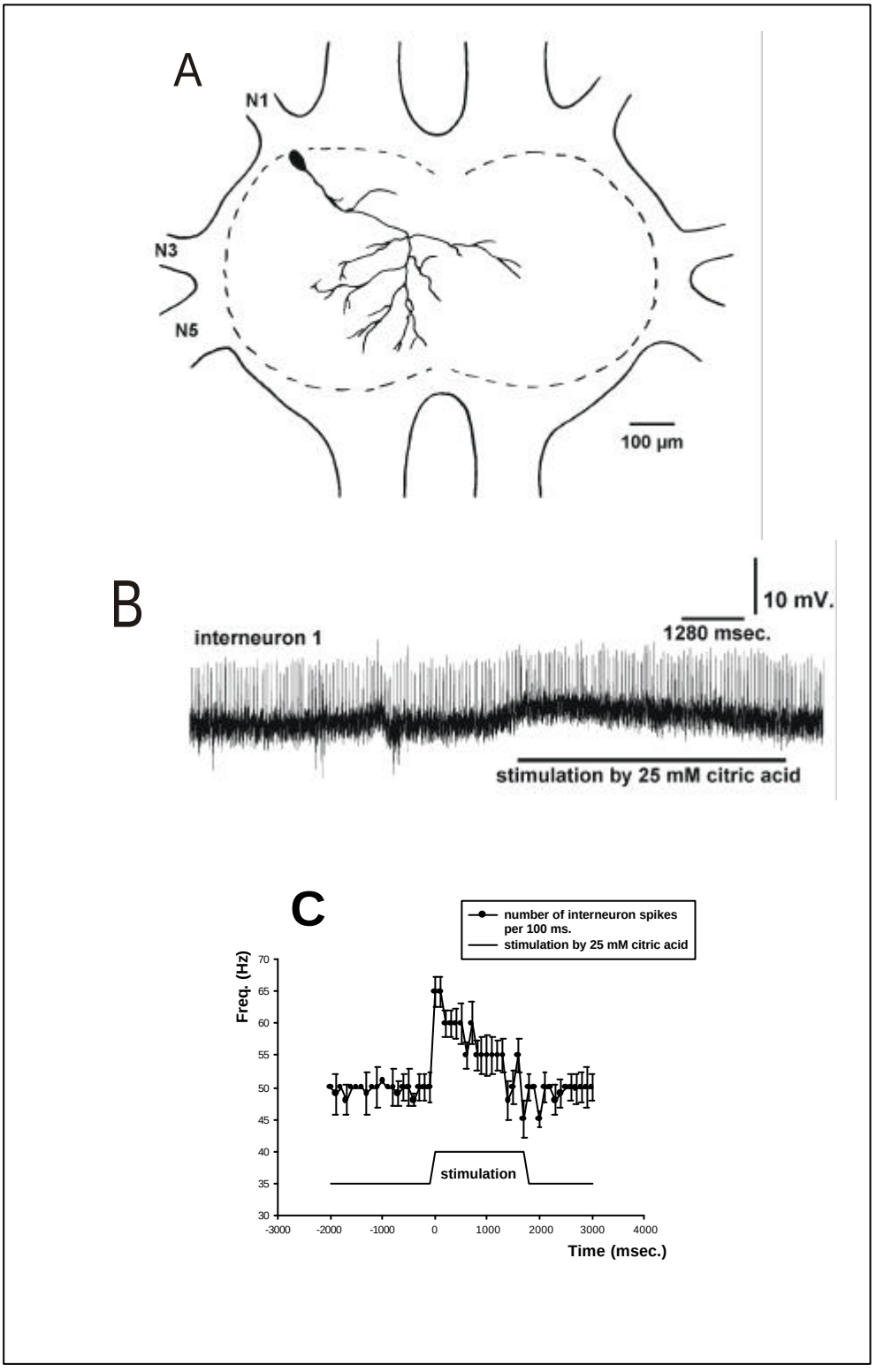


Fig. (41 i) Legend: next page.

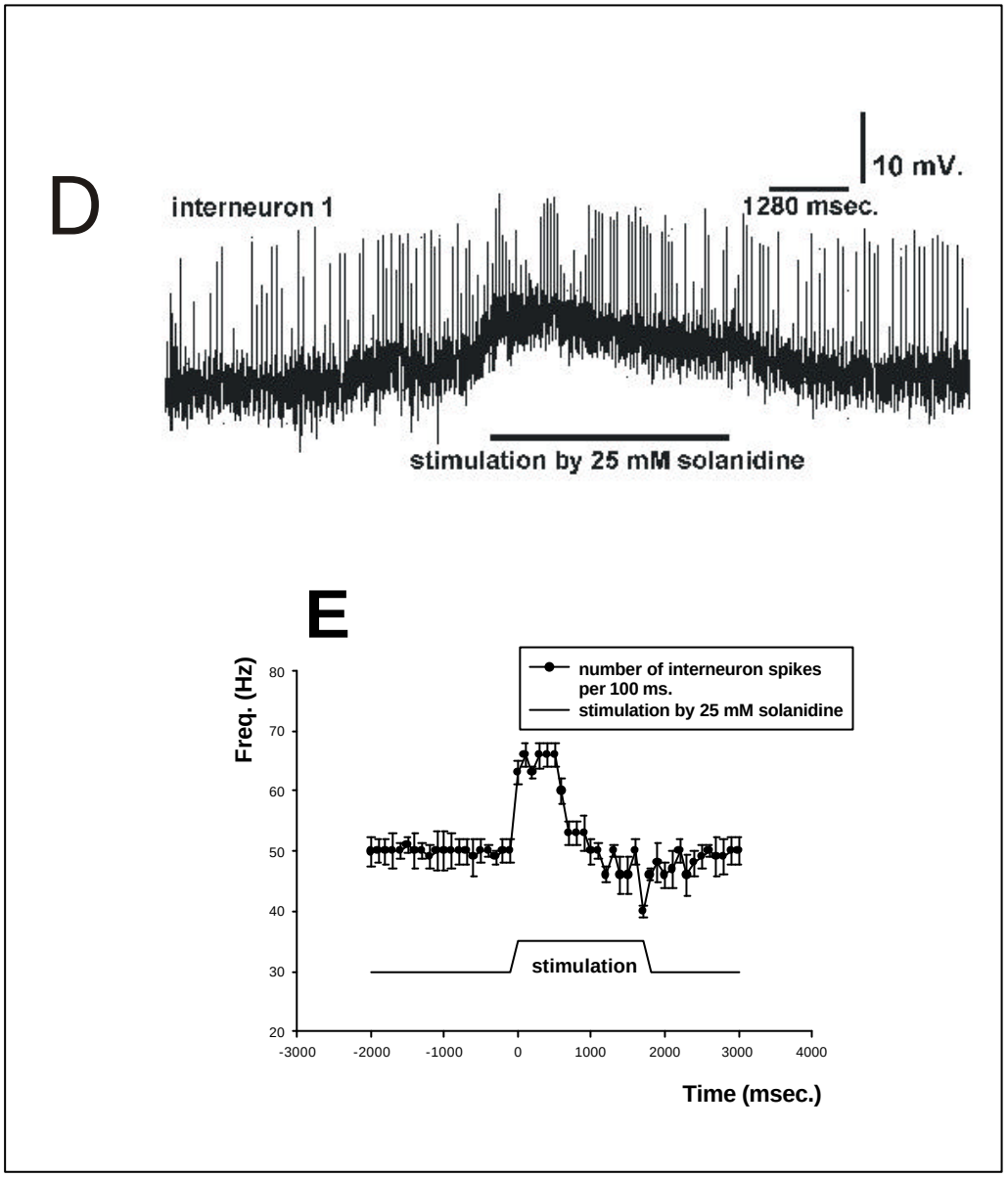


Fig.(41ii) Functional and morphological features of interneuron 1 (INT1) in the mesothoracic ganglion, responsive to contact chemosensory input from one pulvillar basiconic sensillum. Responses to citric acid and solanidine (B-D) no responses to glucose and sucrose (tested but not shown). (A) arborizations of interneuron 1, (B) response to 25 mM citric acid, (D) response to 25 mM solanidine, (C) Peristimulus frequency changes of interneuron 1 with 25 mM citric acid applied at time zero. (E) Peristimulus frequency changes of interneuron 1 with 25 mM solanidine applied at time zero. Frequency measured as spikes per 100 msec. Each point represents the mean and ± SE (Standard deviation) of 5 trials.

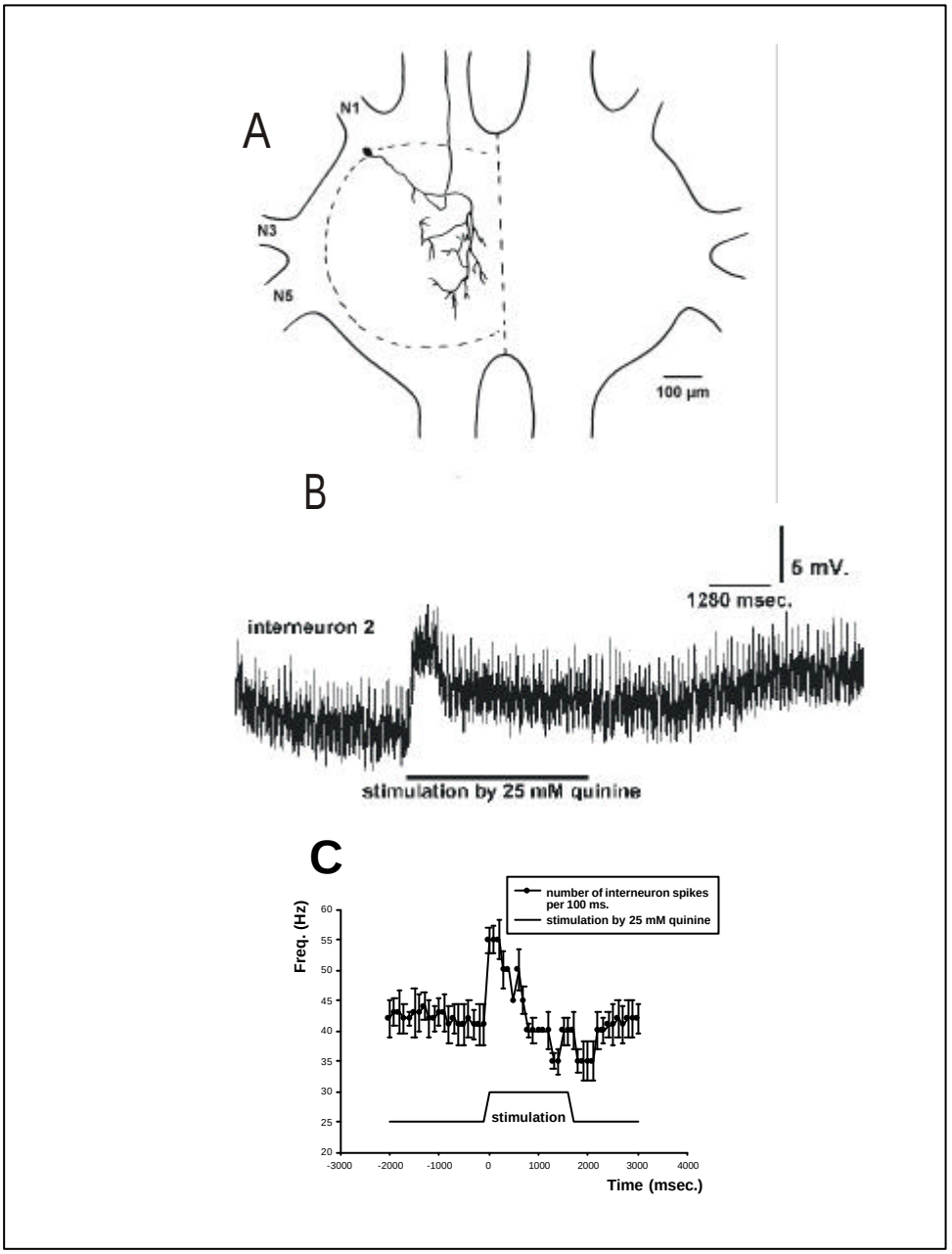


Fig. (42) Functional and morphological features of interneuron 2 (INT2) in the mesothoracic ganglion, responsive to contact chemosensory input from one pulvillar basiconic sensillum. Responses to quinine (B) no responses to glucose and sucrose (tested but not shown). (A) arborizations of interneuron 2, (B) response to 25 mM quinine, (C) Peristimulus frequency changes of interneuron 2 with 25 mM quinine applied at time zero. Frequency measured as spikes per 100 msec. Each point represents the mean and ± SE (Standard deviation) of 5 trials.

branches are mainly restricted to the ventral region of neuropil to which also pulvillar basiconic sensilla afferents project.

An intersegmental interneurone (INT2 ascending axon) responds to stimulation of pulvillar basiconic sensilla with 25 mM quanin hydrochloride with excitation followed by depression in spikes activity (Fig. 42).

In contrast there is a decrease (depression) in the activity in response to NHT application in a different intersegmental interneurone (3) (Fig. 43), with its axon ascending to the prothoracic ganglion.

A local interneurone (4) has a recurrent projection via the contralateral neuropile. The branches from the neurite extend in the ipsilateral half anteriorly and in the posterior ganglion, they reach the ventral neuropil. Stimulation afferent 25 mM NHT resulted in increasing in the activity (Fig. 44).

A different bilateral local interneurone (5), extends over the middle line of the ganglion and in the ipsilateral half it reaches the posterior ventral neuropil. Ist activity decreases when applying by 25 mM quinine or citric acid (Fig. 45 i, ii).

Fig. (43) Functional and morphological features of interneuron 3 (INT3) in the mesothoracic ganglion, responsive to contact chemosensory input from one pulvillar basiconic sensillum. Responses to NHT (B) no responses to glucose and sucrose (tested but not shown). (A) arborizations of interneuron 3, (B) response to 25 mM NHT, (C) Peristimulus frequency changes of interneuron 3 with 25 mM NHT applied at time zero. Frequency measured as spikes per 100 msec. Each point represents the mean and ± SE (Standard deviation) of 5 trials.

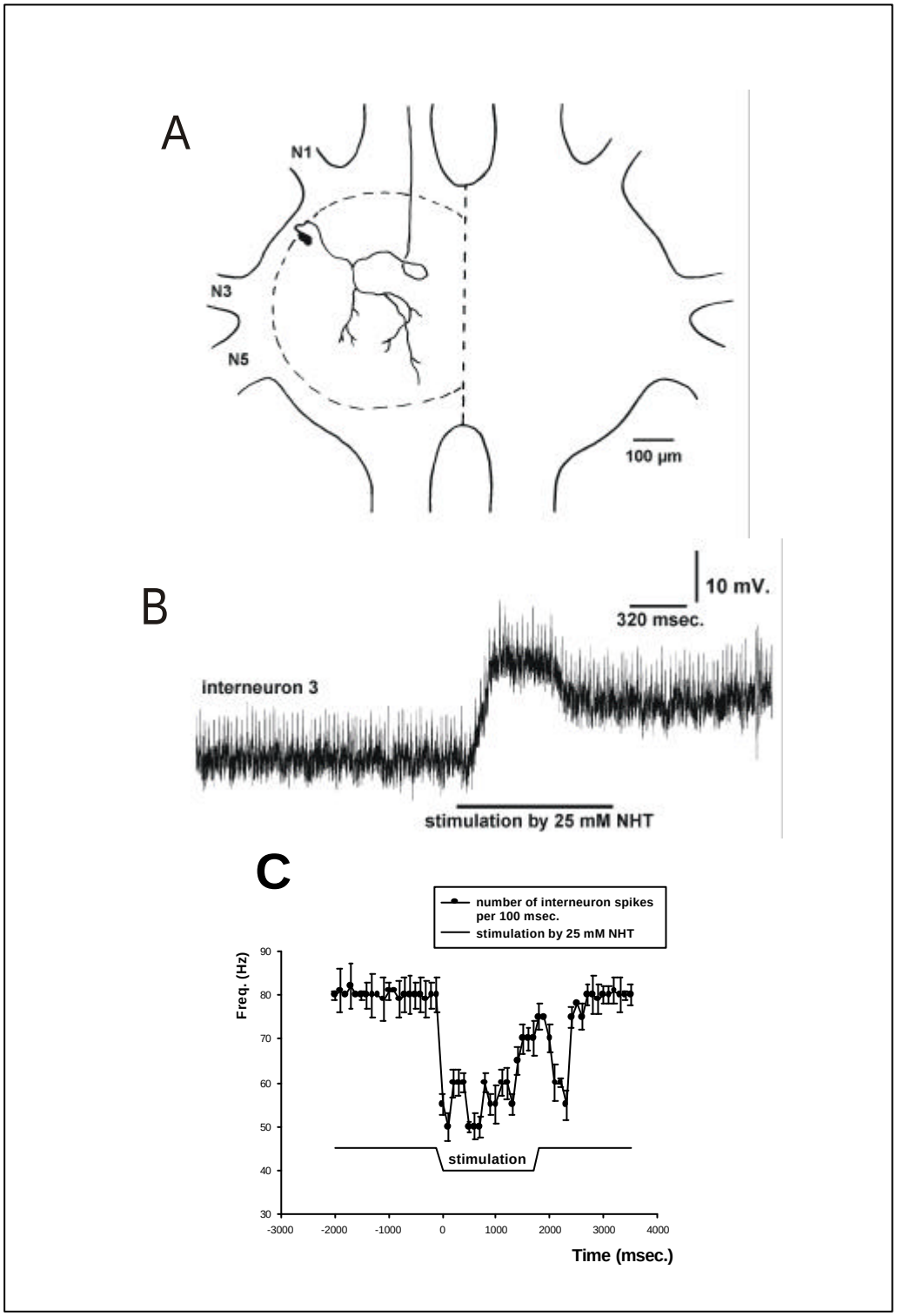

A
N1
N3
N5
100 µm
B
10 mV.
320 msec.
interneuron 3
stimulation by 25 mM NHT
C
number of interneuron spikes per 100 msec.
stimulation by 25 mM NHT
Freq. (Hz)
90
80
70
60
50
40
30
stimulation
-3000
-2000
-1000
0
1000
2000
3000
4000
Time (msec.)

Fig. (43)

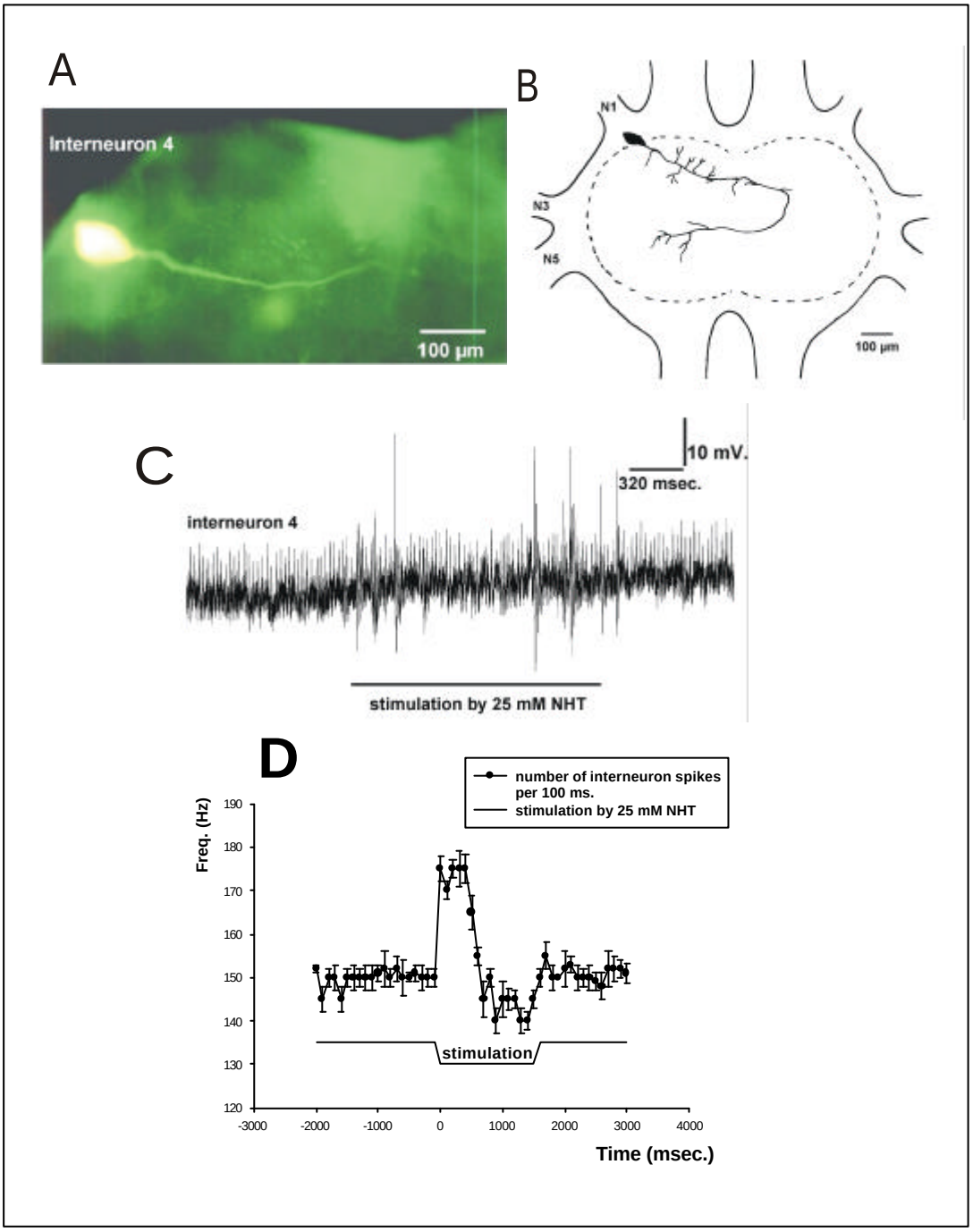


Fig. (44) Functional and morphological features of interneuron 4 (INT4) in the mesothoracic ganglion, responsive to contact chemosensory input from one pulvillar basiconic sensillum. Responses to citric acid and NHT (C) no responses to glucose and sucrose (tested but not shown). (A) Photographs of Lucifer Yellow filling of interneuron 4, (B) arborizations of interneuron 4, (C) response to 25 mM NHT, (D) Peristimulus frequency changes of interneuron 4 with NHT applied at time zero. Fequency measured as spikes per 100 msec. Each point represents the mean and ± SE (Standard deviation) of 5 trials.

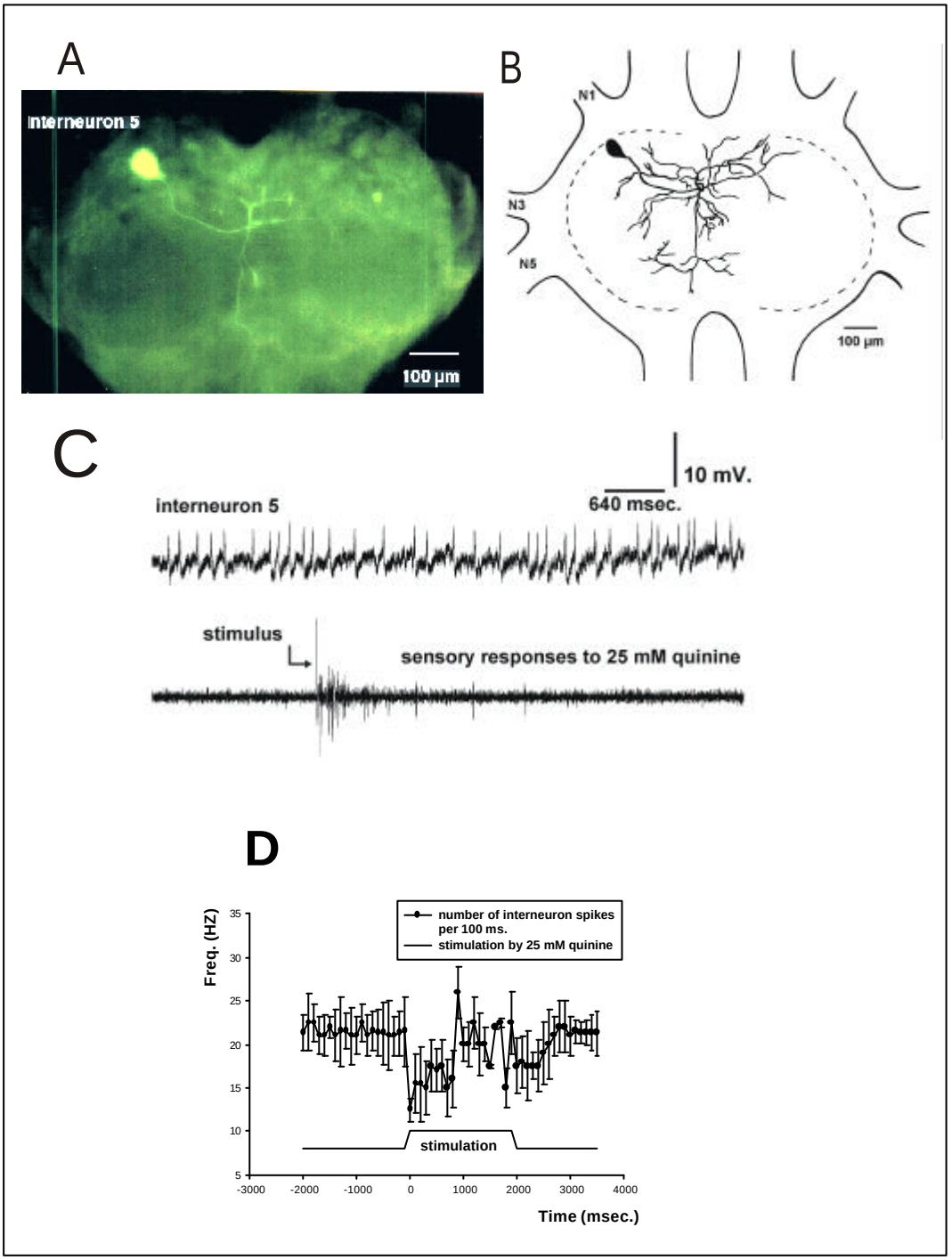


Fig (45i) Legend : next page

Fig. (45 i, ii) Functional and morphological features of interneuron 5 (INT5) in the mesothoracic ganglion, responsive to contact chemosensory input from one pulvillar basiconic sensillum. Responses to citric acid and quinine hydrochloride (C-E) no responses to glucose and sucrose (tested but not shown). (A) Photographs of Lucifer Yellow filling of interneuron 5, (B) arborizations of interneuron 5, (C) response to quinine, (E) response to citric acid, (D) Peristimulus frequency changes of interneuron 5 with quinine applied at time zero. (F) Peristimulus frequency changes of interneuron 5 with citric acid applied at time zero. Frequency measured as spikes per 100 msec. Each point represents the mean and ± SE (Standard deviation) of 5 trials.

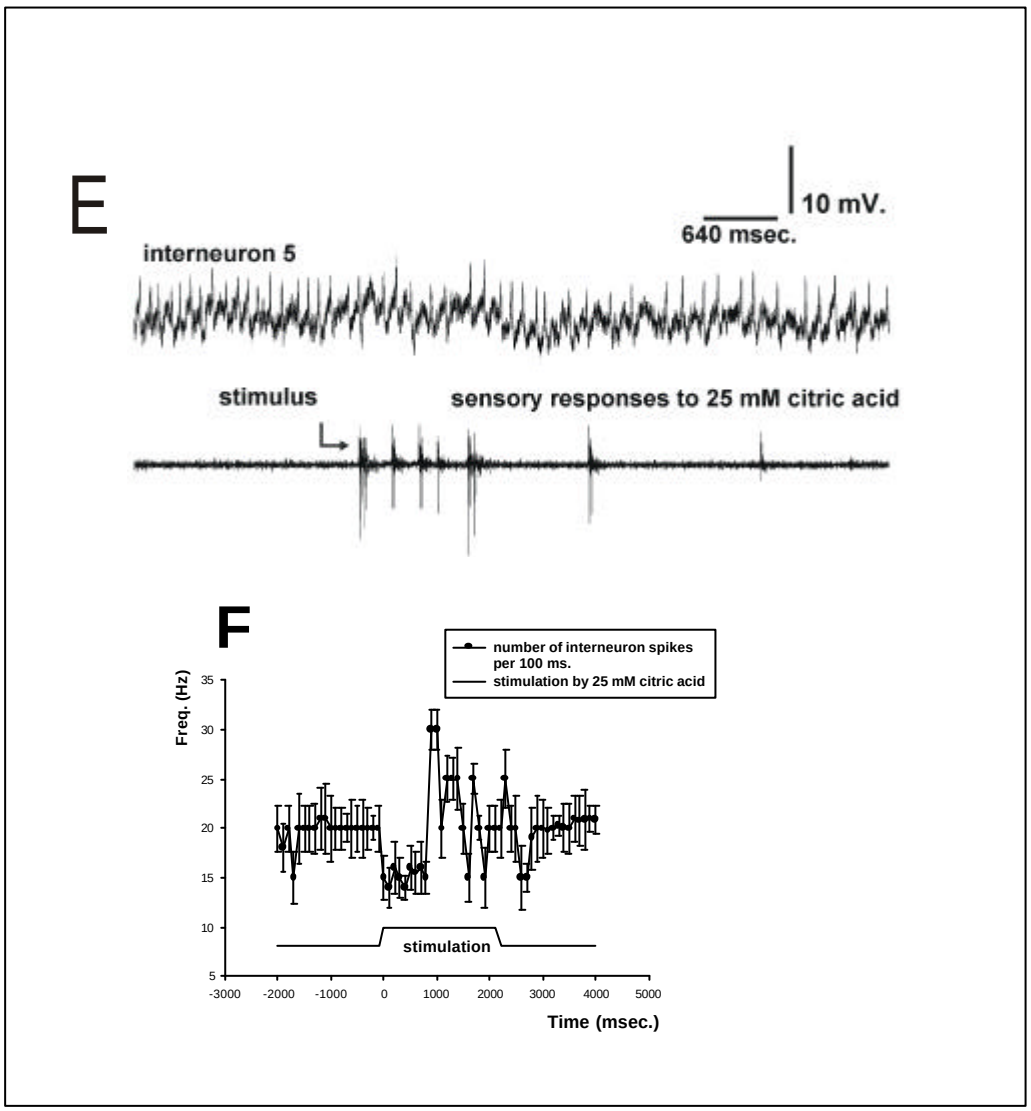


Fig. (45 ii)

3.7 Behavioural responses

The behavioural responses of desert locusts (*Schistoceraca gregaria*) to solutions of two behaviourally relevant chemicals (sodium chloride and sucrose) applied as droplets to the dorsal side of the fore leg tarsus, the hindleg tarsus and the hindleg femur were studied.

3.7.1 Categories of response:

All responses occurring within 1 second (s) of the application of a droplet and consisted of the locust moving its leg away from the stimulus site on the tarsus. Lifting of the leg occurred separately and was not part of a larger motor pattern involving the other limbs. These behavioural patterns can be categorised into two major groups.

The first of these, replacement behaviour, started with lifting of the leg of the tarsus in a new location on the substratum in a continuous motion. The second major category of response, withdrawal behaviour, was more stereotyped in execution and consisted of a sequence of movements starting with levation of the femur, flexion of the tibia and, frequently, adduction of the femur to the side of the abdomen, after which the tarsus was held clear of the substratum for a period of not less than 400 ms and frequently for much longer.

3.7.2 Frequencies of response to Nacl and sucrose solutions:

The proportion of the locusts within 5 groups each of 12 animals responding to applied chemical solutions was strongly correlated with the concentration of the chemical in the droplet for the tested substances (Fig. 46 and Fig. 47). However, there was considerable variation in effective concentrations between the different chemicals, as shown in (Fig. 46 and Fig. 47).

The fore leg is more responsive than hindleg for stimulation by NaCl especially at high concentrations (75 mM, 4600 mM Nacl) (Table 1, Fig. 46). In contrast the responsiveness to high sucrose concentration (1 M, 2M sucrose) in both fore and hindleg was similar (Table 2, Fig. 47). The concentration of a chemical in a droplet sufficient to evoke a response (within 1s) in 50% of the locusts in each group ranged from approximately (50 mM NaCl to 75 mM NaCl) in case of the fore leg and (75 mM NaCl to 100 mM NaCl) in case of the

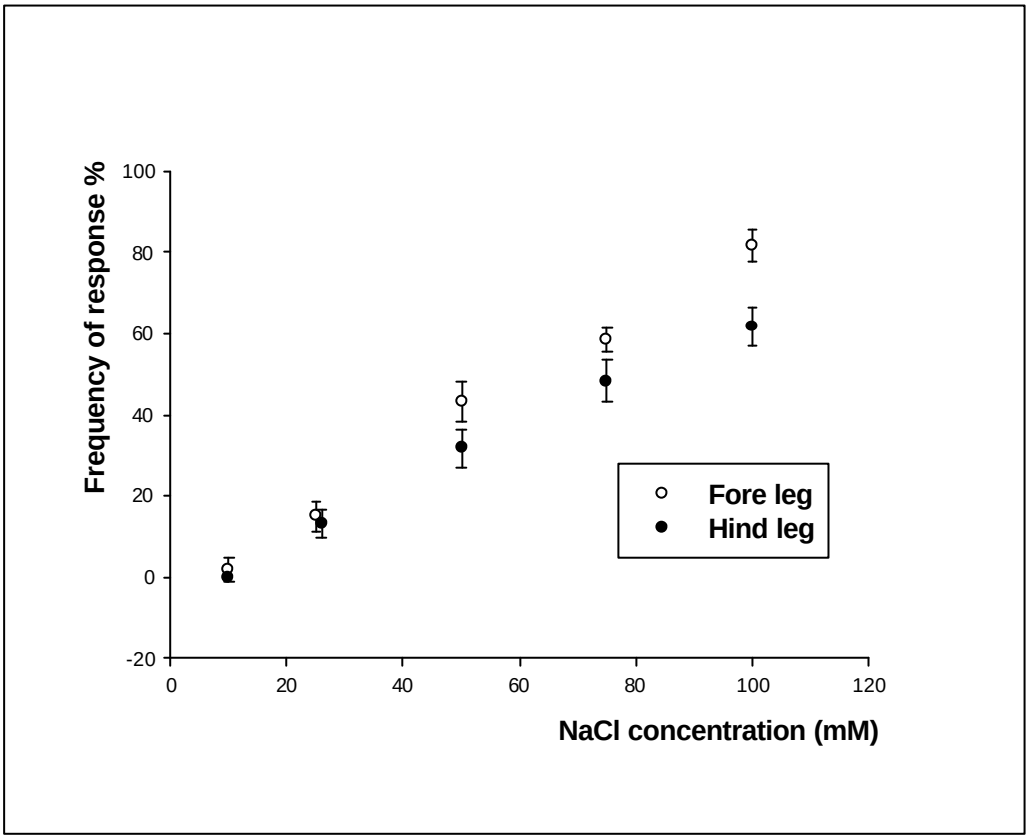


Fig. (46) The frequencies of replacement or withdrawal responses pooled vary with NaCl concentration on the fore and hindleg tarsus. Values are mean + S.E.M Each frequency was calculated from the number of locusts in 5 group of each12 that responded within 1 s to each of the solutions, and each point is the mean of five replicates.

Table (1) Results of an analysis testing the effects of different chemical concentration. Groups were tested as to the frequencies of responses to different NaCl concentrations on the fore and hindleg tarsus.

NaCl	Type III Sum Of Squares	df	Mean Square	F
Corrected model	482.311[a]	2	241.156	254.310**
Intercept	4.844	1	4.844	5.109*
Conc. comparison	474.311	1	474.311	500.184**
Leg comparison	8.000	1	8.000	8.436**
Error	44.569	47	.948	

a. R Squared = .915 (Adjusted R Squared= .912)

Locusts were tested in groups of 12 animals, and the numbers of animals in each group responding to various concentrations of a particular chemical were counted. There were five replicate test groups for each chemical. Concentration was $\log_{e}$-transformed for the analysis to allow for a linear regression fit.
Significant results are marked as follows: **P<0.05; **P<0.01; ***P<0.001*.

Table (2) Results of an analysis testing the effects of different chemical concentration.

Groups were tested as to the frequencies of responses to different sucrose concentrations on the fore and hindleg tarsus.

Sucrose	Type III Sum Of Squares	df	Mean Square	F
Corrected model	115.532[a]	2	57.766	46.331**
Intercept	301.109	1	301.109	241.505**
Conc.	104.266	1	104.266	83.626**
Leg	11.267	1	11.267	9.036**
Error	71.068	57	1.247	

a. R Squared = .619 (Adjusted R Squared= .606)

Locusts were tested in groups of 12 animals, and the numbers of animals in each group responding to various concentrations of a particular chemical were counted. There were five replicate test groups for each chemical. Concentration was $\log_{e}$-transformed for the analysis to allow for a linear regression fit.
Significant results are marked as follows: **P<0.05; **P<0.01; ***P<0.001*.

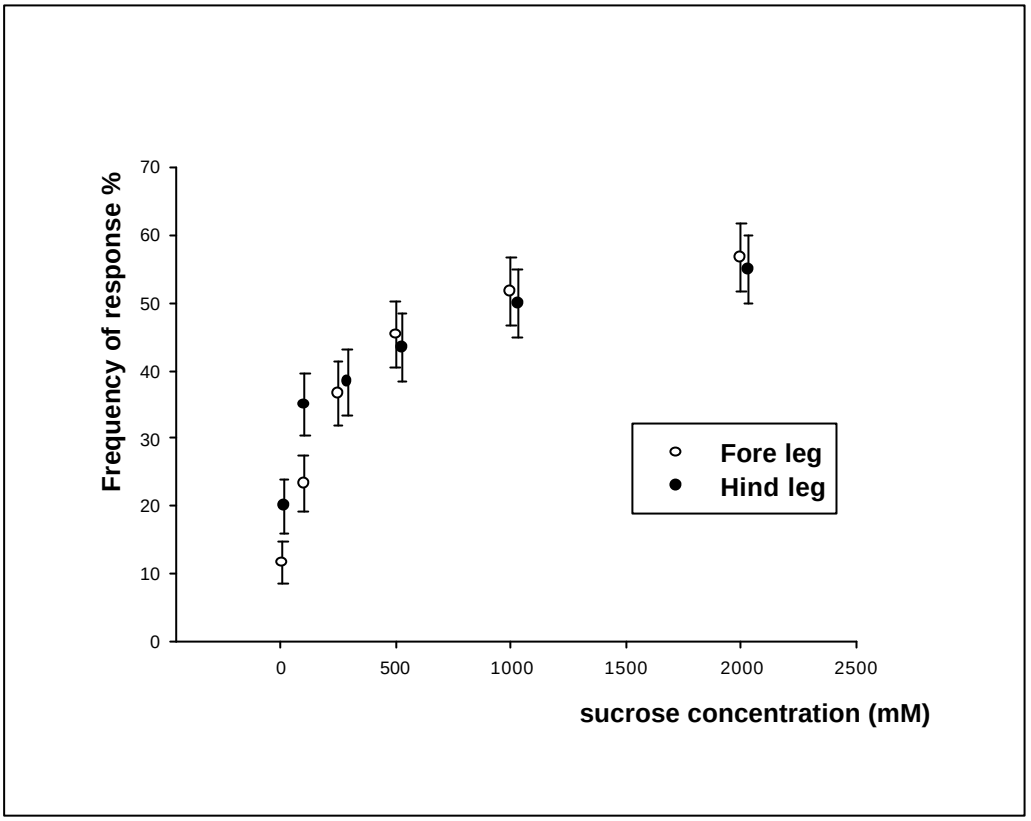


Fig. (47) The frequencies of replacement or withdrawal responses pooled vary with sucrose concentration on the fore and hindleg tarsus. Values are mean + S.E.M. Each frequency was calculated from the number of locusts in 5 group of each12 that responded within 1 s to each of the solutions, and each point is the mean of five replicates.

Table (3) Results of an analysis of testing the effects of 50 mM NaCl and test group on tarsus and femur hindleg .

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Tarsus	6.3333	6	.5164	.2108
	Femur	3.6667	6	.5164	.2108

T- Test (paired) comparing numbers of locusts responding to 50 mM NaCl Droplets on tarsus and femur for each replicate

hindleg. With sucrose however locusts ranged to evoke avoidance of 50% (1 M sucrose to 2 M Sucrose) for the fore leg and (1 M sucrose to 2 M Sucrose) for the hindleg. The duration's of the avoidance behaviour decreased with increasing concentration for NaCl and weaker for sucrose. Responses evoked due to water droplets used as a control solution amounted to an overall percentage of 0.01% among all tested animals.

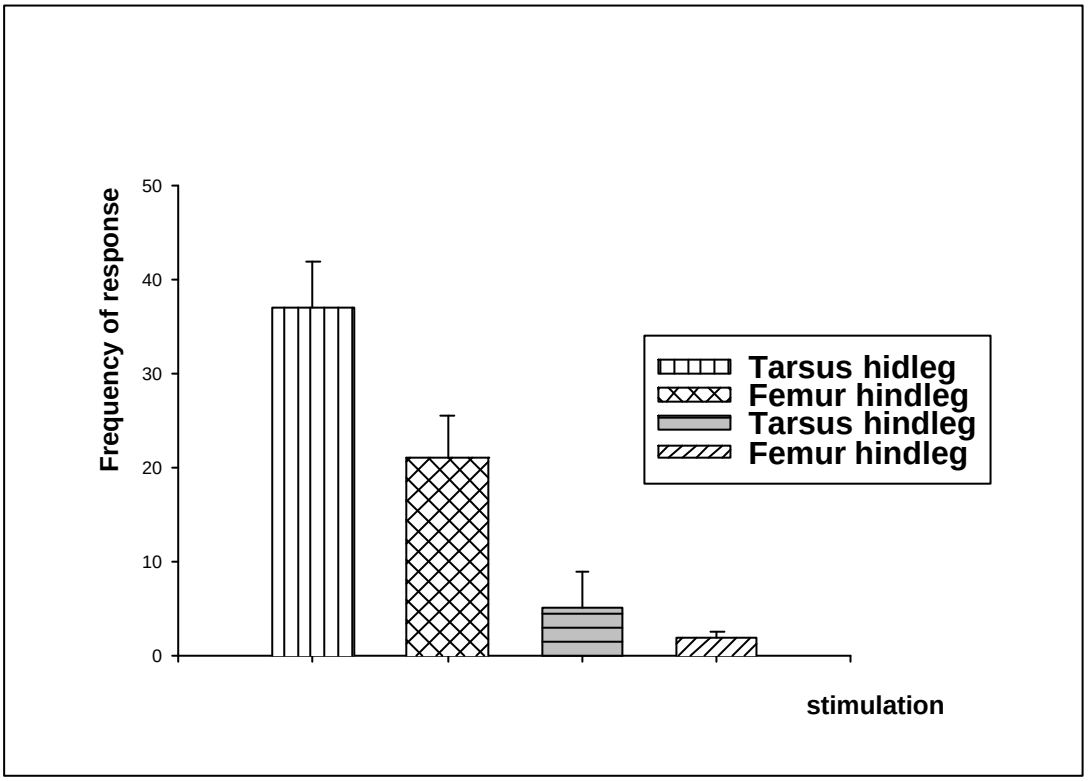


Fig. (48) The frequencies of replacement or withdrawal responses pooled vary with 50 mM NaCl concentration and water on the hindleg tarsus and femur. Values are mean + S.E.M Each frequency was calculated from the number of locusts in 5 group of each12 that responded within 1 s to each of the solutions, and each point is the mean of five replicates.

3.7.3 Frequencies of response for the hindleg tarsus and femur to Nacl solution:

The response of locusts to NaCl solution at a concentration of 50 mM NaCl and water applied as a droplet on both the tarsus and femur of the hindleg was studied. Results (Table 3 and Fig. 48) indicated a significantly higher avoidance response expressed by the tarsus in comparison to the femur, (t value=8*). Responses evoked by water droplets used as a control solution amounted to an overall percentage of .06% among all tested animals, no significant differences were recorded between both the tarsus and femur (t value= 1.46) (Table 4).

Table (4) Results of an analysis of testing the effects of water and test group on tarsus and femur hindleg.

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Tarsus	.8333	6	.7528	.3073
	Femur	.3333	6	.5164	.2108

T- Test (paired) comparing numbers of locusts responding to water Droplets on tarsus and femur for each replicate

4.0 Discussion

This work in its first part is the first investigation of the central projection of leg contact chemoreceptors in locusts or other insects. It focussed on the tarsus of the fore-and mesothoracic leg of the locust, using neurobiotin. The fine structure and distribution of various types of tarsal sensilla in the desert locust were investigated with scanning electron microscope. Another focus was on the processing of different chemical stimuli by the local circuits that control the leg movement in the prothoracic and mesothoracic ganglion of *L. migratoria* and *S. gregaria*. Specifically the antagonism between the levator and depressor tarsi was studied. Behavioural as well as physiological responses of the pro and hind leg were compared. The type of response of pulvillar basiconic sensilla to different chemicals were analysed.

General and specific features of the central projections of chemoreceptors from the pulvillus, a highly specialized region of locust legs could be revealed only after a method of staining very thin axons from neurons of basiconic sensilla had been recently established through this work.

The extent of the somatosensory map of tactile hair afferents and contact chemoreceptors on the middle leg and mesothorax as revealed by this study, extends the scope of earlier work by Mücke and Lakes-Harlan (1995) in which only the mechanoreceptors of 3 middle leg segments were analysed without finding the presence of any anterior-posterior organisation central projection.

The basiconic chemoreceptive sensilla of the tarsal pulvilli should record the chemical composition of the surface, of the substrate but it is not known what the adequate stimuli are and which regular behavioural responses occur. Good understanding of how different tastes are coded at the level of individual receptors (Blaney, 1974, 1975; Maes and Harms,

1986; Maes and Rufiok, 1986) has been achieved, but little is known of how and where chemosensory information from the contact chemoreceptors of the basiconic sensilla is processed, how different tastes are coded in the CNS, or how chemosensory information is integrated with signals coding other senses. This work has increased our knowledge regarding these points.

4.1 Mapping of sensory neurones

We have demonstrated that the sensory neurons from both basiconic sensilla, and canal sensilla and trichoid sensilla on the middle legs of locusts are organized into parallel and largely overlapping somatosensory maps within the mesothoracic ganglion and that the position of the sensilla on the leg is the major correlate of the destination of its sensory projection. Furthermore, because all the sensory neurons from individual basiconic sensilla terminate within the same region of neuropil it appears that not only are all mechanosensory neurons arranged somatotopically, but that the gustatory neurons from these sensilla also follow a closely similar organisation (Newland et al, 2000).

The extent of the somatosensory map of tactile hair afferents on the middle leg and mesothorax revealed by this study extends the scope of earlier work by Mücke and Lakes-Harlan (1995) in which only the distal most 3 leg segments were analysed and was unable to demonstrate the presence of any anterior-posterior organisation. It has now been established that there is a complete 3-dimensional mapping of tactile hair location on the middle leg encompassing proximo-distal, anterior-posterior and dorso-ventral axes, all of which are faithfully represented in the mesothoracic ganglion.

In this respect the arrangement of tactile hair afferents from the middle leg closely resemble that of tactile hair afferents on the hind leg as described

by Newland (1991) and follows the well-established pattern for leg bristle afferent sensory projections described in the study of other insects (e.g. Johnson and Murphey, 1985; Murphey et al., 1989b; Pflüger et al., 1981). Establishing this framework for the sensory projections of tactile hair afferents allowed us to then compare this map with the unknown projections from the bimodal basiconic sensilla, which we have shown to be organisationally and spatially similar to that of the tactile hairs. This has implications for the organisation of both exteroceptive and contact-chemosensory processing within the thoracic ganglia.

4.1.1 Exteroceptive organisation and processing

Previous physiological studies had already provided some evidence to support a close spatial association between the processes of mechanosensory neurons from both trichoid and basiconic sensilla. Spiking local interneurons, which are responsible for much of the initial processing of sensory signals in the thoracic local circuits of insects (Burrows and Siegler, 1982, 1984), receive monosynaptic inputs from the mechanosensory neurons innervating both basiconic and trichoid sensilla (Siegler and Burrows, 1983; Newland and Burrows, 1994; Burrows and Newland 1994). These local interneurons have specific receptive fields, determined by the pattern of sensory inputs they receive from mechanosensory afferents on different parts of the leg. Both types of sensilla are freely intermingled over the surface of the leg and the receptive field of any given spiking local interneuron is similar for both classes of mechanosensory afferent. The input branches of spiking local interneurons are largely restricted to the same regions of the neuropil as the tactile hair somatosensory map (Newland, 1991), and their receptive field properties are strongly correlated with the pattern and degree of overlap their branches make with the tactile hair afferents (Burrows and Newland, 1993). Since both tactile hair afferents and their target postsynaptic neurons follow a somatotopic organisation and since these

same interneurons also receive mechanosensory inputs from basiconic sensilla, then a similar somatotopic projection pattern of at least the mechanosensory afferents from basiconic sensilla would be the most parsimonious arrangement possible.

4.1.2 Chemosensory afferent organisation

The more surprising finding of this study was that all the sensory neurons from basiconic sensilla, both mechano- and chemo-sensory, projected to the same regions of the lVAC as determined by the location of the sensilla on the leg. We could find no anatomical evidence to support either a spatial partitioning, or any other differences in sensory neuron structure, such as neurites with different diameters or branching patterns, consistent with a differentiation of the two modalities of neuron.

The few studies that have examined the responses of basiconic sensilla on the legs of insects suggest that there is no systematic variation in the chemosensory responses of the sensory neurons of basiconic sensilla from different locations on the leg (Blaney and Winstanley, 1980; White and Chapman, 1990). It is therefore likely that the organization we describe is somatotopic and does not arise coincidentally from differences in chemosensory specificity of receptors on different locations of the leg. Anatomical techniques alone, however, cannot rule out specificity of synaptic connections within apparently similar fields of branches.

Much less is known about the initial stages of chemosensory integration by the local circuits in the thoracic ganglia compared to mechanosensory processing, but there is some new evidence that at least some chemical stimuli may be processed by the same neurons as exteroceptive stimuli. First, the mechanosensory receptive field of spiking local interneurons largely coincide with the chemosensory receptive field determined by targeting acidic vapours to different parts of the leg (Newland 1999).

Second, for any particular interneuron the polarity of both mechano- and chemo-sensory inputs (excitatory/ inhibitory) from receptors on specific regions of the leg is always the same (Newland 1999). It has also been shown that just as spiking local interneurons receive monosynaptic mechanosensory inputs (Burrows, 1992) they also appear to receive monosynaptic inputs from chemosensory neurons from the same bimodal basiconic sensilla (Newland 1999). The leg withdrawal reflex performed by locusts on stimulation with acidic vapours (Newland, 1998) or a droplet containing sufficient concentrations of other chemicals (Gaaboub and Hustert, 1998; Rogers and Newland 2000) is closely similar to the withdrawal reflex following tactile stimulation (Pflüger, 1980; Siegler and Burrows, 1986) and suggests that there may be a similar underlying neural organisation. It is still not clear, from physiological data however, whether all chemosensory stimuli are processed by these same interneurons or whether only certain classes of chemosensory neuron synapse onto the midline spiking local interneuron population. The somatotopic projections of all basiconic sensilla afferents to the same region would tend to support the former proposition.

4.1.3 Comparison with other insects

The data shown here contrasts with the data presented in other studies examining the sensory projections of bimodal sensilla on the legs or mouthparts of other insects. Few systematic analyses have been performed on other insects, and such data as is available is sometimes contradictory. Murphey et al. (1989a) suggested that in *Drosophila,* afferents from tactile bristles formed a clear somatotopic map of the proximo-distal leg axis in the mesothoracic ganglion. In a separate analysis of the central projections of contact chemoreceptors (bimodal sensilla) on the tarsi of the fore leg, Murphey et al. (1989b) suggested that there was a spatial segregation of sensory neurons with different modalities. This study did not address the problem of the proximo-distal

mapping of sensory neurons from contact chemoreceptors over the entire leg. It did show, however, that that the putative mechanosensory neuron from gustatory sensilla projected to the same area as tactile bristle afferents from the tarsus (Murphey et al., 1989a). It has commonly been found in studies of the sensory projections of bimodal sensilla in Diptera that one of the afferents is of larger diameter than the others (Yetman and Pollack 1986; Murphey et al., 1989b; Edgecomb and Murdock, 1992) and it was this afferent that projected to the same region as tactile bristle afferents.

It is possible that chemosensory projection patterns are organised in a different way in the Orthoptera and Diptera, as there is clear evidence of a spatial separation of presumed mechanosensory and chemosensory neurons on the labella (mouthparts) of flies. There are 11 identifiable long contact chemoreceptors on the labellum of the blowfly, each innervated by a single mechanosensitive afferent and four chemosensory afferents, all with different chemical sensitivities (Dethier 1976). The central projections of the thicker, presumed mechanosensitive, afferents from these contact chemoreceptors formed a discontinuous map that reflected the spatial position of the sensillum on the labellum (Yetman and Pollack, 1986; Edgecomb and Murdoch, 1992). The remaining, presumably chemosensory, neurons projected predominately to a more ventral and medial region of neuropil, although some sent processes to the brain. Sensory projections from contact-chemoreceptors on the mouthparts of locusts, which are involved in making detailed assessments of food quality, may have a different neural organisation than found in the thoracic ganglia and more closely resemble the organisation seen in the Diptera.

4.1.4 Implication for chemosensory processing by the thoracic ganglia of locusts

The repeated representation of chemosensory neurons with similar sensitivities in different spatial locations in a purely somatotopic map shows that gustatory processing in the thoracic ganglia is organised in a very different manner compared to olfaction in locusts. There is increasing evidence that the antennal lobes, the primary olfactory neuropil in insects, are arranged in an odotopic manner (Vickers et al., 1998). Particular functional classes of olfactory receptor neurons with similar sensitivities project to the same compartment, or glomerulus, in the antennal lobe regardless of the position of the olfactory receptor on the antenna. Indeed, in some insects, the antennal lobes also receive sensory projections from subsidiary olfactory organs located elsewhere on the head, such as the maxillary palps of Diptera (de Bruyne et al., 1999). Different odours are represented by the patterns of activity across the total population of glomeruli.

In contrast to the convergence of all olfactory receptor neurons onto the same integrative region, the initial processing of contact chemosensory signals by the thoracic ganglia appears highly redundant. Chemosensory stimulation of basiconic sensilla on the leg is said to evoke local reflex movements, which are always similar regardless of the chemical used, even if the chemical is a nutrient or other phagostimulant (Rogers and Newland, 2000). Chemical identity and concentration strongly affect the probability of occurrence of this response, and blends of different chemicals may increase or even decrease the likelihood of response compared to the constituents applied individually. Clearly, local circuits controlling leg movements use information about chemical identity and concentration to determine whether to perform a leg withdrawal response, but it is perhaps unlikely that local circuits in the thorax encode individual chemical qualities, and instead use a generic index of aversiveness in

reaching a decision. It is then possible that both mechanosensory and chemosensory information may be combined in the same local circuits at this level.

4.2 Structure and distribution of tarsus sensilla

Four different specific hairs were identified and classified as sensillum, canal sensillum, trichoid sensillum and campaniform sensillum. These sensilla are defined by their sizes, shapes and distributions on the tarsus as contact chemoreceptors or mechanoreceptors. Two types of receptors (Kendall 1970): i) tiny basiconic sensilla distributed on each pulvillus are the contact chemoreceptors with an open pore at their tip and ii) the mechanosensory canal sensilla, which are campaniform sensilla, are embedded deeply in the soft structure of the pulvilli. They lie scattered among the basiconic sensilla. The canal sensilla monitor deformations of a pulvillus depending on the load of the leg (Laurent and Hustert 1988; Gaaboub and Hustert 1998). The number of basiconic sensilla and canal sensilla are higher on the pulvillus of the fore leg than on the pulvillus in the middle leg and hind leg. The number of probable chemoreceptors on the tarsi of *Schistocerca gregaria* and *Tettigonia viridissima* are similar to those in most calyptrate flies and there are about twice as many on the forelegs as on the hind legs. On each foreleg there are about 1000 chemosensitive neurones compared with 500 on each hind leg. This explains that fore leg is more sensitive than hind leg (Kendall, 1970; Chapman, 1982).

The density of basiconic sensilla in both fore and middle pulvillus is higher in the middle of the pulvillus. But, the canal sensilla is more dense in the lateral side. This means that the chemoreceptive stimulation is stronger in the middle of the pulvillus and the mechanoreceptive stimulation is stronger on the lateral side.

4.3 Chemosensory stimulation

Stimulation of the pulvillar basiconic sensilla by different types of chemicals such as salts (NaCl), sugars (sucrose or glucose), acid (citric acid), alkaloid (quinin, soladinin and NHT) elicits a response from 1, 2 or 3 neurons that are distinct from the cell that responds to nicotine hydrogen tartrate. This verifies the study by (White and Chapman, 1990). The afferent and higher order neuron impulse trains, which normally have certain regularity in their interspike time intervals, are temporarily distorted after the application of higher concentration chemicals and unusual highly irregular firing frequencies or bursting activities occur (especially with citric acid). After a few seconds of stimulation, the firing pattern often develops into low burstlike activity. This irregular impulse patterns or bursting activity in sensory neurons are generally considered to reflect injury effects on sensory neurones.

Our observation shows that the stimulation with citric acid vapours appears to activate the sensory neurones in advance of contact. It is assumed that these uniporous chemoreceptors (pulvillar basiconic sensilla) detect some chemicals by olfaction.

According to the study of (Newland, 1998) noxious acidic vapours appear to activate the sensory neurones that respond also to salt solutions, based on the similarity in spike amplitudes evoked during both odour stimulation and contact with salt solution. Also (Dethier, 1972) found that basiconic sensilla on the legs of locusts can be stimulated with vapours of acids in common basiconic sensilla on the mouthparts and legs of blowflies. The above results and the result of (Städler and Hanson, 1975) who showed that contact chemoreceptors on the maxillae of Manduca also respond to odours of food plants contradicts the findings of (Laurent and Naraghi, 1994) that the basiconic sensilla do not have olfactory capabilities similar to olfactory receptors on the antennae.

Although we have a good understanding of how different tastes are coded at the level of individual receptors (Blaney, 1974, 975; Maes and Harms, 1986; Maes and Rufiok, 1986), we still know little of how and where chemosensory information from the contact chemoreceptors of the basiconic sensilla is processed, how different tastes are coded in the CNS, or how chemosensory information is integrated with signals coding other senses (Newland, 1998). Part of the underlying problem in trying to analyse taste reception is rapid adaptation of the sensory neurones. This adaptation means that it is not possible to consistently evoke spikes in the chemosensory neurones over periods sufficiently long to analyse the patterns of their central connections. But, now, through the chemosensory mapping of the basiconic sensilla, our knowledge regarding the above mentioned points has increased. The input branches of spiking local interneurones are largely restricted to the same regions of the neuropil as the contact chemoreceptors and their receptive field properties are strongly correlated with the pattern and degree of overlap their branches make with the chemosensory afferent.

It has been shown that the contact chemoreceptors tarsal sensilla of the locust have an important role in many aspects of the insect's life. They help it in the assessment of food materials, of oviposition site, or mating and these roles can be investigated morphologically and correlated with the insect behaviour (Blaney and Simmonds, 1990).

4.4 Neuronal pathways producing the avoidance reflex

An important feature of the gustatory avoidance reflexes is that the amplitude of the response in a motor neurone and interneuron changes depending on the type and concentrations of the chemical, which evoked the greatest effects in a motor neurone and interneuron. It is difficult to precisely quantify the responses of the motor neurones. Depressor motor neurones are, however, consistently depolarised more by chemical

stimulation. This could result from a number of factors that merit further investigation.

My preliminary data suggests that reflex response to specific chemical stimuli by applying small droplets onto single basiconic sensilla indicated that not all motor neurones of the depressor muscles in mesothoracic ganglia show similar responses. Some responded only to NaCl, others only to sugars, others responded to both stimuli at the same time. Of the local interneurones responding to tarsal chemical stimuli, several were sensitive to NaCl and glucose solutions. A different class responded only to a repellent agent (quinine hydrochloride). It is likely that this differential sensitivity of the sensory neurones themselves will contribute to changes in the strength of the reflex. Thus the greater the number of spikes in the sensory neurones the bigger the depolarization or hyperpolarisation in a motor neurone. Further explanations of this phenomena could be that 1) the density of receptors may be different on different areas of the leg, 2) the sensitivity of individual receptors may be different on different areas on the leg, 3) the strength of input from the sensory neurones onto central neurones may differ (White and Chapman, 1990; Newland, 1998). This means that receptors in one area will have a strong effect, whereas receptors in another surrounding area have a lesser effect at exciting an interneurone (Burrows, 1992; Burrows and Newland, 1994).

The different pools of tarsal motor neurones can be identified by their mechanosensory receptive fields (Laurent and Hustert, 1988). Similarly, the receptive fields of motor neurones could be identified by chemosensory stimulation. These receptive fields comprise excitatory and inhibitory response.

The excitatory response in some motor neurones occur due to direct excitation from the sensory afferents. The central projections of these afferents and motor neurones, which they excite overlap in the neuropil,

making possible the synaptic contact. Thus pulvillar basiconic afferents can make direct contacts with depressor tarsi motor neurones in the locust. This is similar to mechanosensory afferents in locusts and cockroaches (Pearson et al, 1976; Burrows, 1987a; Laurent and Hustert, 1988).

The inhibitory response in some motor neurones and interneurones could be explained by the time needed for a spike to be evoked in one interposed interneuron and its transmitter to be released. The most likely candidates for this inhibitory role are spiking local interneurons (Laurent and Hustert, 1988). They receive direct inputs from extero-and proprioceptors (Siegler and Burrows, 1983; Burrows, 1987a) and make direct inhibitory connections with certain motor neurones (Burrows and Siegler, 1982) and nonspiking local (Burrows 1987b; Burrows et al., 1988; Laurent and Burrows, 1988) and intersegmental interneurons (Laurent, 1987b).

The relationship between the chemosensory afferent and locomtion, oviposition, assessment of food materials is known. But, it is also found that a relationship between the chemosensory afferents and the flight motor neuron exists.

The chemical stimulation of the pulvillar basiconic sensilla causes antagonistic reflexes between the levator and depressor tarsi. We found that the levator is excited and the depressor is inhibited at the beginning of the stimulation (with sugar, salt and low concentration citric acid), but later the activity of the depressor tarsi is higher than the levator. In contrast, stimulation with high concentration citric acid inhibited the levator and excited the depressor tarsi. According to Laurent and Hustert (1988), the depressor motor neurones, active during the stance phase, are excited by ventral tarsal contact or an imposed levation and are inhibited by dorsal contact or an imposed depression. Partial differentiation of the anterior

tarsus reduces this stance phase depressor activity. The levator motor neuron, active during the swing phase, has the opposite receptive field. The retractor unguis motor neurones, synergistic to the depressors, are like them, excited by ventral contact but, like the levator, are inhibited by afferents which can signal the end of the stance phase of the inhibition of the retractors, could constitute a preparation for the swing phase, by reducing the grip on the substrate. The motor neuronal receptive fields thus appear to support the patterns of muscular activity recorded during walking.

4.5 Behavioural responses to stimulation with chemical solutions

Stimulation of the pro and hind legs by different concentration of NaCl or sucrose leads to a local avoidance movement with a short latency, produces high-velocity movement of several segments and responses that are present in the leg motor neurones. All observed behavioural responses occurring within 1s of stimulation by chemical solutions were avoidance response, and the likelihood of evoking a response was strongly linked to both chemical identity and concentration. Moreover, the frequency of response appeared to be a function of the combination of chemicals present in the stimulus and could not be simply predicted from the response to its individual constituents. The responses were broadly similar regardless of the chemical used in the droplet.

The sensitivity of the withdrawal reflex in prothoracic leg is higher than in the hind leg for stimulation by NaCl or sucrose, and the sensitivity of the withdrawal reflex in tarsus of the hind leg is higher than in the femur of the hind leg for stimulation by NaCl. Neuroanatomical data, obtained by backfilling with neurobiotin from single basiconic sensilla of the tarsus may hint at one possible cause: a considerably denser branching and termination density of the chemosensory afferents is seen in the prothoracic ganglion than in meso and metathoracic ganglion (the same tendency is seen for prothoracic afferents from mechanosensory canal

sensilla). Apparently, the prothoracic legs are more adapted to identifying chemicals than middle and hind leg, and possibly help the function of the highly chemosensory palps of the mouthparts (Gaaboub and Hustert, 2000).

There have been few investigations of chemoreceptors on the legs of insects, but the information available show that nearly all such sensilla are concentrated on the tarsi. In *Phormia regina*, (Grabowski and Dethier, 1954) found no chemoreceptors on the femora and only 9% of the total numbers on the legs were on the tibia; in *Musca domestica* 2% and 8% on the femora and tibia respectively (Dethier, 1955). (Sutcliffe and Mclver, 1976) record some chemoreceptors distally on the tibiae of four *Simulium* species, but the majority are on the tarsi, and (Owen, 1971) observed none on the femora or tibia of *Anopheles atroparvus.* The number of probable chemoreceptors on the tarsi of *Schistocerca gregaria* and *Tettigonia viridissima* are similar to those in most calyptrate flies and there are about twice as many on the forelegs as on the hind legs. On each foreleg there are about 1000 chemosensitive neurones compared with 500 on each hind leg. This explains that fore leg is more sensitive than hind leg (Kendall, 1970; Chapman, 1982).

Sodium chloride differed from the other test chemicals in that higher concentrations led to a large decrease in both the latency to response and the duration of the movement. The duration of the avoidance response to acetic acid odour found by (Newland, 1998) was 193$\pm$11.6ms, which compares closely with the mean duration of responses to 100 mm NaCl of 170$\pm$20ms. The mean movement duration in response to the other chemicals was over 100 ms longer. It remains to be seen whether these apparent differences in response dynamics, between rapid responses to NaCl (and possibly acetic acid) and the longer time course of responses to the other chemicals, reflect differences in the central processing off these chemical stimuli (Rogers and Newland, 2000)

The form of the avoidance behaviour evoked by chemosensory stimulation differs from the leg movements that take into account the spatial location of the stimulus. For example, mechanical stimulation of basiconic sensilla afferents on the dorsal tarsus result in an avoidance reflex of the leg in which the trochanter/femur, the tibia flexed and the tarsus levated. Conversely, when the ventral tarsus is stimulated the trochanter/femur is again levated but this is accompanied by an extension of the tibia and a depression of the tarsus (Siegler and Burrows, 1986). Different avoidance reflexes are produced when other leg surfaces are stimulated. The movements caused by stimulation of the chemoreceptors, however, are always the same irrespective of where the stimulus is applied to the leg.

(Blaney and Ducket, 1975; Cook, 1977) suggested that high concentrations of salt solutions act as an antifeedant to locusts. Moreover, (White and Chapman, 1990) showed that a locust will move its leg away from antifeedants such as nicotine hydrogen tartrate and salt solutions, and held or waved above the substrate for longer periods when compared to similar tests using sucrose or water. Stimulation by NaCl or sucrose to the dorsal tarsus of hind leg of a locust, which was free to move caused the locust to raise that leg rapidly, by levating the tarsus, flexing the tibia and levating the femur. Withdrawal of the leg occurred with a very short latency and with high-velocity movements of the different leg segments (Newland, 1998). This response may therefore represent a more extreme form of the aversive behaviour previously described for the locust (White and Chapman, 1990).

The applied droplets unavoidably combined both mechanical and chemical stimuli and may be expected to have activated exteroceptive and proprioceptive mechanosensory neurones. Stimulation of individual tactile hairs is a fairly weak stimulus and only infrequently evokes withdrawal movements (Pflüger, 1981). It seems likely that the response frequency to water and to the lowest concentrations of the test chemicals

(approximately 10-20% of cases) represents a baseline level of response to the mechanical component of the stimulus. It is possible, however, that there is some hygroreceptive or other sensory input onto the neural pathways controlling the reflex, but this does not appear to have a strong influence on withdrawal behaviour. The movements evoked by chemical stimulation are very similar to the responses to tactile stimulation (Rogers and Newland, 2000).

5.0 References

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List of Abbreviations used

AVC	anterior ventral commissure
Ba	basiconic sensilla
Ca	canal sensilla
D	dorsal
DC I-VI	dorsal commissures I-VI
dDCI	dorsal part of DCI
vDCI	ventral part of DCI
Dep	depressor
DIT	dorsal intermediate tract
DMT	dorsal median tract
dDMT	dorsal part of DMT
vDMT	ventral part of DMT
DuMtr	tract of dorsal unpaird median (DUM) cells
FETi	fast extensor tibiae (motoneuron)
IN	interneuron
LAC	lateral association centre
aLAC	anterior LAC
pLAC	posterior LAC
LDT	lateral dorsal tract
Lev	lavtor
LVT	lateral ventral tract
iLVT	inner LVT
oLVT	outer LVT
MDT	median dorsal tract
Mesh	mechanoreceptor
MESO	mesothoracic ganglion
META	metathoracic ganglion
MN	motoneuron
mtr	midline trachea
MVT	media ventral tract
N	Nerve
N 1-5	peripheral nerves 1-5 (different roots are indicated i-v)
PRO	prothoracic ganglion
PT	perpendicular tract
PVC	posterior ventral commissure
Pul	pulvillus
RT	ring tract
aRT	anterior part of ring tract
SMC	supra median commissure
TT	T-tract
Ta	tarsus

Tr	trichoid sensilla
V	ventral
VAC	ventral association centre
aVAC	anterior VAC
lVAC	lateral VAC
mVAC	medial VAC
dmVAC	dorsal part of mVAC
vmVAC	ventral part of mVAC
vVAC	ventral most VAC
VC I-II	ventral commissures I-II
VCLII	ventral commissural loop II
dVCLII	dorsal part of VCLII
vVCLII	ventral part of VCLII
VIT	ventral intermediate tract
VLT	ventral lateral tract

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