Institut für Zoology und Anthropology
Georg-August Universität
Göttingen - Germany

Neural Processing of Chemosensory Information from the Locust Ovipositor

Dissertation
zur Erlangung des Doktorgrades
der Mathematisch – Naturwissenschaftlichen Fakultäten
der Georg – August – Universität zu Göttingen

Vorgelegt von

Ehab Mostafa Tousson

Aus Damanhour / Ägypten

Göttingen 2001

D7

Referent : Prof. Dr. R. Hustert

Korrefent : Dr. R. Lakes – Harlan

Tag der mündlichen Prüfung : 03.05.2001

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Abbreviations:

272	depressor muscle of the ventral valve
273	adductor muscle of the ventral valve
5-HT	serotonin
7^{th} Ag	7^{th} abdominal ganglion
A	anterior
ax	axon
Ab8	abdominal neuromere 8
Ab9	abdominal neuromere 9
Ab10	abdominal neuromere 10
Ab11	abdominal neuromere 11
AS	abdominal segment
Asp.N	spermathecal aperture
AVC	anterior ventral commissure
Axb	afferent axons of basiconic sensilla
Chem. r.	contact chemoreceptors
ChSIN	chemosensitive interneuron
Cn	common nerve
CNS	central nervous system
cm, mm	centimeter, millimeter
CP	central projection
D	dendrite
DAB	3,3`-diaminobenzidine tetrahydrochloride
Db	dendrites of basiconic sensilla
DC I -VI	dorsal commissure I -VI
DIT	dorsal intermediate tract
D.Ov.Va	dorsal ovipositor valve
Dsp. n	spermathecal duct
hr	hour
HS	hair shaft
IN	interneuron
IV	intermediate ovipositor valve
L	lateral
µm	micrometer
ml	millitre
M, mM	molar, millimolar
NaCl	sodium chloride
Mech. r.	mechanoreceptor

MS	multipolar cells
ms	millisecond
mv	millivolt
MVT	median ventral tract
R8st	right 8th sternal nerve
R8tg	right 8th tergal nerve
R8Vn	right 8th ventral nerve
R9Vn	right 9th ventral nerve
S	sensory
s	second
SEM	scanning electron microscope
SMC	supermedian commissure
So	single soma
Sob	sensory somata of basiconic sensilla
Tg	terminal abdominal ganglion
TT	T – tract
V	ventral
VAC	ventral association centre
VIT	ventral intermediate tract
VLT	ventral lateral tract
VMT	ventral median tract
V.Ov.Va	ventral ovipositor valve

Abstract

Contact chemoreceptors (basiconic sensilla) located on the ovipositor and genital segments of the locust serve to control the chemical features of the substrate before and during oviposition. They occur dispersed and also crowded in fields between mechanosensory exteroceptors sensitive to touch or wind (trichoid and filiform sensilla). The central nervous projections of their four chemosensory and one mechanosensory neurons from single basiconic sensilla were stained selectively, focussing on receptors on the ovipositor valves, which usually contact the substrate during the pre oviposition probing movements. All axons and neurites from one contact chemoreceptor usually stay close together in most of their projections. Segregation occurs mainly when single axons terminate in one neuromere while the others proceed to a different neuromere or ganglion. For projections from one chemoreceptor, there is evidence neither for functional segregation of mechanosensory from chemosensory afferent terminals nor for specific segregation between different chemosensory afferents. The projections from sensilla of dorsal cuticle tend to project rather uniformly along the midline of the terminal ganglion. Comparative staining of touch and wind sensitive hair receptor neurons shows mostly central projections, similar to those of neighbouring contact chemoreceptors. From the typical intersegmental projections of most primary afferents and from the lack of segregation into glomerular structures, it is concluded that integration of chemosensory information from the genital segments is distributed in the terminal and the 7th abdominal ganglion.

Signals from the ovipositor receptors could influence the various modes of behaviour of ovipositing locusts. The basiconic sensilla on the ventral ovipositor valve responses to different attractant or repellent chemicals. Responses were seen to aqueous solutions of salts (NaCl, 0.01 M to 3,0 M), sugar (glucose, 0.01 M to 3,0 M), acids (citric acid, 0.01M to 1,0 M), oviposition aggregation pheromones (veratrole and acetophenone

at 1,0% and 0,1%), neurotransmitters (GABA, 0,1%), neuromodulators (serotonin, and octopamine at 0,1%), alkaloides (quinine and tomatine at 0,1%) and phenolic compounds (salicin, 0,1%). With the classical tip recording and stimulation method (Hodgson et al 1955) usually three sensory neurons of these sensilla were excited, followed by rapid adaptation at lower concentrations. Local and ascending interneurones of the terminal abdominal ganglion process chemosensory information from the ventral ovipositor. This study focussed on interneurons extending in the anterolateral region of the eighth abdominal neuromere with some of their collaterals ascending to more anterior abdominal ganglia. The projecting interneurons of these respond only to one or two chemical substances (sugars or salts, or salts and acids together) with excitatory or inhibitory responses. The physiological and morphological differences between the interneurons suggest that there is no specific center for processing taste information in the locust terminal ganglion

Keywords

Chemoreception · Genital segments · Interganglionic projections · Sensory neurons · Extracellular recording . Interacellular recording . Intrneurones . *Locusta migratoria*

Introduction:

All animals react to chemicals in the environment, initially through a sensory process called chemoreception. The process begins when chemical stimuli make contact with the body and transduce the immediate effects of such substances into nerve impulses. Insects live in an enviroment that continually requires appropriate responses to various types of sensory information. Common stimuli that are available to them include brightness, patterns and colour of light, sound mechanical contact, gravitation, temperature, odours, tastes and textures. An insect's response to these depends on their specific requirements for food, shelter, escape reactions, mating, or oviposition. Some insects are able to use only a limited number of stimuli whereas others have more complex capabilities and often utilise combinations of stimuli to initiate or reinforce behavioural patterns. Some stimuli, however are used almost universally by insects, as evidenced by the widespread occurrence of the special structures required to receive these stimuli. Chemical stimuli belong to this group, and one can find evidence of specialised receptors for chemical recognition in any insect studied. The antennae are the most obvious location for chemical receptors, and they bear numerous smell receptors especially adapted for receiving specific chemical information. Chemically sensitive sensilla are not limited to antennae but are also found on all body parts or restricted to legs, mouth parts and ovipositor. The wide distribution and diversity of chemosensory sensilla in other animals besides insects have led to speculation that chemical recognition was the earliest sensory system evolved (Zacharuk, 1980).

In insects chemoreception plays a major role in a number of behaviours, including avoidance (White and Chapman, 1990), the detection and the selection of food (Dethier, 1976; Newland et al., 2000) the selection of egg-laying sites (Ma and Schoonhoven, 1973; Roessingh, et al. 1992; Stadler, et al. 1994, 1995; Dougherty, et al., 1995; Tousson and Hustert, 2000). Insects, like other animals have evolved chemical sensing devices

for detecting stimuli that have adaptive value, so that most of their receptors are sensitive to a specific range of available stimuli. Chemosensilla are primarily olfactory or gustatory, and these two categories have well defined and quite different structures. The differences are sufficiently marked to allow one to predict the function of a sensillum from its external and internal morphological structure. It was once thought that the olfactory and gustatory categories were mutually, exclusive but Dethier (1972) and Städler and Hanson, (1975) have shown that this is not invariably so. In fact, the transition from exclusively gustatory to exclusively olfactory sensilla includes sensilla sensitive to both kinds of stimuli.

Most olfactory receptors of insects are on the antennae, though some have been found on maxillary and labial palps. Those sensilla responsive to airborne chemicals typically have thin cuticular walls (Slifer, 1970) with many pores that allow contact between the dendrite within and the stimulus molecules. These multiporous sensilla (Kaissling, 1971; Boeckh, 1980; Zacharuk, 1980; Gaaboub, 1990) come in many shapes and sizes: hairs, domes, cones, and pegs, either projecting from the cuticle or surrounded by deep or shallow cuticular pits. The surfaces of these sensilla may have grooves or pits.

Gustatory chemosensilla are similar to olfactory receptors, but have two notable differences. The two kinds of receptor have in common terminal or subterminal, single or multiple openings which allow chemical communcation between dendrites and stimulus but in addition gustatory sensilla often have a mechanosensitive neuron associated with them. These sensilla generally have thick walls and because of their single opening are called uniporous sensilla. 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 (Zacharuk, 1980; Chapman, 1982).

Gustatory or contact chemosensory sensilla are short peg-like structures that are typically much shorter than the tactile hairs amongst which they are often intermingled and act as multimodal receptors by responding to mechanical stimuli and to contact with chemicals through their terminal pore (Chapman, 1982; Dethier and Bowdan, 1989; Newland, 1998). 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* 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 sensory neurons of pure mechanoreceptors and of chemoreceptors with olfactory function have been studied extensively as to their central projections mainly in orthopteran, lepidopteran insects. Projection patterns of mechanosensory neurons in the central nervous system (CNS), often follow topological rules that can be related the distribution of the sensilla on the body, especially from the appendages (Pflüger et al., 1981; Johnson and Murphey, 1985; Newland and Burrows, 1994). In contrast, olfactory chemoreceptors of the insect antennae segregate their projections according to functional principles into a few or many glomerular centers of the brain (review: Hildebrand and Shepherd, 1997), often corresponding to the specificity of the sensory neurons for classes of chemicals or smells.

In locusts, contact chemoreceptors develop everywhere on their cuticle, but only the type of basiconic sensilla is seen on the body and appendages interspersed between other sensory, mostly mechanosensory hairs. Projections from single insect contact chemoreceptors are known only from circumstantial or preliminary data (Murphey et al., 1989; Newland, 1998; Tousson and Hustert, 1998) mostly due to technical problems. Basiconic sensilla are very short (10-30 µm) and their afferent axons have diameters below 1µm, and each is supplied with several chemosensory neurons and one additional mechanosensory neuron. Stimulation with acids and alkaloids elicits grooming and avoidance behaviour (Levefbre, 1981; White and Chapman, 1990; Newland and Burrows, 1994). Many sites of regular contact with the substrate usually have dense fields of basiconic sensilla: the mouthparts with the palps (Fudalewicz-Niemcyk. et al 1980), the ventral tarsus (Kendal, 1970; Laurent and Hustert, 1988; Gaaboub and Hustert, 1998) and the ovipositor valves (Kalogianni, 1995, 1996; Tousson and Hustert, 1998) as well as adjacent sclerites and cerci. The present study was begnin to find out about rules that might apply for the central projections of the sensory neurons from locust contact chemoreceptors on the genital segments, a region of the body that is important for detecting the chemical composition of the soil before and during oviposition (Kalogianni and Burrows, 1996).

In *Locusta migratoria*, the ovipositor is a highly specialized structure comprising heavy cuticular appendges, hinges and large muscles. It extends beyond the tip of the female abdomen, where it appears externally as two pairs of shovel – shaped structures called ovipositor valves (Thomas, 1965; Thompson, 1986a, b). Snodgrass (1935) has shown that the two pairs of valves are hinged at their bases to each other and to a prominent pair of internal apodemes and that only ten pairs of muscles are involved in ovipositor movement. Thompson and Schabtach (1986) have found that, in the embryo of grasshoppers all of the

abdominal segments have pairs of ventral appendages, most of these disappear by hatching but the pairs on segments eight and nine of female are retained and modified throughout larval and adult development to become the ventral and the dorsal ovipositor valves respectively. Seabrook in 1968 showed that the ventral valve are innervated by the eighth ventral abdominal nerve and the dorsal valves are innervated by ninth ventral abdominal nerve and later in 1970 he described the general structure of the terminal ganglion of the locust *Schistocerca gregaria* as well as a number of neuronal tracts within this structure.

Bharadwaj and Banerjee (1971) described the nervous system of *Schistocerca gregaria* with a discussion on muscle innervation. The different receptor systems of the ovipositor of insects have been subject to extensive morphological and physiological studies. Though the locust has often been chosen for experiments, a detailed description of the innervation of the ovipositor valves and the distribution of the different sensilla and receptor organs as for *Schistocerca gregaria* (Seabrook, 1968) and other insects are still lacking. Detailed information is available only for the locust ovipositor muscles (Snodgrass, 1935; Thompson, 1986a, b; Belanger and Orchard, 1992, 1993 ; Facciponte, et al., 1995) and oviducts (Kalogianni and Pflüger, 1992; Kalogianni and Theophilidis, 1993).

Locusts search for suitable substrate before starting oviposition with the tarsal contact chemoreceptors of fore- and middle legs (prevailing chemosensory neurons for water, sugar, salt and alkaloids, White and Chapman, 1990) and of the tip of the abdomen. Then they probe and dig with their ovipositors into soil as deep as three inches, before the eggs are deposited. If soil conditions are unfavourable down there a female will retain from depositing its eggs and withdraw its abdomen (Kennedy, 1949; Choudhuri, 1956; Popov, 1958; Woodrow, 1964, 1965; Norris,

1968; Rose et al., 2000; Tousson and Hustert, 2000) and start digging again until suitable soil conditions are encountered at every stage. One condition which elicits this rejection response is unfavourable chemical composition of the soil (Woodrow, 1965). The discrimination probably depends upon chemoreceptors in the ovipositor valves (Dethier and Chadwick, 1948).

In the locust, contact chemoreception in the ovipositoir is attributed to basiconic sensilla and plays a decisive role in oviposition behaviour at all stages (Kalogianni and Burrows, 1996; Tousson and Hustert, 1998; Tousson et al., 1999)). The majority of the ovipositor hair receptors are mechanically sensitive either to air currents or touch. The locust ovipositor bears a number of extroreceptors which respond to different sensory modalities, that can be classified as mechanosensilla and chemosensilla (Kalogianni, 1995, 96).

Insect sensory receptors encode in their signals different types of information about the environment which can modify the animal's behaviour, these signals are integrated and distributed to several segmental ganglion by projection interneurones. Signals from the ovipositor receptors could influence the various modes of behaviour of ovipositing locusts. Tarsal contact chemoreceptors of fore – and middle legs (White and Chapman, 1990; Gaaboub and Hustert, 1998; Tousson et al., 1999) and consecutively the contact chemoreceptors of the abdomen and ovipositor valves inform the locust about suitable substrate for starting and maintaining digging and egg laying. The basiconic sensilla on the ovipositor of migratory locust are thier contact chemoreceptors and their five afferent sensory neurons project intersegmentally in the terminal abdominal ganglion and further into the 7^{th} abdominal ganglion (Tousson and Hustert, 1998).

In contrast to most other sensory systems the neuronal pathways processing tastes were poorly understood. Although the general pathways involved in the processing of taste signals were known, as are the modes of sensory transduction in the gustatory receptors themselves, we do not yet known how different chemicals, or tastes are coded and represented in the central nervous system or which interneurones are responsible for their processing. Part of the underlying problem is the relative inaccessibility and small size of the taste cells and the control neurones that process their signals (Roper, 1989) and their physiological properties. Nevertheless, a detailed understanding of chemoreception is essential, not only for a greater understanding of sensory processing and integration but also, in the context of insects, for an understanding of how insects select suitable oviposition site or the control of oviposition during digging and egg laying.

A technique to record neural responses from the tips of insect taste sensilla was first described by Hodgson et al. (1955). Ever since, several aspects of peripheral sensitivity of contact chemoreceptors have been studied. Recording from the single receptors have also been used to study differences in specificity between various receptor. The chemosensory afferents of the basiconic sensilla code different qualities of a chemical stimulus. In the fly, for example, different chemosensory afferents in one sensillum respond selectively to water, anions, salts and sugars (Dethier, 1976). More natural stimuli, such as plant extracts, appear to be encoded in an across fibre pattern in the responses of many afferents (Blaney, 1974, 1975). The signals from these chemosensory play a key role in eliciting various behavioural responses. For example in the fly stimulation of contact chemoreceptors on the tarsi leads to an extension of the proboscis (Dethier, 1976), and in the locust, stimulation of the chemoreceptors on the tarsi with an antifeedant leads to leg waving (White and Chapman, 1990). The

contact chemoreceptors can discriminate between different chemical cues and this information can generate different behavioural responses.

In 1965 Den Otter and Van Der Poel reported that in some labellar taste hairs of *Calliphora vicina* 0.5 M solutions of LiCl, NaCl, NH4Cl, KJ, KBr, and KNO3 evoked impulses of three different size classes, two of these impulse types originated in salt-sensitive cells, whereas the third type was supposed to originate from a water receptor. Dethier (1974) found four chemoreceptors neurons of the labellar setae of the blowfly *Phormia regina*. These have been termed sugar, water, salt (cation) and salt (anion) respectively. For the tarsi of insects, four types of chemosensory neurones for water, sugar, salt and alkaloids (White and Chapman, 1990) have been identified. Insects, like other animals have evolved chemical sensing devices for detecting stimuli that have adaptive value, so that most of their receptors are sensitive to a specific selection of stimuli available. Haskell and Schoonhoven (1969), Winstantely and Blaney (1978) and Blaney (1980) have shown that the sensilla on the tips of the palps and those in the epipharyngeal group respond to a range of salts, sugars. Haskell and Schoonhoven (1969) suggested that specific neurones were sensitive to each class of compounds, including one with maximal sensitivity to grass chemicals and one to repellent compounds. However Blaney (1974, 1975) concluded that most tested compounds caused a number of neurones in each sensillum to fire and that each neurone probably responded to a range of chemicals. She suggested that the sensillum is the sensory unit and showed that a measure of specificity occurred amongst sensilla. Winstanley and Blaney (1978) suggested that the sensilla on the palp tips of *Schistocerca gregaria* which respond positively to nicotine might be particularly sensitive to deterrent chemicals and so might provide labelled lines to the brain.

Almost allprevious 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.

Several electrophysiological studies of tarsal chemoreceptors have been carried out on flies although no surveys of responsiveness to a range of chemicals have been attempted. Ma and Schoonhoven (1973) studied the tarsal contact chemosensory hairs of the large white butterfly *Pieris brassicae* and their possible role in oviposition behaviour and established that *Pieris brassicae* females are stimulated at their ovipositor by some glucosinolates (Sinalbin, Sinigrin and Glucotroaeolin), they also identified the sensilla on the ventral side of the tarsus that contain a receptor sensitive to the three glucosinolates. In *Phormia regina* and *Delia brassicae* the results of McCutchan (1969) and Städler (1978) suggest the presence of separate cells responding to NaCl, Sucrose and in *Delia brassicae*, to water. McCutchan sometimes obtained a response in two cells when a sensillum was stimulated by NaCl, while Van der Starre (1972), working with *Calliphora vicina*, found that both water and sucrose stimulated a number of cells and considered that the tarsal chemoreceptors neurones exhibited a lack of specificity. Ramaswamy (1987) reported

that stimulating the tarsal sensilla of female *Heliothis virescens* with the sugars sucrose, fructose and glucose resulted in proboscis extension, this finding and earlier studies with *Pieris rapae* (Kusano and Sato, 1980) suggest that the tarsal sensilla of these species should contain neurones sensitive to different sugars.

Szentesi and Bernays (1984) observed that stimulation of the tarsi of *Schistocerca gregaria* by nicotine hydrogen tartrate elicited a behavioural response whilst Chapman et al (1988) suggested that detection of the host-specific chemical by tarsal chemoreceptors may be important in host-plant recognition by the monophagous grasshopper *Bootettix argentatus*. Newland and Burrows (1994) have shown that the mechanosensory neurones from the basiconic sensilla respond phasically to deflections of the receptor shaft and make excitatory monosynaptic connections with spiking local interneurones in the metathoracic ganglion. In 1998, Gaaboub and Hustert studied the tarsal contact chemosensory hairs of the migratory locust had shown their motor responses to chemical stimulation while Newland (1998) investigated in more detail the responses of basiconic sensilla on locust legs to stimulation with different odours, with acids being the most effective.

Several electrophysiological studies of ovipositior chemoreceptors have been carried out in insects. Wallis (1962a, 1962b) working on the blowfly *Phormia* has shown by electrophysiological methods that pegs on the ovipositor are sensitive to chemical stimuli and that they are important in oviposition. The ovipositor of sheep blowfly *Lucilia cuprina*, is provided with a number of chemosensilla containing at least three neurons sensitive to various chemicals (Rice 1976). One of these neurons was found to be specially sensitive to monovalent cations (Rice 1977). Rice suggested that these receptors play a role in the selection of suitable egg laying sites. Waladde (1983) found

chemosensilla, present on the ovipositor of *Chilo partellus*, to be sensitive to various salt solutions.

Responses of basiconic sensilla on locust ovipositor to stimulation with different odours could also be associated with pheromones. Acetophenone and veratole have been identified as two major behaviourally active components of the oviposition aggregation pheromone of the desert locust, *Schistcerca gregaria*. Rai et al. (1997) identified both compounds from the volatiles of egg pod froth by using gas chromatography-electroantennographic and gas chromatography-mass spectrometriy. They reported that, both acetophenone and veratole compounds can elicit aggregation of gravid females in oviposition bioassays therefore, each is a compenent of the oviposition phermone. Chapman and Ascoli-Christensen (1999) suggested that all the grasshoppers that have been examined electrophysiologically appear to have deterrent-sensitive neurons comparable with those present in some other phytophagous insects, and they conclude that the gustatory sensilla of grasshoppers contain neurons that provide qualitatively different information to the central nervous system and in this respect they are comparable with those of other insects.

Projection interneurones originating from the terminal abdominal ganglion and ascending to the anterior ganglia are known in crickets (Edwends and Palha, 1974; Kämper, 1984; Jacobs and Murphey, 1987 ; Kohstall and Gras, 1995; Kohstall, 1996; Gras and Kohstall, 1995,98), cockroaches (Dagan and parnas, 1970; Westin et al., 1977; Daley et al., 1981; Ritzman and Pollack, 1981), locusts (Boyan and Williams, 1989a; Boyan et al., 1989). They respond to different types of mechanical stimuli such as wind and sound applied to the cercal hair receptors. In the progress of the study presented here it became clear that taste receptors have also a large array of interneurones that integrate neurals aspects of contact chemicals and transfer their information to other ganglion. These,

as in the locust terminal ganglion itself decisions for resulting behaviour can be elicited.

The present work aims to illustrate:

1. The sensory innervation of ventral and dorsal locust ovipositor valves.
2. Types of receptors in the locust ovipositor valves.
3. The number and the distribution of ovipositor receptors.
4. The central projections of afferents from contact chemoreceptors of the ovipositor valves and surrounding structures.
5. The central projections of afferents from mechanoreceptors of the ovipositor valves and surrounding structures.
6. The stimulation effect of some chemicals on single chemosensory receptor.
7. Identification of chemosensory interneurones integrating chemosensory information of ventral ovipositor valves.

2.0 Materials and Methods

2.1 Animals:

All experiments were performed on sexually mature females of *Locusta migratoria* taken from our crowded laboratory cultures at 25 °C. on 12h light / 12h dark regime, and fed fresh wheat seedlings supplemented. Prior to dissection they were anaesthetized by cooling the preparation and all physiological experiments were performed at 26-28 °C.

2.2 Scanning electron micrographs:

To identify the chemoreceptors and the mechanoreceptors sensilla, scanning electron micrographs of the cuticle surface were taken. The terminal abdominal segments were usually rinsed 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 on a scanning electron microscope (SEM).

2.3 Neuroanatomical studies:

2.3.1 Cobalt chloride backfill

The distribution and peripheral innervation of locust contact chemoreceptors on the ovipositor valves and adjacent cuticle were revealed in whole-mount preparations with the cobalt chloride backfilling technique (Pitman et al., 1972), and consecutive silver intensification (Bacon and Altman, 1977).

An intact insect was anaesthetised by chilling on ice and then was mounted side down on a piece of non-toxic plasticine in Petri dish. The abdomen was dissected ventrally by an incision in the midline and the two sides of the body wall were pinned down laterally so that the abdominal cavity formed a pool which was filled with locust saline (Clements and May, 1974).

The two caudal ganglia in the abdomen (Seventh and terminal ganglia, also called the genital ganglia, which innervate the ovipositor valves), were exposed by an incision along the ventral midline. The oviducts were cut at their distal attachment site and fixed with pins at the anterior end of the abdominal cavity. The gut was stretched anteriorly and flattened with pins or removed, and the cut ends of the gut must be sealed with Vaseline to avoid leakage of its contents into the body cavity.

In order to backfill the peripheral nerves and the sensory neurons of the receptors on the valves, the cut ends of eighth ventral abdominal nerve innervating the ventral ovipositor valve (or the ninth ventral abdominal nerve innervating the dorsal ovipositor valve) was exposed to the cobalt solution. The stump was sealed in asaline-filled Vaseline pool and by using a hypodermic syringe, a droplet of distilled water are placed the saline to open the axons before applying 3-6% aqueous cobalt chloride (Fig. 1)

The exposed tissues must be sealed off to prevent desiccation, which was easily done with a layer of Vaseline. Only highly purified white Vaseline should be used, as standard Vaseline tend to contain toxic and oil impurities.

In these preparations, care has to be taken with localization and interpretation because the cobalt ions can migrate through leaks under the Vaseline pool and be picked up by other nerves and tissues. After applying the cobalt, the animals were kept restrained in a moist chamber at 4 °C for 3-4 day or in room temperature (approximately 25 °C) for 48-72 hours.

Before the cobalt solution is removed the animal may be placed in the cold for a few minutes to harden the Vaseline. The ventral and / or the dorsal

ovipositor valves are dissected free and rinsed in Ringer solution for 5 minutes.

The preparations were then transferred to ringer solution containing approximately one drop of concentrated (44%) ammonium sulphide per millilitre, for 10-20 minutes. A single preparation can be examined to see if the cobalt perception reaction has proceeded for enough. If the reaction was completed, all the remaining specimens in the ammonium sulphide solution could be removed and washed well in several changes of saline. If a longer reaction time was necessary, the preparations could be returned to the ammonium sulphide solution for addition reaction.

$$Co^{2+} + S^{2-} \longrightarrow CoS$$

Ammonium sulphide deteriorates with exposure to air, because NH4 was lost as ammonia, it is essential to used fresh ammonium sulphide. In the solution, first polysulphides could combine with cobalt to produce a soluble compound from cobalt sulphide.

The cobalt in the tissue was precipitated as cobalt sulphide, which was brownish-black and insoluble, and the cells that had taken up cobalt were displayed as black silhouettes on a clear background.

The valves were fixed either in Carnoy`s for 5-10 minutes or in alcoholic Bouin`s for at least 6 hours. The fixed specimens were dehydrated through a standard ascending alcohol series, starting at 30% after aqueous fixative and 70% after the alcoholic fixatives, 10 minutes in each concentration were sufficient and then the valves were cleared in methyl salicylate.

2.3.2 Timm`s sulphide-silver intensification (Bacon and Altman, 1977):

The specimens were brought to water through a descending alcoholic series, 5 minutes in each concentration was sufficient, then the specimens were warmed to 60 °C in distilled water for 5 minutes.

The specimens were soaked in the stock developer (20g Sacrose, 1.6g Citric acid, 0.34g Hydrochinon and 6g Gum arabic) at 60 °C for 1 hour and then transferred to 9 parts developer and 1 part 1% silver nitrate. This development was in the dark incubator at 60 °C. The specimens were transferred to a fresh solution every 20 minutes or as soon as a silver mirror begins to appear on the surface of the solution.

Preparations can be examined to see if the intensification has proceeded long enough. If the reaction was complete, all the remaining specimens in the silver nitrate developer solution could be removed. If a longer reaction time is necessary, the preparations could remain in the silver nitrate developer solution for additional incubation.

After intensification, the intensive by stained specimens were transferred to warm distilled water for 5 minutes to stopped the reaction, then cooled, and dehydrated through a ascending alcohol series, 20 minutes in each concentration was sufficient and then the valves were cleared in methyl salcylate. The specimens were drawn and photographed by using a camera Lucida attachment on a Zeiss compound microscope.

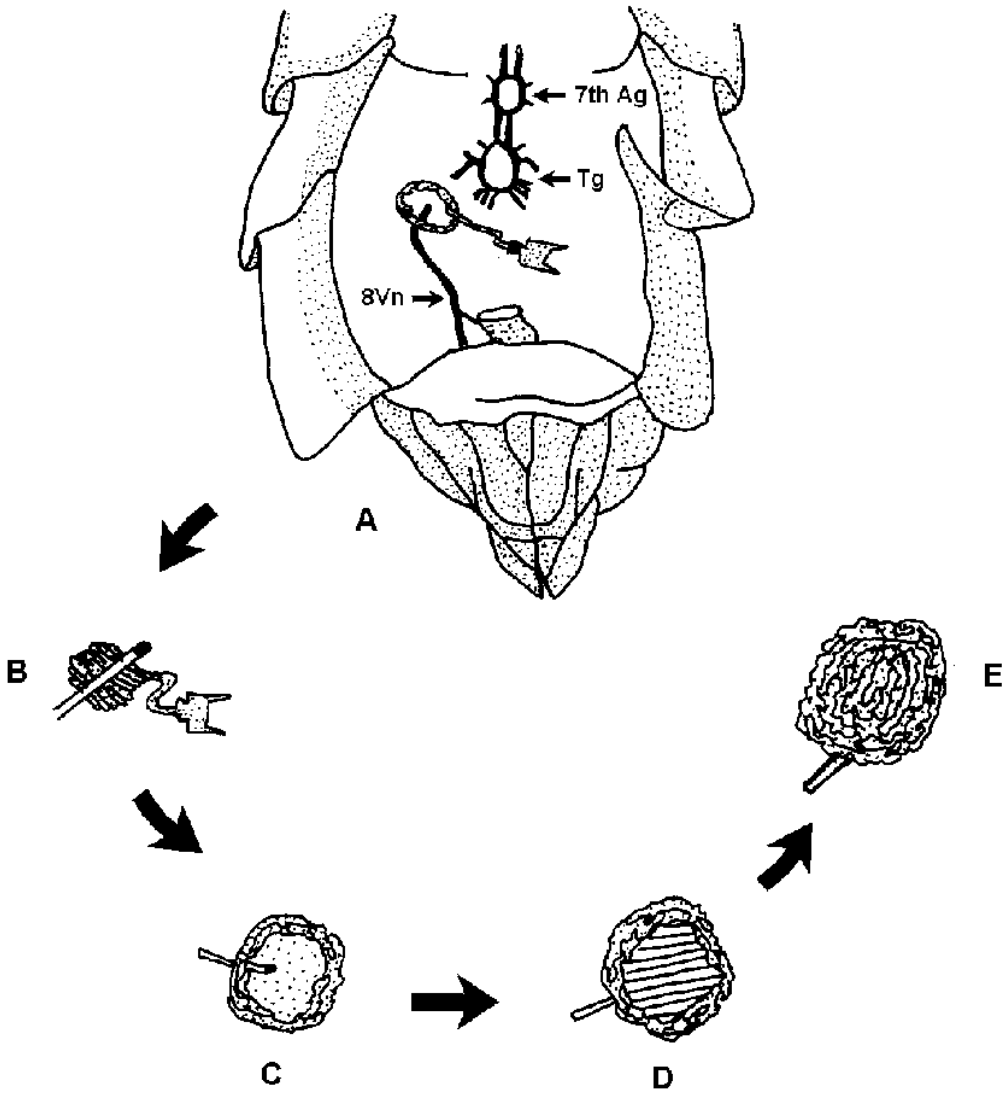


Fig. 1, A-E. The method for in vivo filling of neurones through peripheral nerves. A: A small quantity of vaseline, dispensed from a hypodermic syringe with shortened needle, is used to build a cup around the selected nerve (8Vn) within the animal. The sequence of the construction of the cup is shown in B-E. B: Vaseline is injected under the intact nerve; C: The nerve is cut, and a wall of Vaseline is built up to surround the cut end to be filled; D: A drop of cobalt chloride solution is pipetted into the cup; E: The drop is sealed over with more Vaseline. Exposed tissues in the animal are also covered with Vaseline to prevent their drying out.

2.3.3 Biotin backfill:

The central projection of both contact chemoreceptor and mechanoreceptor neurons of ovipositor valves and adjacent sclerites 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.

2.3.3.1 In vivo preparation:

The chemosensitive and the mechanosensitive sensilla from different location on the terminal abdominal sclerites (ventral and dorsal ovipositor valves, sternum, epiproct, paraproct and cerci) were stained by surrounding the receptor with a wall of wax or vaseline. Then a droplet of distilled water was placed on the cuticle within this enclosure, the chemosensitive or mechanosensitive sensillum was shaved off with a fine blade or with a broken glass microelectrodes, and the distilled water was replaced with a droplet of 3% aqueous Biocytin solution (Fig. 2). In these preparations, care has to be taken with localization and interpretation because the Biocytin ions can migrate under the Vaseline pool (Biotin creep) and be picked up by other neurones, both motor and sensory. After applying Biocytin, the animals were kept restrained in a moist chamber at 4 °C for 48-72 hours or in room temperature (approximately 25 °C) for 36-48 hours.

2.3.3.2 Biotin visualisation:

The two caudal ganglia (seventh and terminal ganglion, which innervate the terminal abdominal segments), were dissected from the insect, with the nerve containing the axons of interest left as long as possible.

The labelled ganglia were fixed in 4% paraformaldhyde for 5 hours, then dehydrated through a standard ascending alcohol series, 10 minutes in each concentration was sufficient and then the ganglia were cleared in xylene for 20 minutes.

The preparations were brought to water through a descending alcohol series, 10 minutes in each concentration was sufficient.

Incubation of the labelled ganglia for 1 hour at 37 °C in solution from 1 mg collagenase, 1 mg hyaluronidase, and 1 ml 0.1 M phosphate buffer. Then the ganglia were rinsed in 0.1 M phosphate buffer with two changes of 15 minutes then three changes of 15 minutes with 0.5% Triton X-100 added. The peroxidase binding to neurobiotin was best by using the Avidin-Biotin complex (Vectastain ABC kit PX400 standard, Vector Laboratories, Burlingame) in buffer, incubated for 5-12 hours at room temperature. Rinsing followed by two changes of buffer with 0.5% Triton X-100 and finally by pure 0.1 M phosphate buffer (each 15 minutes). Peroxidase bound to neurobotin in the central afferent projection was localized with the black chromogen DAB (3,3`-diaminobenzidine tetrahydrochloride) reaction.

For the DAB reaction, the preparations were incubated in solution from 30 mg 3,3`-diaminobenzidine tetrahydrochloride (Sigma Chemical Co.) and 45 µl hydrogen peroxide (30%) in 100 ml 0.1 phosphate buffer for 5-15 minutes. To stop the reaction with two changes of 0.1 phosphate buffer were required for 5 minutes.

Everey single preparation had to be examined to see if the DAB reaction has proceeded long enough. If the reaction was complete, all the remaining ganglia in the DAB-hydrogen peroxide solution could be removed and rinsed in 0.1 M phosphate buffer. If a longer reaction time is necessary, the preparations could remain in the DAB–hydrogen peroxide solution for addition incubation.

The preparations were dehydrated through an ascending alcohol series with 20 minutes in each concentration and then the ganglia were cleared in methyl salicylate.

From the basiconic sensilla of every characteristic site at least 5 successful stains were made generally and one was selected to be used for every representative figure in this study.

The major tracts and commissures of the central nervous system (terminal ganglion) were studied in the neurobiotin-stained whole-mounts which were embedded in soft Durcupan (Fluka – Chemie) or polyester wax (Sigma) 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 terminal ganglion of *Schistocerca gregaria* from Watson and Pflüger (1987) and Kalogianni (1995). Peripheral nerves and muscles were named as by Seabrook (1968) and Thompson (1986 a, b).

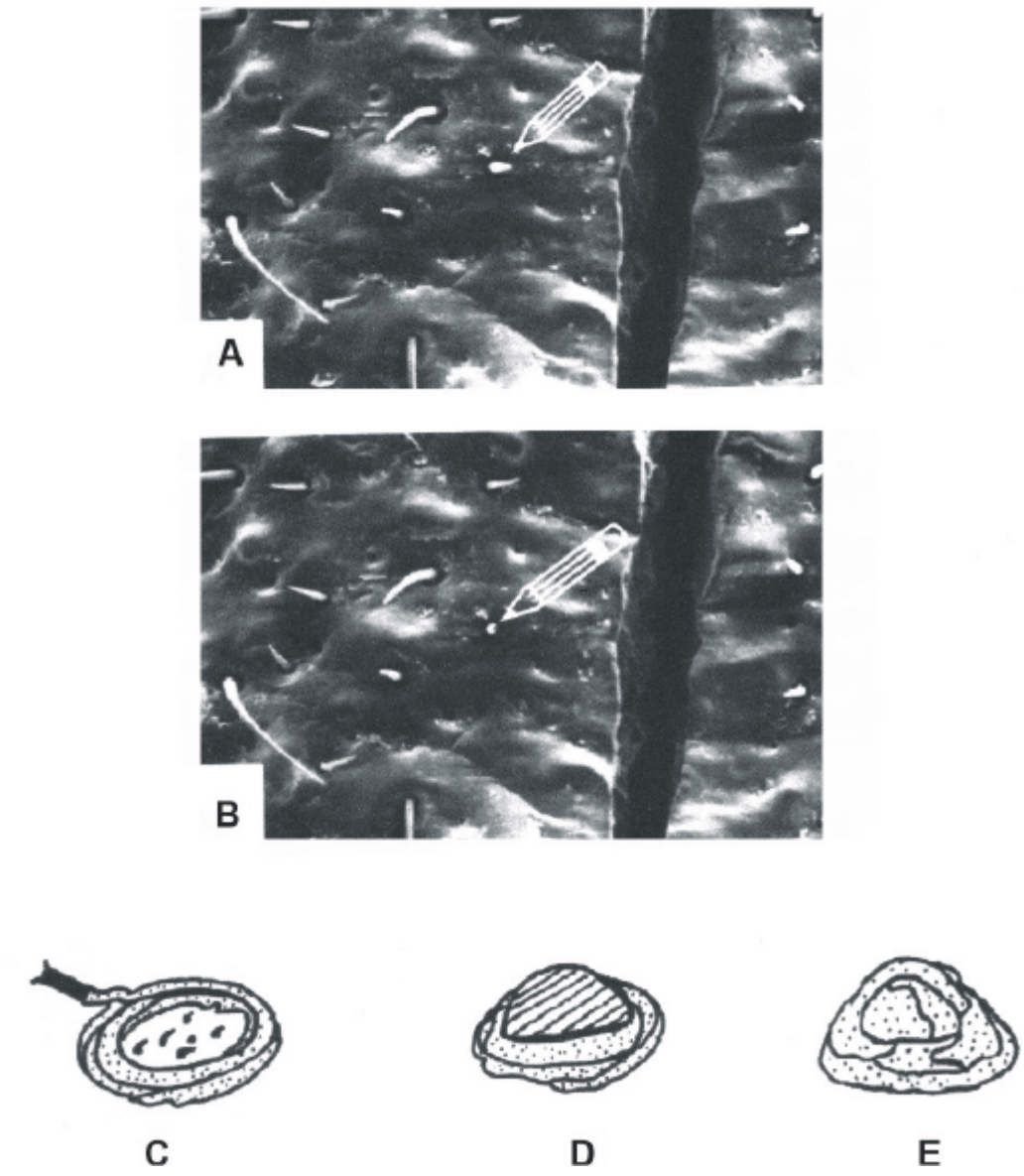


Fig. 2. A-E. The method used for in vivo filling from receptor cells innervating cuticular sensilla. The sequence. A: Chemosensitive sensillum in ventral view of locust ventral ovipositor valve; B: The chemosensitive sensillum is cut or crushed by a microglass electrode; C: A ring of vaseline is made around the sensillum to be cut; D: A drop of 3% biotin solution is placed within the Vaseline ring; E: A layer of vaseline is built up to cover the biotin drop, to prevent evaporation.

2.4 Electrophysiological studies:

2.4.1 Extracellular recording:

2.4.1.1 Stmulation and sensory responses

Responses from individual sensilla (basiconic sensilla) to chemical stimuli on the ventral side of the locust ventral ovipositor valve were recorded using the tip recording technique (Hodgson *et al.* 1955). The potentials were amplified and filtered using AC amplifiers. A blunt glass microelectrode or the plastic tip of a suction electrode, filled with different solutions was placed over the shaft of the sensillum (Fig. 3).

Electrodes containing salt (0.1M NaCl), sugar (0.1M glucose), acid (0.1M citric acid), alkaloid (0.01 M nicotine hydrogen tartrate and 0.1% quinine hydrochloride), oviposition aggregation pheromones (0.1% veratrole and 0.1% acetophenone) and phenolic compounds (0.1% salicin). All these chemicals substance were diluted in 0.01 M NaCl and used to stimulate and record from 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 mechanically and record the spikes of the afferents. The displacement of a sensillum did not deform its short and stout shaft.

2.4.1.2 Stimulation and Interneuron responses

For recording from the left or right abdominal connective between the 7th and the terminal abdominal ganglia large diameter suction electrodes were used. Stimulation with different chemicals was applied to a distinct single basiconic sensillum (contact chemosensitive sensillum) in the ventral region of the ipsilateral ventral ovipositor valve. To avoid the mechanical stimulation of other sensory neurons, all other sensilla in the terminal abdominal segments and on the ovipositor valves were

immobilised with vaseline. In addition, large mechanosensitive sensilla near the basiconic sensillum selected for recording were shaved off with fine razor blade and then, these mechanosensitive sensilla and the chemosensitive sensillum were usually surrounded by a ring of a soft, low temperature melting wax (Cenco Softseal Tackiwax) in which drops of different chemical stimulants could be applied selectively during recording. Sometimes a droplet of the stimulant solution deflected the basiconic sensillum initially and elicited spikes phasically for up to 20ms in the mechanosensory afferent.

Each stimulus was repeated 8-10 times for each stimulant chemical. For testing the specific response of the stimulants all basic classes of stimulating chemicals (salts, acids, sugar, alkaloids) diluted in water only were applied consecutively with interspersed pauses of several minutes in each experiment. This type of stimulation had the great advantage of being unbiased by an additional electrolyte which was required for recording and stimulating chemicals simultaneously at the terminal (and only) pore of the gustatory sensilla.

The recording stimulating electrodes contained different concentrations of salts (NaCl, 0.01 M to 3.0 M), sugar (glucose, 0.01 M to 3.0 M), acids (citric acid, 0.01M, 0.1 M and 1.0 M), oviposition aggregation pheromones as veratrole and acetophenone (1.0% and 0.1%), alkaloides as quinine and tomatine (0.1%), phenolic compounds as salicin (0.1%), neurotransmitters as GABA (0.1%) and neuromodulators as 5-HT (serotonin) and octopamine (0.1%).

The terminal abdominal segments were continuously superperfused with locust saline (Clements and May, 1974) at 22-25°C throughout an experiment.

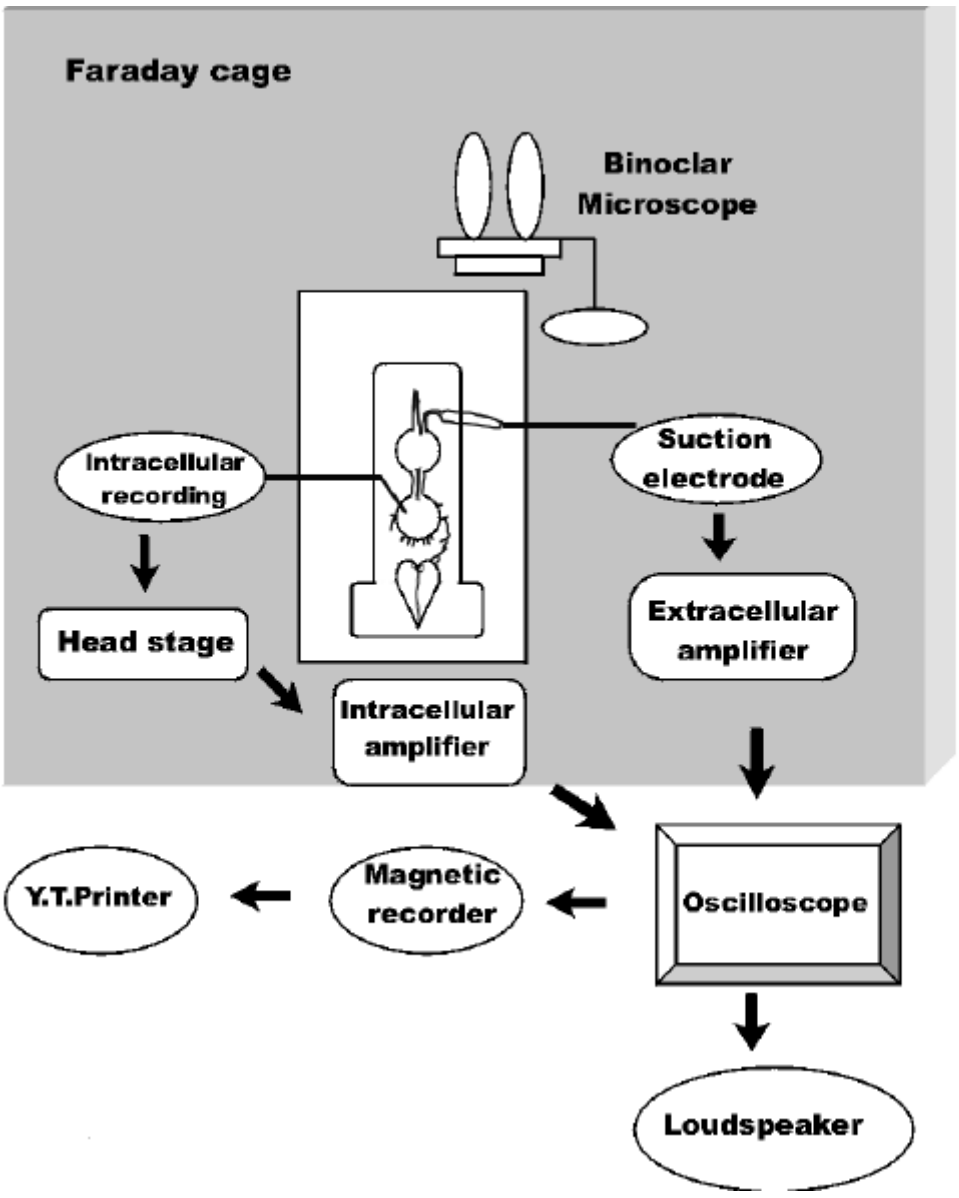


Fig. 3 Diagram of the electrophysiological setup for extra and intracellular recording.

2.4.2 Intracellular recording:

To obtain intracellular recordings, the ventral nerve cord (abdominal ganglia) was exposed by ventral dissection of the cuticle. The oviducts were cut at their distal attachment sites and fixed with pins near the anterior end of the abdominal cavity. All terminal abdominal nerves were cut with the exception of the eighth ventral abdominal nerve (8Vn). The 8Vn was freed of surrounding tissue and all side branches were severed except for the input from the ventral ovipositor valve.

The abdominal ganglia were isolated with the ventral ovipositor valve and fixed in a specific dish with non-toxic plasticine with of ventral ovipositor valve in dorsal orientation (Fig. 4) and the ovipositor apodemes immobilised. The abdominal ganglia were constantly perfused with locust saline (temperature 20-22°C).

On a wax-covered stainless steel platform on a micromanipulator, the terminal ganglion was mounted and the sheath was treated with about a 1% solution of protease (Sigma XIV) to facilitate intracellular recording from interneurones.

Intracellular microelectrodes were pulled from single tube capillary glass (World Precision Instruments). Their tips were filled with a solution of 4% Lucifer Yellow CH (Sigma) in 0.1 M lithium acetate (Sigma). The shaft of the electrode was back-filled with 1.0 M Lithium acetate. Resistances consistently ranged from 60 to 80 Mega ohm.

The tip of the electrode was positioned near the midline of the terminal ganglion just anterior to 8^{th} ventral nerve in the eighth abdominal neuromere. The stimulus apparatus was mounted on a micromanipulator such that the ventral region of the ventral ovipositor valve could be reached without disturbing the extracellular or the intracellular recordings. The projection chemosensitive interneurones in the terminal ganglia were

characterised physiologically by correlation spikes recorded intracellulary in their somata with those recorded extracellulary from the anterior connectives of the seventh abdominal ganglion.

All potentials were amplified by a DC- amplifier with current passing facility, displayed on an osciloscope, and stored on magnetic or recorder tape recorder along with the extracellulary recorded activity (Fig. 3).

2.4.2.1 Identification of chemosensitive interneurones:

Individual chemosensitive interneurones could be identified based on their physiology and their responses to chemical stimuli. For the completion of every experiment Lucifer Yellow dye was injected into each recorded cell. Lucifer Yellow is a fluorescent dye, with a molecular weight of 457,3 and by applying negative current a small amount of it could be injected into a cell could produce a brightly stained cell.

The Lucifer Yellow ions were injected into the interneurones by passing hyperpolarising current pulses for 500 msec at 1 HZ for 20 minutes. Later, the preparation was left in saline for 1 hour to allow the dye to diffuse into the internal arborizations within the terminal abdominal ganglion. The ganglia were removed from the preparation and fixed for 30 minutes in a buffered (pH7.4) 4% formaldehyde, dehydrated through an ascending alcohol series, in the 20 minutes in each concentration, and then the ganglia were cleared in methyl salicylate.

Ganglia containing stained interneurones were viewed first as whole-mounts under an epifluorescence microscope, photographed (35 mm or digital camera, Nikon Coolpix 950) and the interneurone was then either drawn directly by using a camera Lucida attachment on a the compound microscope (Leitz Aristoplan) or reconstructed from negatives or computer printouts.

3.0 Results:

3.1 Peripheral sensory innervation:

The peripheral innervation of the locust ovipositor valves have been revealed by whole-mount preparations after using the cobalt chloride backfill techniques. The ovipositor, as a highly specialized structure at the tip of the female abdomen, consists of three pairs of valves (Fig. 6B, C). The dorsal and the ventral pairs are strongly sclerotised and form prong-like processes with sharp curved tips, while the third pairs (intermediate valves) are very short and concealed between the other two pairs (Fig. 6C).

The ventral ovipositor valve is innervated by the posterior branch of the eighth ventral nerve (8Vn) of the terminal abdominal ganglion (Fig. 4A). The eighth ventral nerve arises lateral from the terminal ganglion, proceeds ventrad, and immediately divides into a posterior and a vventral branch with the ventral branch innervating the eighth abdominal sternite. The posterior, thicker nerve proceeds without branching for a considerable distance and gives rise to a mesial branch that distributes over the muscles of the spermathecal duct (dsp.n.). Adjacent to this branch another nerve branches laterad to innervate the depressor muscle of the ventral valve (272). The posterior nerve continues without branching until its about to enter the ventral ovipositor valve, at this point a mesial branch innervates the spermathecal duct in the region of the spermathecal aperture (asp.n.). The posterior nerve continues to enter within the ventral ovipositor valve where it branches progressively, receiving sensory axons from different areas within the ventral valve (Fig. 5A, 6A).

The dorsal ovipositor valve and the intermediate valve are innervated by the ninth abdominal ventral nerve (9Vn) of the terminal abdominal ganglion (Fig. 4B). This nerve arises from the posterolateral side of the terminal ganglion immediately beneath the ninth dorsal nerve (9D.n.). It

proceeds posteriorly either ventral beneath or between the epiproct and the cercal nerve. The mesial branch extends to the posterior region of the 9th apodeme (not shown in figure 4B) and turns lateral at this point. Here a branch arises to innervate the muscle of the intermediate valve while the lateral branch continues posteriorly where a small nerve branches off to the adductor muscle of the ventral valve (273). Then the main branch reaches the base of the dorsal valve where first a short side branch supplies a large plexus of multipolar cells while the main sensory nerve branches in the valve progressively, receiving sensory fibres from different axons within the valve(Fig.4B, 5B).

3.2 Sensilla and their innervation:

The sensory receptors associated with the ovipositor valves can be divided into mechanoreceptors and chemoreceptors. Contact chemoreceptors on the external ovipositor valves and on adjacent sclerites (Fig. 6, 7) occur widespread and also in dense fields, but usually with interspersed sensilla of different types. The types we could recognize additionally were (a) trichoid hairs of the mechanosensory type, both bristle type and wind-sensitive filiform type, (b) campaniform sensilla identified by their cuticular cap (Fig. 8C, D, E) and (c) pits serving either a sensory function or as outlets of cuticular glands (Fig.8C, D). Cobalt staining of the peripheral nerves and sensory neurons (Fig. 5, 6) confirmed the identity both mechanosensory hairs and campaniform sensilla with one neuron (Fig. 5C) below their cuticular structure and contact chemoreceptors (basiconic sensilla) with five neurons (Fig. 5C) gathered below the small and blunt hair with a terminal pore.

3.2.1 Basiconic sensilla:

Basiconic sensilla are peg-like structures (Fig. 7, 8) with a shaft that is typically much shorter (20 - 40 µm) than that of the trichoid sensilla and which has a pore at its tip (1,19 µm in diameter) . The pore provides access for contact of chemicals. Cobalt backfilling of the basiconic sensilla

on the ovipositor 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. 9D). The ovoid-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 locust ovipositor 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 the group to form a small nerve that finally enters larger nerves which also converge and leadto the terminal ganglia.

3.2.2 Trichoid sensilla:

The trichoid sensilla act as mechanoreceptors signalling tactile stimulation (bristle type) or they respond to air currents as the wind-sensitive filiform type. The trichoid sensilla are arbitrarily divided by size, shape and type of socket into two main types, the long sensilla (Fig. 8A, C, D) are over 100 µm in length and about 4.5 µm in diameter at their base and medium sensilla (Fig. 6, 8) are 40-100 µm long and about 3.5 µm in basal diameter, many of this type are straight (Fig. 6F), some are slightly curved anteriorly (Fig. 8H). The innervation of the trichoid sensilla is similar in the two types, each hair has a single sensory neuron (Tousson and Hustert 1998) with a large ovoid–shaped cell body that is about (4–5 µm) in diameter below the socket (Fig. 9A, B). The dendrite of this sensory neuron type inserts at the base of the shaft and transduces 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 (as in the cerci, the paraproct and the marginal edge of ovipositor valve) and at the other, bristle hair (as in the epiproct, the ovipositor valves and the 8th sternum). Correlated with these differences in the shafts are variations

in the stiffness of the socket. These factors set limits on the mechanical responses of the hairs and on the spike coding of their sensory neurons, although this is also influenced by the membrane properties of the sensory neurons themselves.

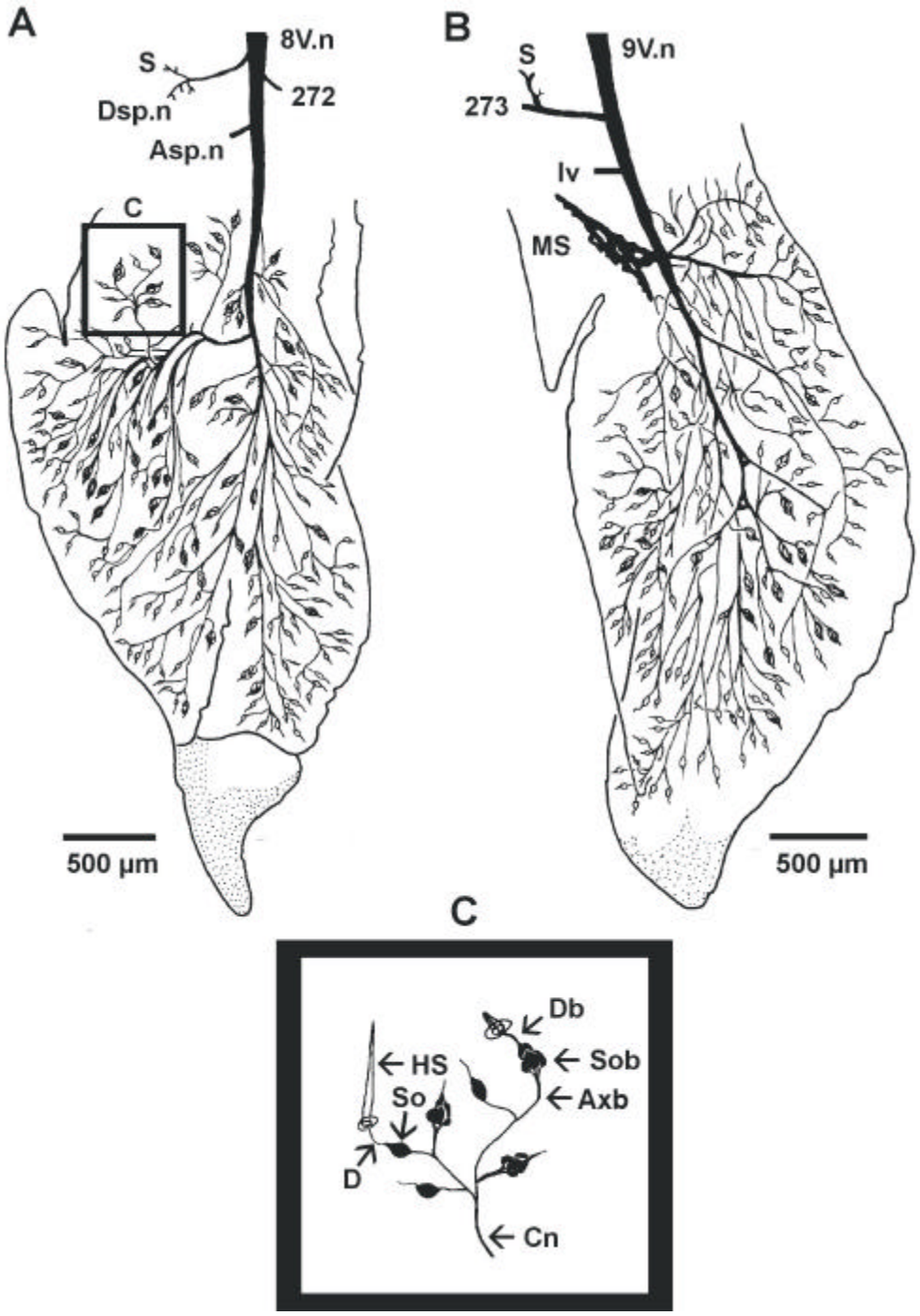


Fig. 4 Peripheral branching of the sensory nerve with sensory neurones and dendrites indicated in the ventral and dorsal ovipositor valve. Silver-

intensified cobalt staining. Single sensory neurons supply mechanosensory hairs and campaniform sensilla and each basiconic sensillum is supplied by a group five neurons. A. Ventral face of a ventral valve with the terminal sensory branch of the whole valve arising from the eighth ventral nerve (8Vn).The last distal branches before the nerve enters the valve are the dorsal spermathecal nerve (Dsp.n), the motor nerve to the depressor muscle of the ventral valve (272), and the anterior spermathecal nerve (Asp.n.). The heavily sclerotized tip of the valve (dotting) bears no receptors. B. Innervation of the dorsal face of the dorsal valve by the terminal branch of the ninth ventral nerve (9Vn), previously branching off nerves to the adductor muscle of the ventral valve (273), to the intermediate valve (Iv) and to a field of large multipolar cells (MS). The heavily sclerotized tip of the valve (dotting) bears no receptors. C. Inset from a proximal site of the ventral ovipositor (marked in A) shows terminal branching of the common nerve (Cn). To the right a complete basiconic sensillum with its dendrites (Db), the sensory somata (Sob) and the afferent axons (Axb) is seen. The left branch shows a complete mechanosensory hair sensillum with its hair shaft (HS), dendrite (D) and its single soma (So).

3.2.3 Campaniform sensilla :

Campaniform organs occur on the cerci, and the ovipositor valves of the adult female locust. The external parts of the campaniform organs are, in some cases, small dome-like papillae, others (Fig. 8C, D, E) are minute discs slightly sunken into the body wall, resembling in surface view vacant hair follicles, though they are usually distinguishable from the circular hair sockets by a more elliptical or oval form. The dome or disc in typical examples consists of a very thin outer lamella of the cuticle and of an endocuticular layer, generally having the form of an inverted cup. The inner layer is perforated by a central opening or by an axial slit through which the distal end of the sense cell process is inserted on the under surface of the outer lamella. Beneath the cap is the usual canal of the cuticula. It innervated by a single neuron with a large avoid cell body (4-6 µm in diameter) and a short dendrite (Fig. 9C), similar to those of the trichoid sensilla.

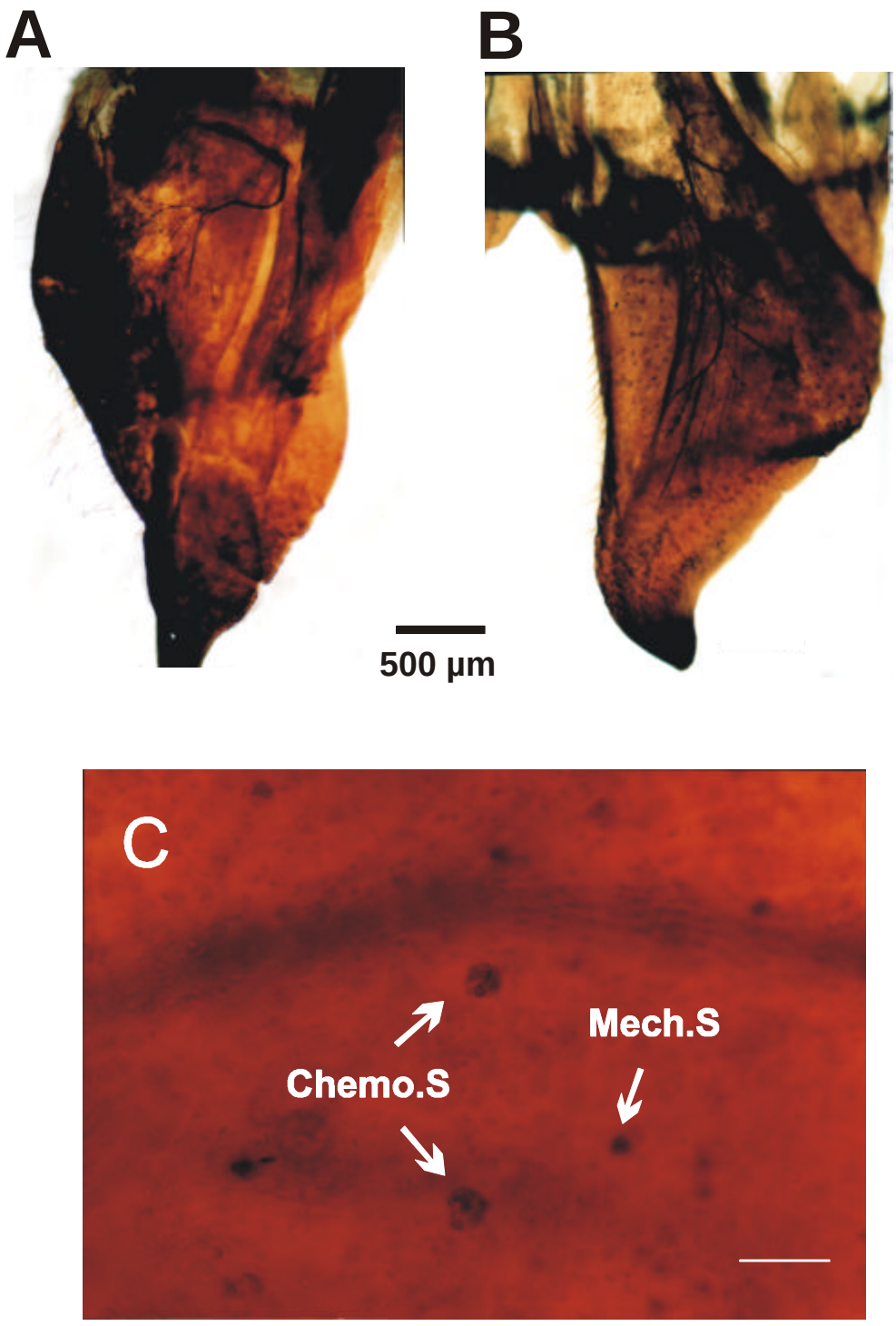


Fig. 5 The peripheral branching of the sensory nerve with sensory neurones and dendrites indicated in the ventral and dorsal ovipositor valve. Silver-intensified cobalt staining. A. Ventral face of a ventral valve with the terminal sensory branch of the whole valve arising from the eighth ventral nerve. B. Innervation of the dorsal face of the dorsal valve by the terminal branch of the ninth ventral nerve. C. Single sensory neurons supply the mechanosensory sensillum and a group of five neurons supply the chemosensory sensillum scale= 100 µm.

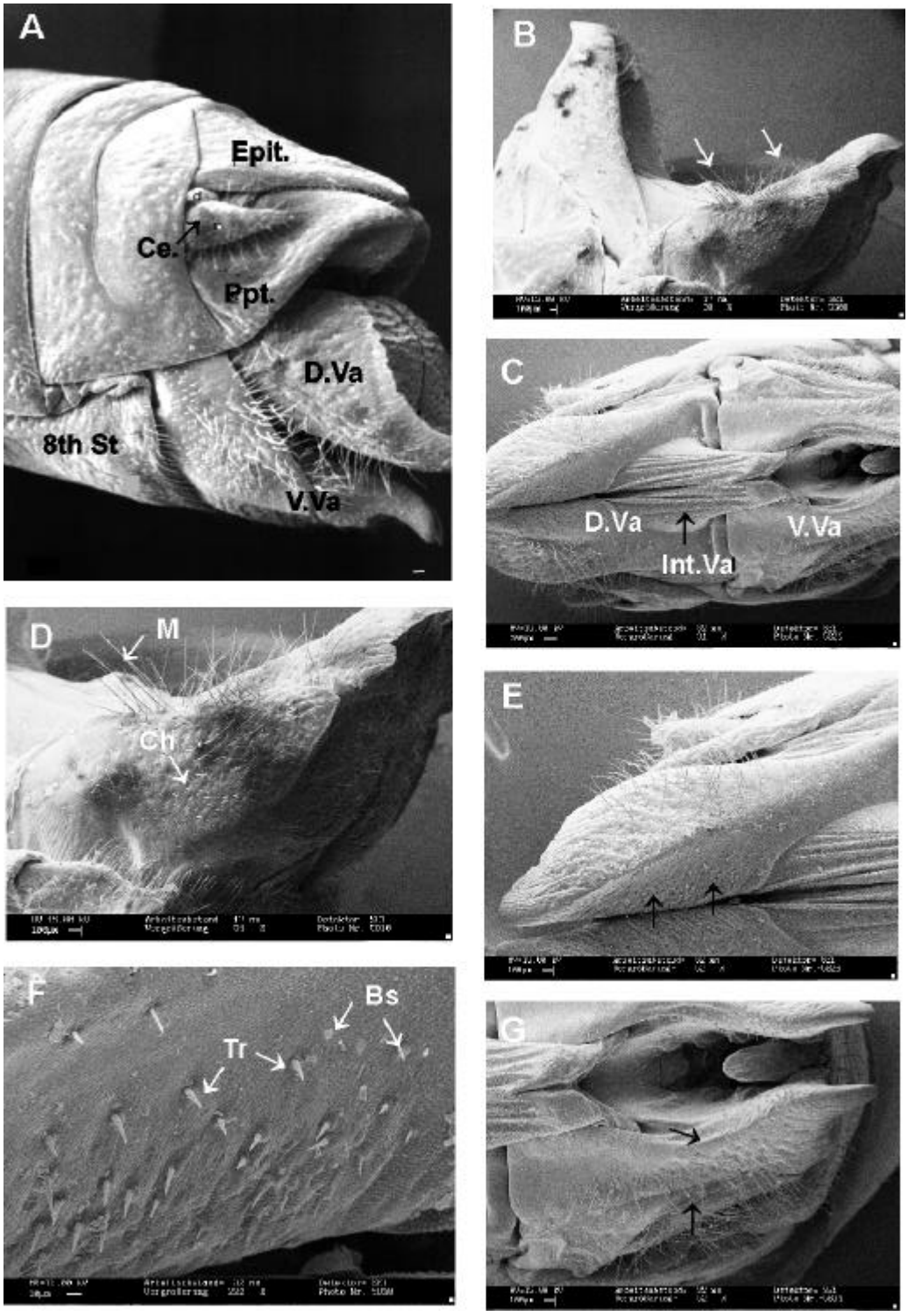


Fig. 6 Scanning micrograph of the locust female genital segments. A. Postero-lateral view of the external dorsal (D.Va) and ventral (V.Va) valves and the neighboring sclerites, the subgenital plate (8^{th}St), the paraproct (Ppt.), the epiproct (Epit.), and the cercus (Ce.): note that the ovipositor valves are closed and the dorsal valves overlap the ventral valves; scale=100µm. B. Lateral view of the dorsal and ventral pairs of ovipositor

valves wide open; in the open position the small pair of inner valves are visible with their tips in grooves on the inner surface of the ventral valves (arrows) scale = 100 µm. C. Posterior end of abdomen showing the ovipositor valve are wide opened, note the three pairs of ovipositor valves scale= 200 µm. D. Lateral view of the external ventral ovipositor valve with a large number of mechanosensory sensilla and chemosensory sensilla; scale=100µm. E. Internal-lateral view of the dorsal ovipositor valve; scale=100µm. F. View of the internal side of dorsal ovipositor valve with large number of mechanoreceptors (trichoid sensilla approximately 40-65 µm) and scattered contact chemoreceptors (basiconic sensilla); scale=30µm. G. View of the internal side of ventral ovipositor valve (arrowhead); scale= 100 µm. M: Mechanosensory sensilla; Ch: Chemosensory sensilla; Tr: Trichoid sensilla.

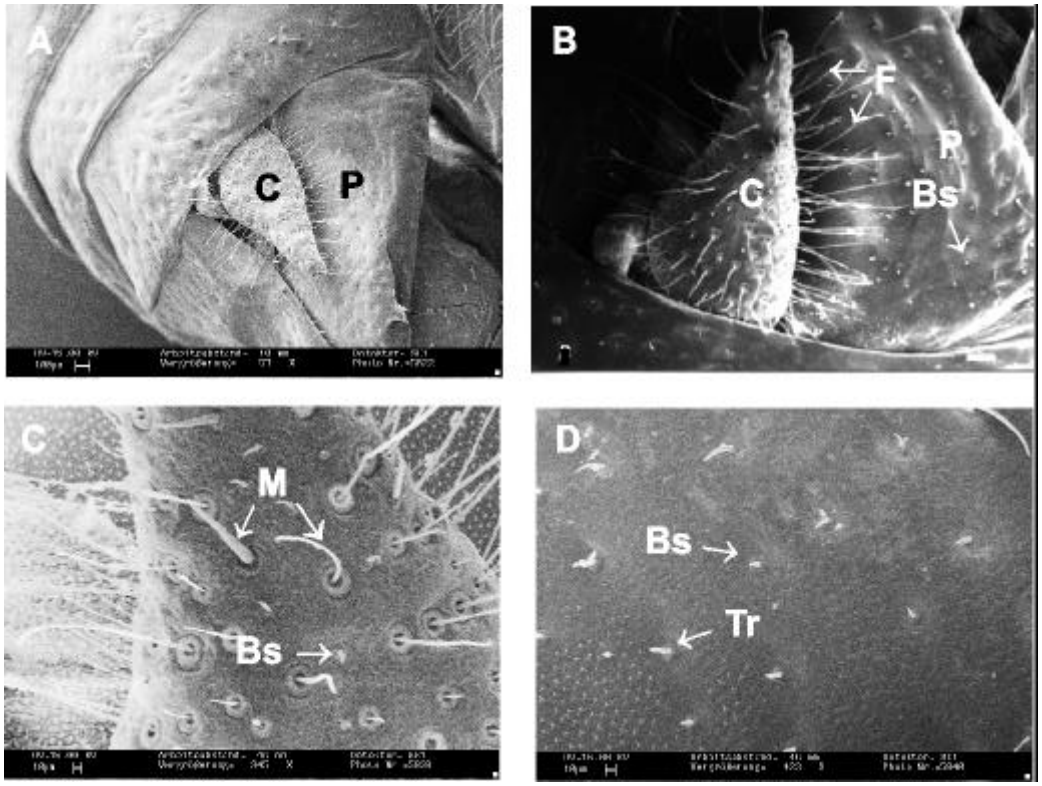


Fig. 7. Scanning micrograph of the cerci on the locust female genital segments. A. The cercal system of female locusta migratoria showing the location of the cerci near the tip of abdomen; scale=100 µm. B. The paraproct and the cercus (ventral side to the left) with its filiform hairs and basiconic sensilla; scale=20µm. C. large magnification of B showing the chemosensory basiconic sensilla scattered between the mechanosensory sensilla; scale=10 µm. D. Cuticle with a large number of short basiconic sensilla and trichoid sensilla on the paraproct; scale= 10 µm. C: Cerci; P: Paraproct; M: Mechanosensory sensilla; Bs: Basiconic sensilla; F: Filiform sensilla; Tr: Trichoid sensilla.

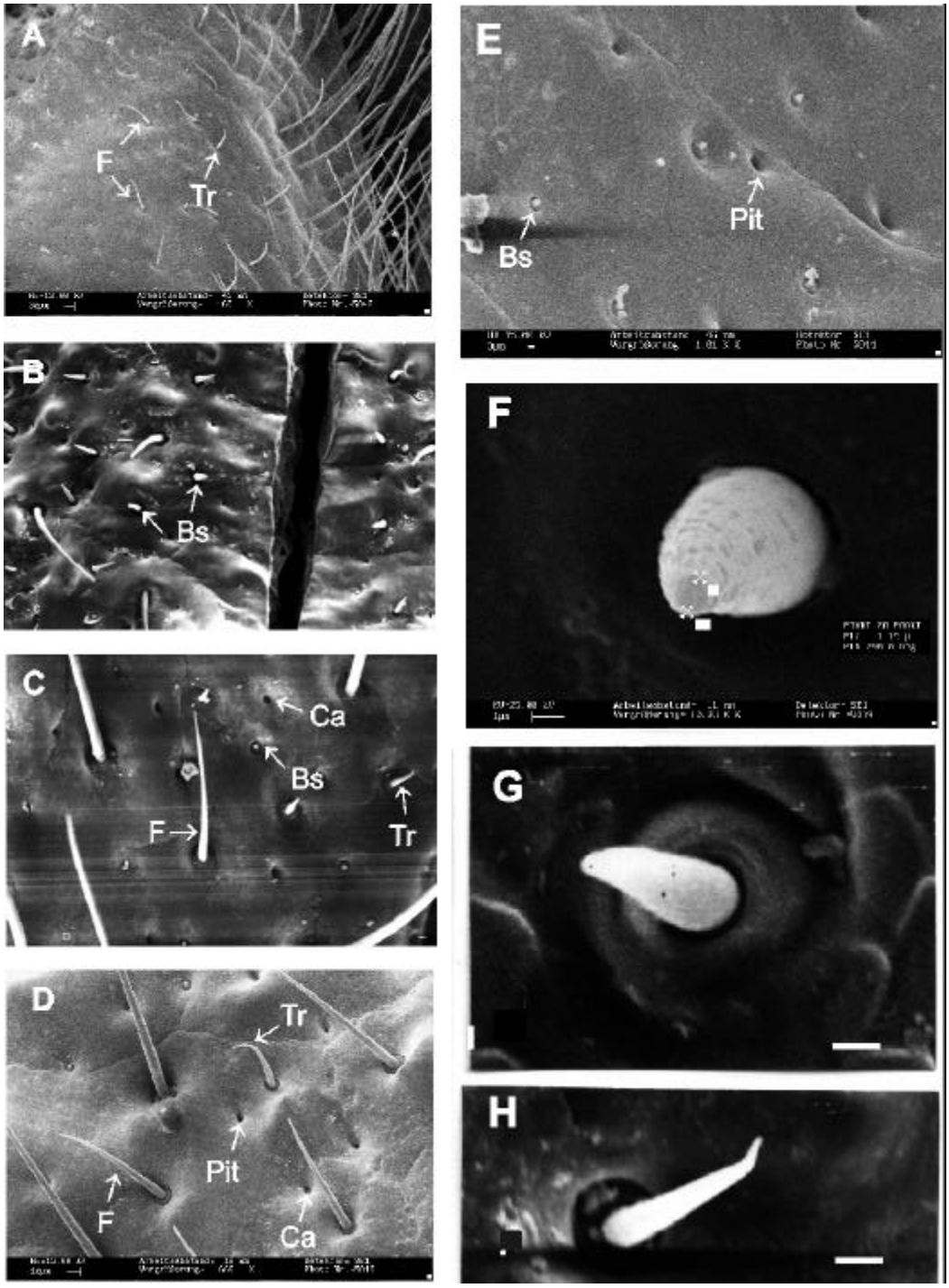


Fig. 8 Scanning micrograph of the locust ovipositor valves. A. View of the margin of the ventral ovipositor valve showing the long filiform and trichoid sensilla with slightly hooked tip; scale=30µm. B. Ventral face of two adjacent ventral valves separated by the groove in the midline. The short blunt hairs (arrows, chemosensory basiconic sensilla) were stained and stimulated repetitively due to their constant position relative to the sourrounding longer mechanosensory hairs; scale= 5µm. C and D. Cuticle with long filiform, stout basiconic and trichoid sensilla (with slightly hooked

tip) on the ventral (C) and lateral (D) sides (respectively) of the ventral ovipositor valve . There are also possible campaniform sensilla (pits with ‘knobs’), and pits of unknown function; scale=10µm. E. Cuticle with very short basiconic sensilla (approximately 3-5 µm) and campaniform sensilla in the dorsal side of dorsal ovipositor valve; scale= 3µm. F. A contact chemoreceptor (basiconic sensillum with pore approximately 1.19 µm in diameter) in ventral ovipositor valve; scale= 1µm. G. Chemosensory basiconic sensillum with a blunt tip in the ventral ovipositor valve; scale=2µm. H. Mechanosensory hair sensillum with a typical hooked tip; scale=2µm. Tr: Trichoid sensilla; F: Filiform sensilla; Bs: Basiconic sensilla; Ca: Campaniform sensilla; P: Pit.

Fig.9 Light micrograph of a whole mount stained with silver-intensified cobalt staining, showing the ovipositor sensilla and their innervation. A. Innervation of trichoid sensillum (Tr) on the ventral ovipositor valve by single neuron with a small cell body (S), a large axon (Ax) and a short dendrite (De). B. Innervation of filiform sensillum (Fs) on the marginal edge of the dorsal ovipositor valve by single neuron with a small spindle-shaped cell body (S) and a short dendrite (D). C. Innervation of cambaniform sensillum (Cs) by single neuron with large cell body (S) and a very short dendrite (De). D. Innervation of a single basiconic sensilla (Bs) by five neurons with five cell bodies (S) and short axons (Ax); scale = 100 µm.

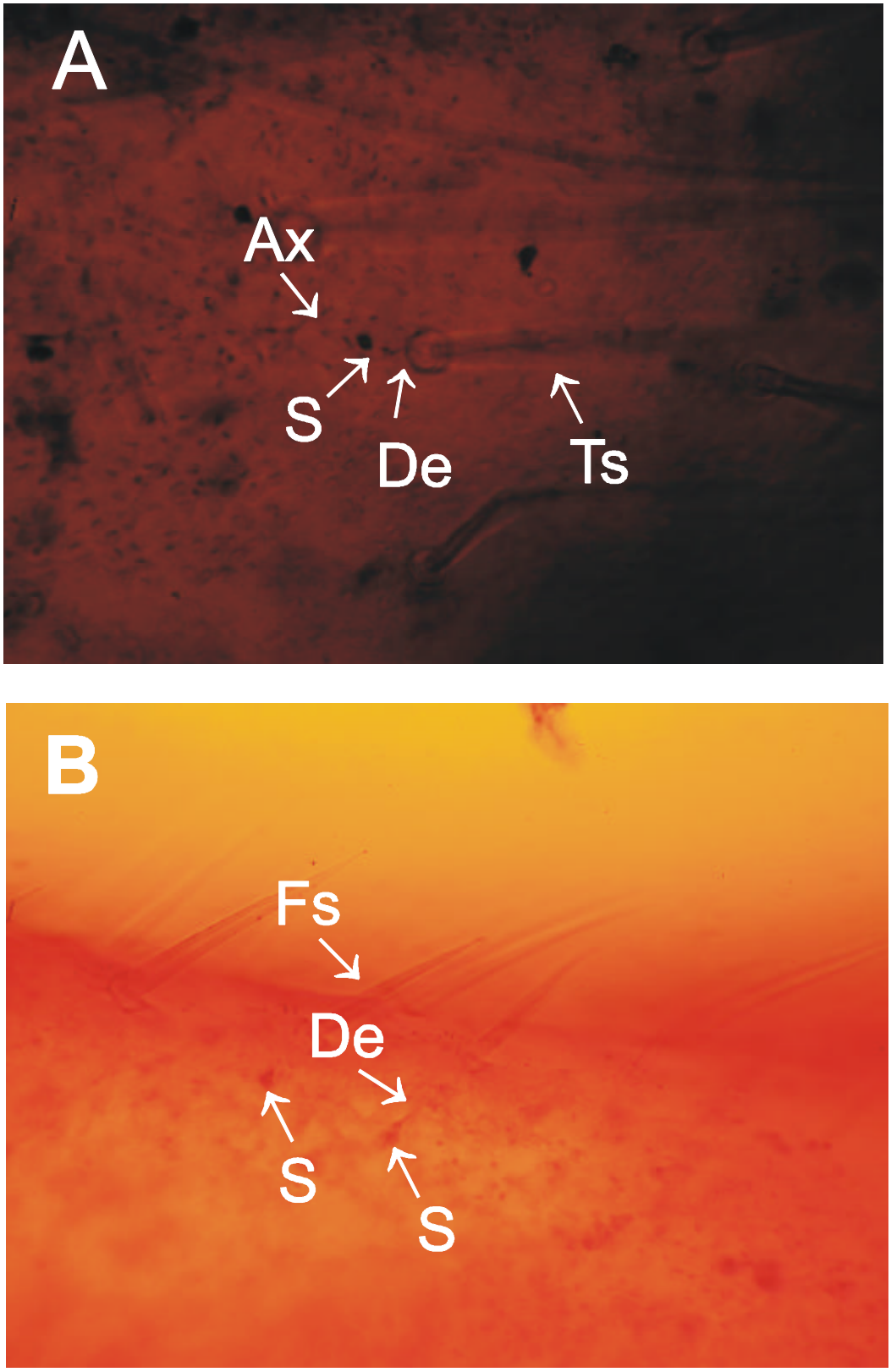


Fig. 9 A and B.

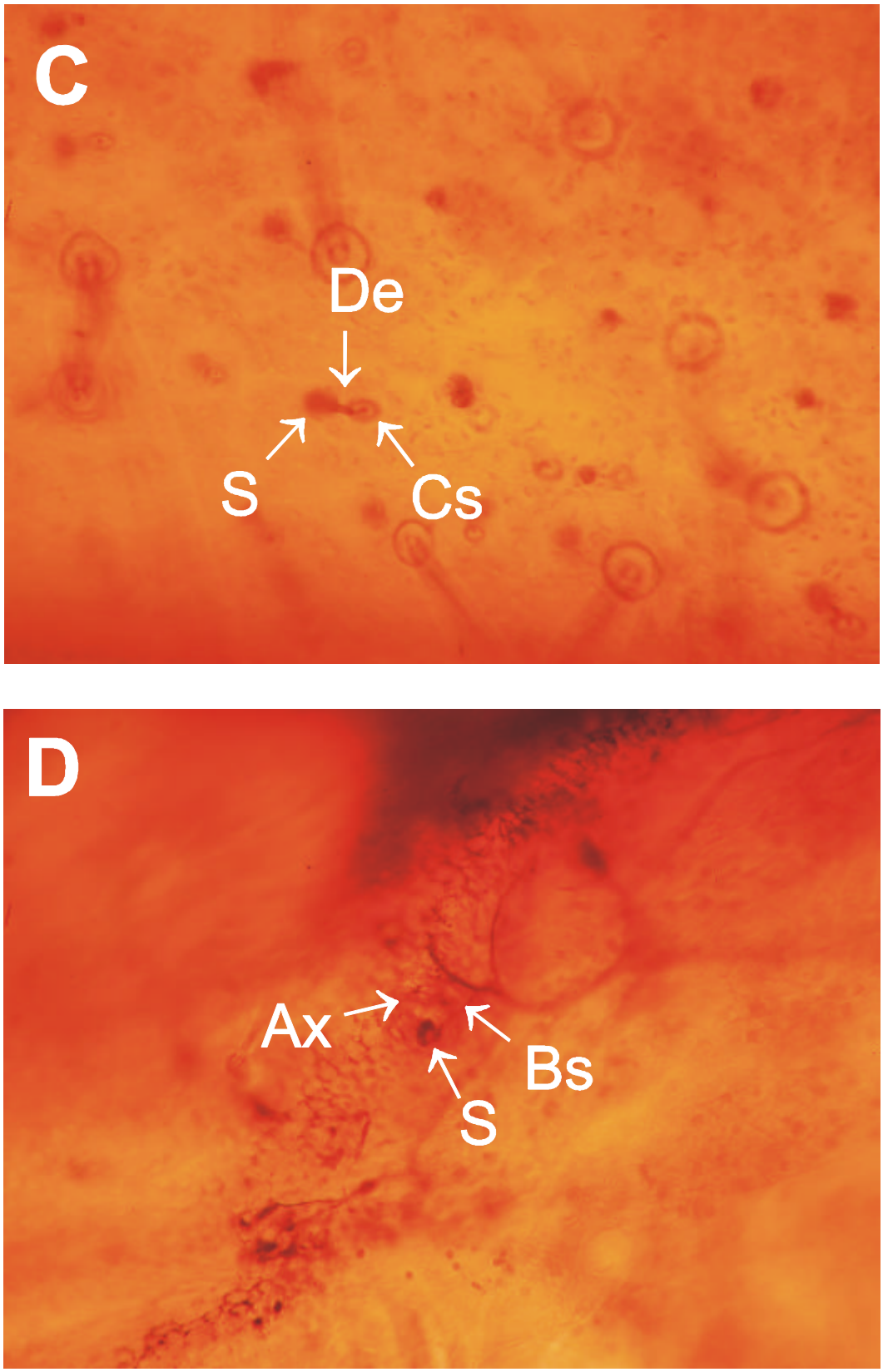


Fig. 9 C and D.

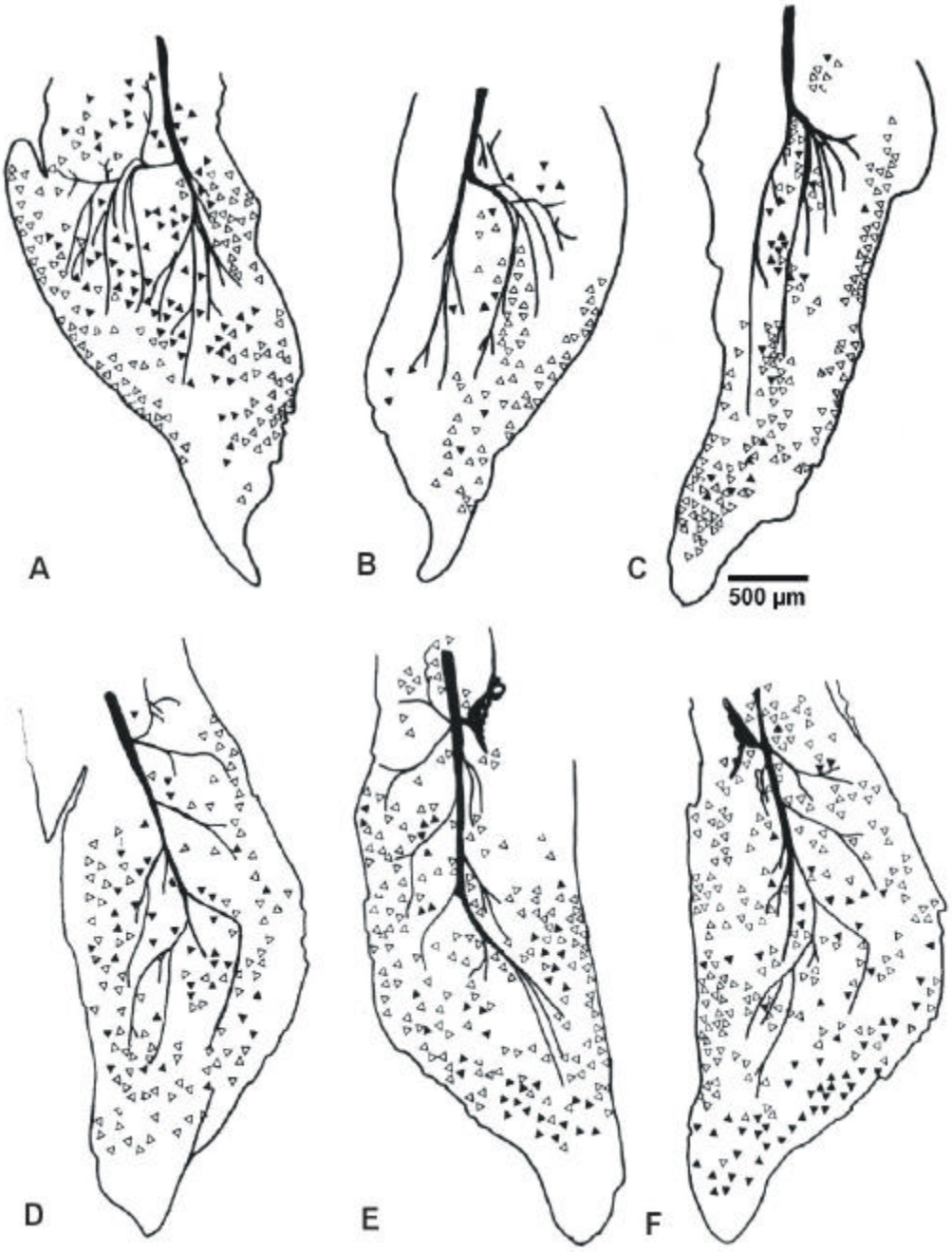


Fig. 10 Distribution of mechanosensory hair receptors (open triangles) and contact chemoreceptors (filled triangles) on the three principal faces of the ventral (A-C) and dorsal (D-F) ovipositor with main nerve branches. A. Ventral face of the ventral ovipositor with mechanosensory hairs crowded near the edges and pure basiconic sensilla fields in the central region. The preferred area of our staining of central projections is marked by a dotted circle. B. Median (internal) face with predominant mechanosensory hairs. C. Lateral face with few and distributed basiconic sensilla. D. Dorsal face

of the dorsal ovipositor. The areas supplied only with basiconic sensilla in the central region is also marked as preferred area of staining central projections (dotted circle). E. Median (internal) face with prevailing mechanosensory hairs and most basiconic sensilla assembled in the posterior area. F. Lateral face with most basiconic sensilla near the posterior rim.

3.3 Number and Distribution of Ovipositor Receptors:

As a means of estimating the total number of receptors that were found on the locust ovipositor valves, five successful silver-intensifed cobalt staining whole mounts were used. On the locust ovipositor, two basic types of receptors are present, contact chemoreceptors (Basiconic sensilla with five neurons) and mechanoreceptors (Trichoid and campaniform sensilla) innervate with a single neuron. On the dorsal valves, with their tetrahedral (but curved and tilted) shape, on all three principal faces (dorsal, lateral, internal) the chemoreceptors (Fig. 10) lie isolated or are assembled in fields. No mixing with mechanoreceptor hairs occurs on the most extensive field in the middle of the ventral valve (Fig. 10A) This site shows the highest density of contact chemoreceptors on the female valves of the locust. The basiconic sensilla of this location were mainly used for our experiments. Mechanosensory hairs crowd closer to the edges of the valves (Fig. 10A-C). The same principles (in mirror image) apply for the dorsal valve (Fig. 10D-F) as a tetrahedral shape with a ventral, lateral and internal face. The contact chemoreceptors in the central region of the ventral face were selected for staining their central projections.

It has approximately 1205 receptors, about 626,8 sensilla on the dorsal valve and about 579 sensilla on the ventral valve. The average number of different types of receptors for different regions of the valves shows a ratio of 1:3 for chemosensory to mechanosensory hairs (Fig. 13) and approximately 44-45% on the ventral side of ventral valve and on the dorsal side of dorsal valve but the average of 22% in internal side of the ventral and the dorsal valves (Fig. 11, 12) and the rest. Complete results are present in table 1.

	Ventral Valve				Dorsal Valve				Ovipositor Valve
	Ventral side	Lateral side	Internal side	total	Dorsal side	Lateral side	Internal side	total	
Chem. Recp.	43,8	66,6	37,8	148,2	53,8	64,6	42,4	160,8	309
Mech. Recp.	100	156,6	174,4	431	125,2	156,6	183,2	465	896
total	143,8	223,2	212,2	579,2	179	221,2	225,6	625,8	1205
total	579,2				625,8				
total	1205								

Table 1. Number and density of receptors in the locust ovipositor valves.

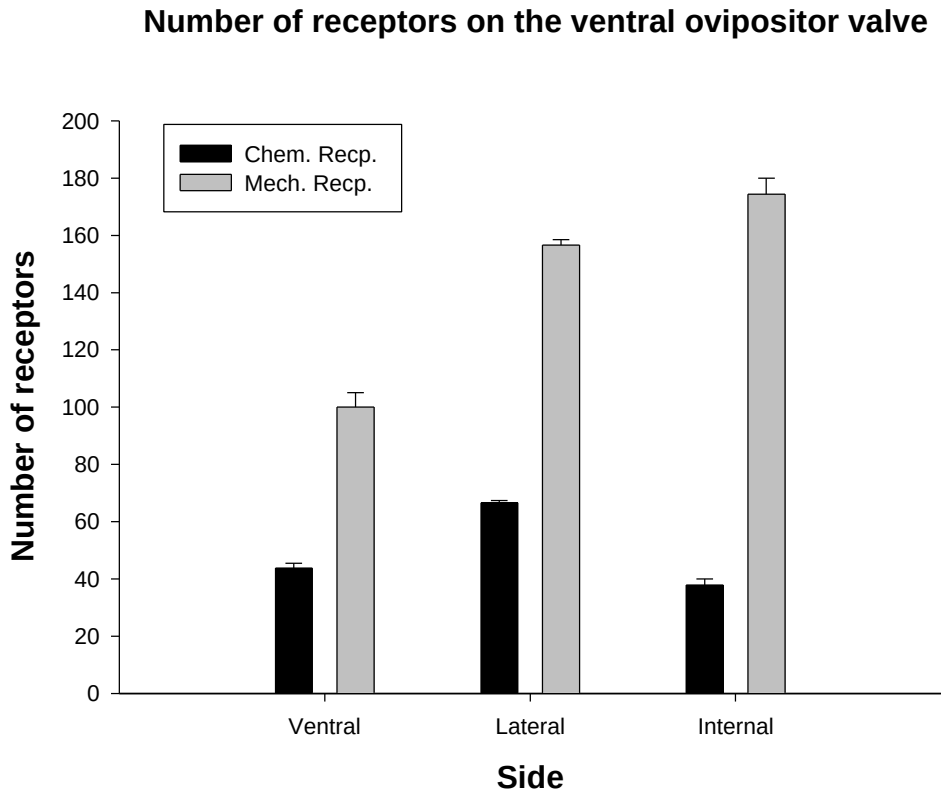


Fig 11. Number and density of receptors on the ventral ovipositor valves in female *Locusta migratoria*.

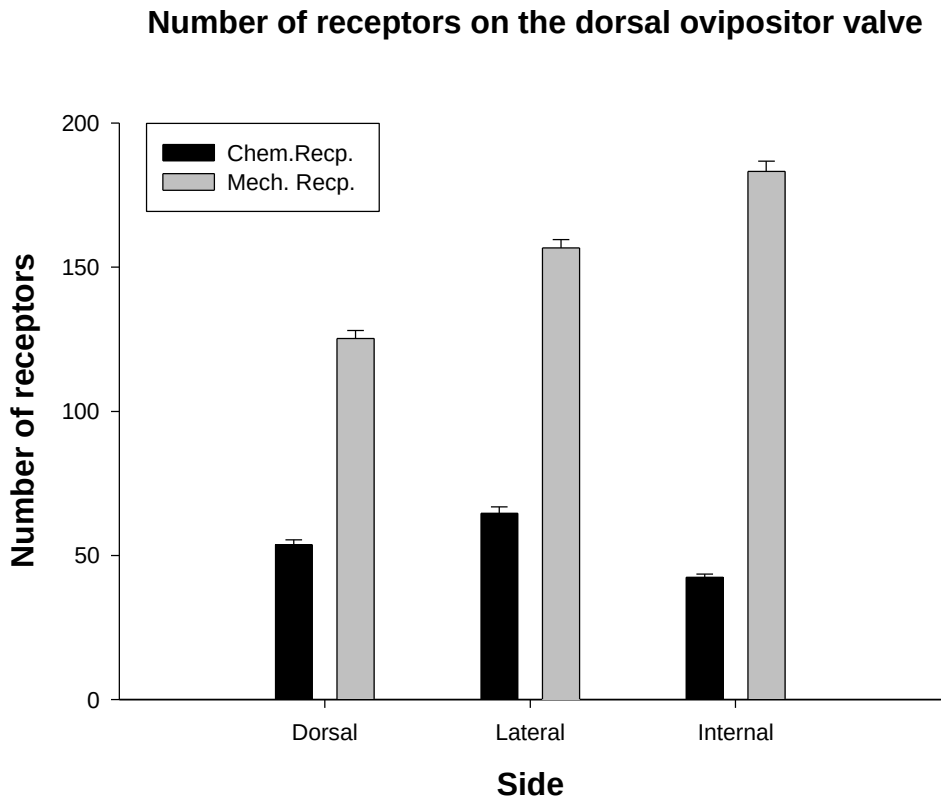


Fig 12. Number and density of receptors in dorsal ovipositor valves in female *Locusta migratoria*.

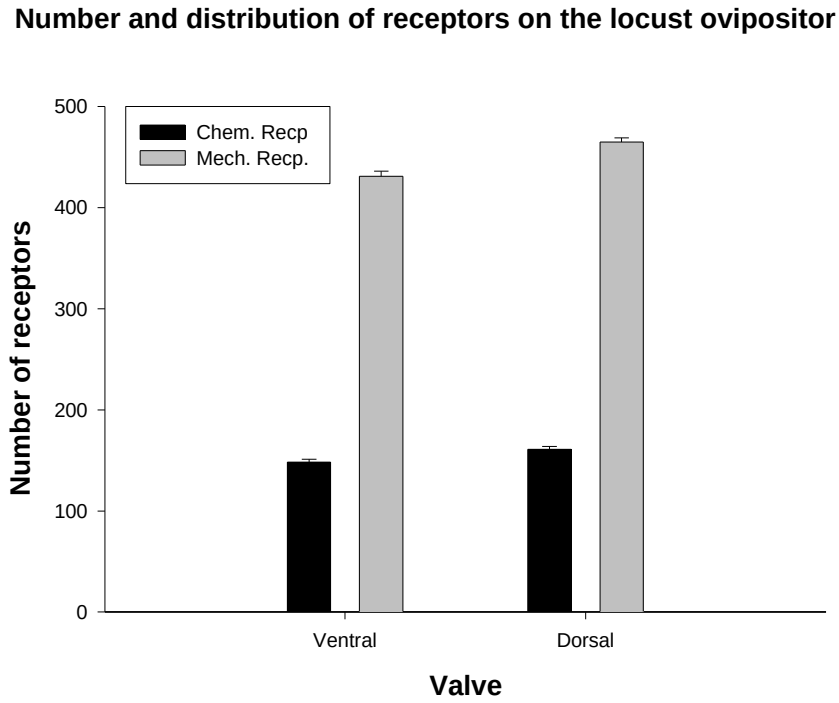


Fig 13. Total number and distribution of chemoreceptors and mechanoreceptors on the ventral and dorsal ovipositor valves of female *Locusta migratoria*.

3.4 The central projections

3.4.1 The central projections of single chemoreceptors:

Afferent sensory neurons from contact chemoreceptors of the posterior abdominal segments may follow general (type-related) and/or specific (site-related) rules as to their central projections. Therefore, all afferents of single contact chemoreceptors and (for comparison) of single mechanoreceptors from different sites on the terminal abdominal segments were backfilled with neurobiotin. In summary, projections from single sensilla on the ovipositor valves, on the 8th sternite (subgenital plate), on the epiproct, on the cerci and on the paraproct were labelled.

Whenever a basiconic sensillum from the ventral ovipositor valve was stained iontophoretically, five neurons; one mechanosensory and four presumably chemosensory were revealed in the whole-mount (Fig. 14; 18A). The axons enter the terminal abdominal ganglion via the eighth ventral abdominal nerve (8Vn) and proceed laterally to the 8th abdominal neuromere. There, all axonal projections branch ventrally and send neurites medially to the ventral associatiation center (VAC) at a median level within the VAC. From there, several branches extend into the contralateral neuropil and one turns posteriorly. From the lateral axons only four collaterals proceed through the anterior ipsilateral connective on a ventral level and further into the seventh abdominal ganglion, where they all terminate medio-ventrally.

From each basiconic sensillum of the dorsal ovipositor valve (Fig.15) five afferent axons enter the terminal ganglion via the ninth ventral abdominal nerve (9 Vn), proceed to the lateral area of the ninth neuromere where primary branches split off into a rather restricted area of the medio-ventral neuropile. Anterior collaterals run antero-medially into the eighth neuromere and develop a second branching region at a medio-ventral level in the VAC. All main axon collaterals proceed ipsilaterally into the preceeding ganglion of the seventh abdominal segment.

When five afferent axons from a single basiconic sensillum were stained, one of them terminated in the ninth neuromere while collaterals of the other four proceeded to the eighth neuromere and further to the seventh abdominal ganglion. When occasionally only four axons were labelled in the whole-mount, presumably as a result of missing the mechanosensory dendrite at the base of the sensillum we found all axons extending to 7th abdominal ganglion.

The central projections of single basiconic sensilla from different sites on the genital segments (from the 8th abdominal sternite, the epiproct, the cerci and the paraproct) were backfilled for comparison with ovipositor sensilla (Fig. 16; 17; 18 B, C, D). Their typical five afferent axons enter the terminal ganglion via the eighth ventral abdominal nerve or the tenth ventral or dorsal nerve (from epiproct, paraproct or cerci) respectively. Projections from the eighth sternite proceed medially to the VAC of the eighth neuromere and at least one neurite extends contralaterally, resembling those of the ventral ovipositor. Similarly, only four of the sternite afferents were found to proceed anteriorly into the ipsilateral connective (Fig. 16A,18B).

The afferents of basiconic sensilla located on tergal sites of the genital segments (epiproct, paraproct, cerci) take a rather median path through the neuromeres into the ipsilateral connective (Figs 16 B,C; 17A; 18 C,D). Branching is very restricted and no branching of the neurons is seen before the ninth neuromere is reached. Only the cercal afferents show dense branching areas at the level of different neuropiles some of which form small tufts of glomerulus-like structure (Fig.17, 18D).

3.4.2 The central projections of single mechanoreceptors:

The central projections of single mechanoreceptors (bristle or filiform type) were backfilled for comparison to the chemosensory and mechanosensory projections of the basiconic sensilla of the genital segments.

Filiform mechanoreceptors on the ventral or dorsal ovipositor valve are innervated by a single sensory neuron greater in diameter than the basiconic axon entering the terminal ganglion via their respective ventral nerves. Ventral valve mechanosensory afferents (Fig.19, 22A) have projection patterns in the eighth neuromere that resemble the neighbouring basiconic afferents but without having contralateral branches in the VAC (Fig.14). The ipsilateral collateral ascends into the preceding ganglion.

Filiform hair projections from the dorsal ovipositor (Fig.20, 22C) are also rather similar to those from adjacent basiconic sensilla, with branching in the VAC of the ninth and eighth neuromere and a projection in the anterior ganglion, but the anterior branching areas tend to be more extensive along the path of the axon than those of adjacent contact chemoreceptor afferents (Fig. 15).

The comparison with mechanosensory hair projections of cuticle neighboring the ovipositor (Fig 21, 22) again shows similarities with their chemosensory counterparts (Figs.16, 17) as to the neuropiles they reach, except for the chemosensory and mechanosensory projections from the cerci and the epiproct. The mechanosensory cercal projections for instance end locally in the cercal neuropile the filiform wind hair afferents whereas the contact chemosensory afferents are intersegmental and show small glomerulus-like tufts of projections in the terminal ganglion.

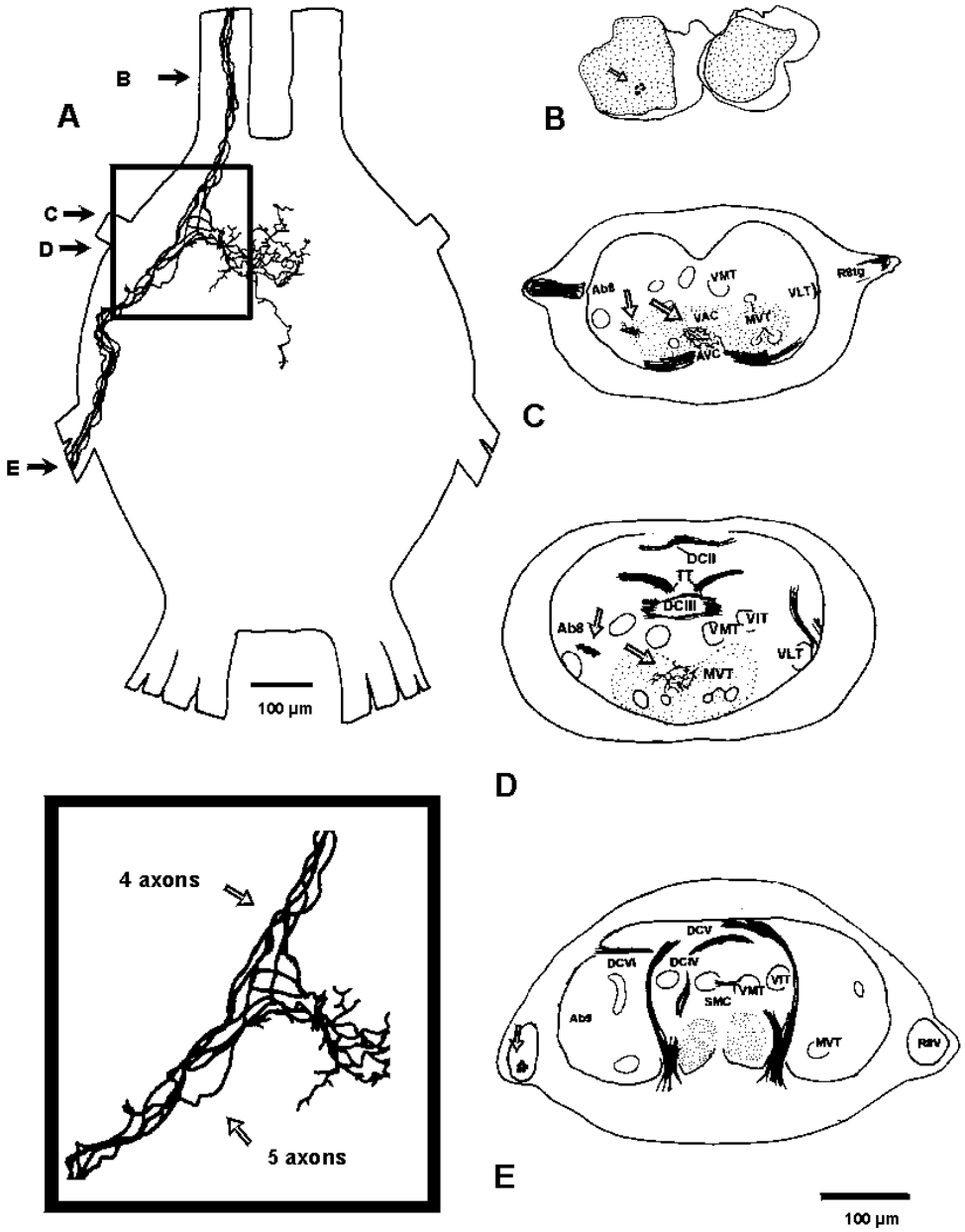


Fig. 14 Central projections from all five neurons of a basiconic sensillum located on the ventral ovipositor stained with neurobiotin. Inset: expanded view from the area marked in A. Arrows point at projections in sections B-D. A. Five afferents enter the terminal ganglion and branch in the lateral and medial VAC of the eighth neuromere where some cross the midline. A single branch descends caudally. Only four collaterals proceed into the ipsilateral connective. A single branch descends caudally. B-E indicate the level of the following cross sections at different levels. B. Four ascending afferents in the ipsilateral connective. C. Lateral, medial and contralateral branching of the afferents in the VAC of the eighth neuromere. D. More branching in the posterior VAC of the eighth neuromere. E. Five afferents entering the terminal ganglion via the nerve root of the eighth ventral nerve (8Vn).

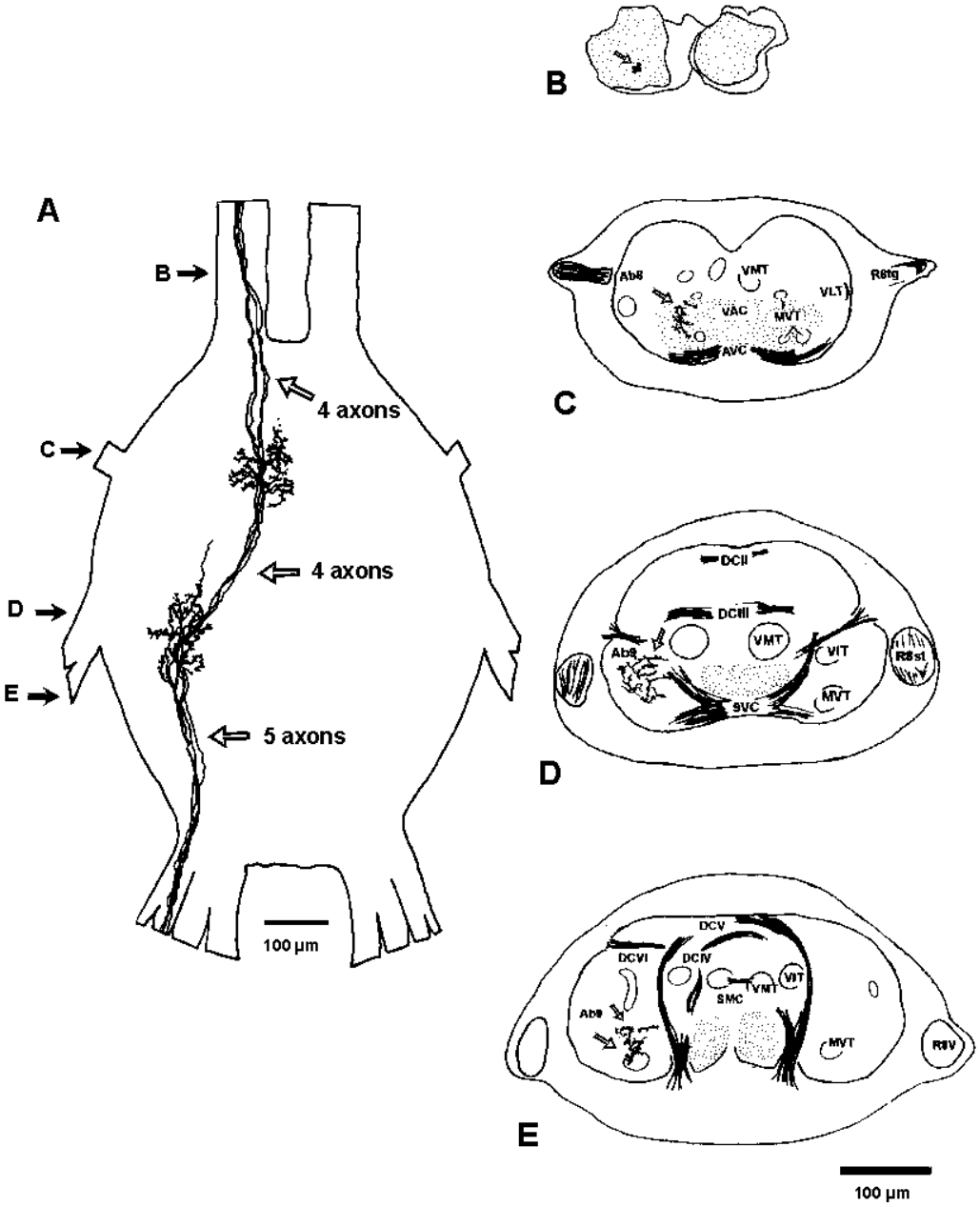


Fig. 15 Central projections from all five neurons of a basiconic sensillum located on the dorsal ovipositor stained with Neurobiotin. A. Five afferents enter the terminal ganglion via the ninth ventral nerve (9Vn) but later, only four enter the eighth neuromere and the ipsilateral connective. Branching occurs laterally in the ninth neuromere and one branch ascends separately. B-E indicate the level of the cross sections shown at different levels. B. Four ascending afferents in the ipsilateral connective. C. Branches in the eighth neuromere from four afferents at a median level in the VAC without contralateral extensions. D. All five afferents from the basiconic sensillum branch in a small area of the ninth neuromere located in the lateral region VAC. E. More branching in the posterior lateral VAC of the ninth neuromere.

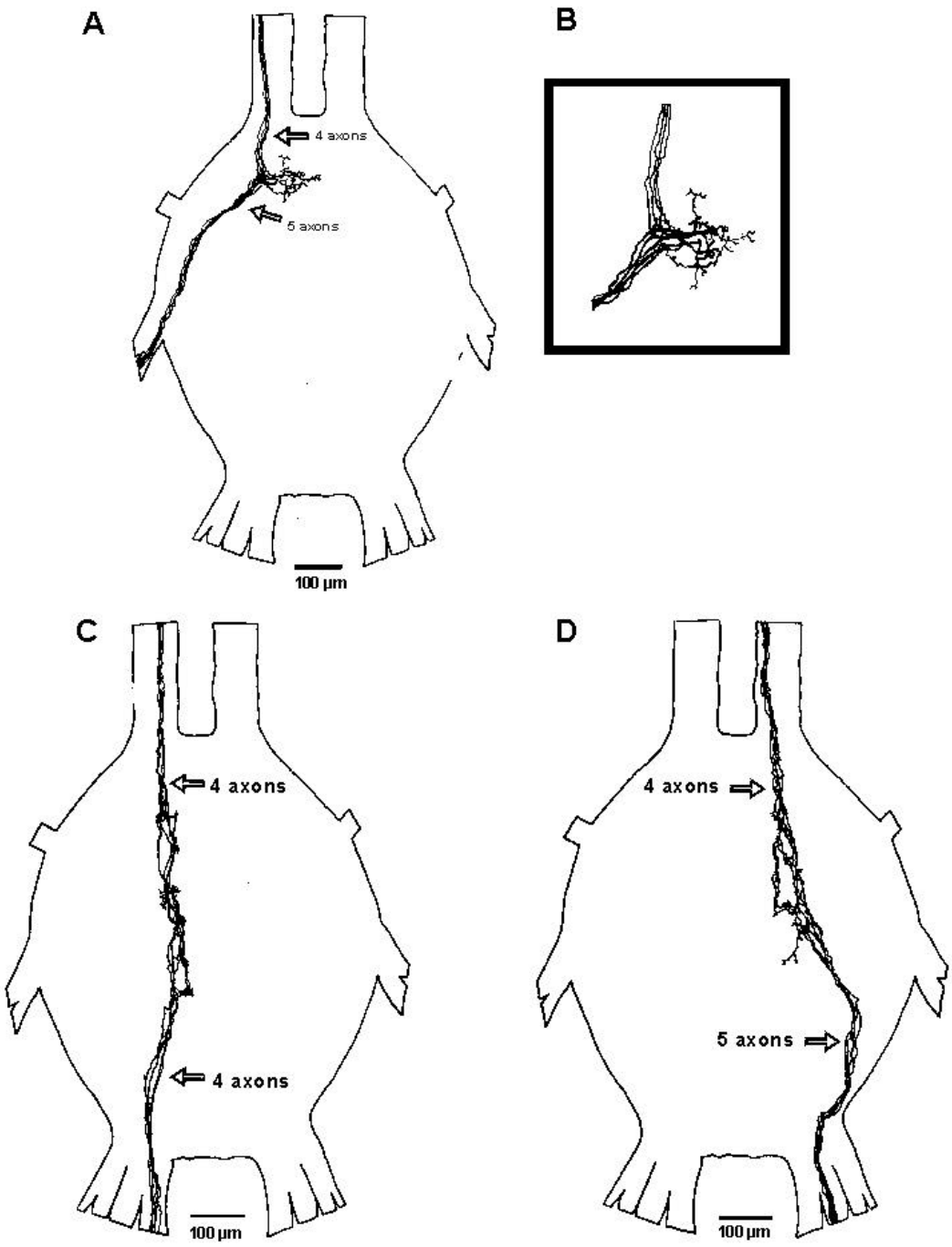


Fig. 16 Neurobiotin staining of central projections from basiconic sensilla located on the subgenital plate (eighth sternite, A), the paraproct (C), and the epiproct (D). Basiconic sensilla on these sites which were suitable for staining their central projections were selected. A. Projections from the subgenital plate branch in the VAC of the eighth neuromere, extend contralaterally and proceed into the ipsilateral connective. Inset: expanded view of the primary branching area with five afferents reaching the

branching area and four afferents ascending further anteriorly. C. From the paraproct basiconic neurons run ventro-medially through the terminal ganglion into the connective, with just a few arborizations in areas of the ninth and the eighth neuromere. D. Epiproctal contact chemoreceptor neurons first head laterally, but then turn towards the middle in the ninth neuromere where some branching occurs, whereas little is seen in the eighth neuromere.

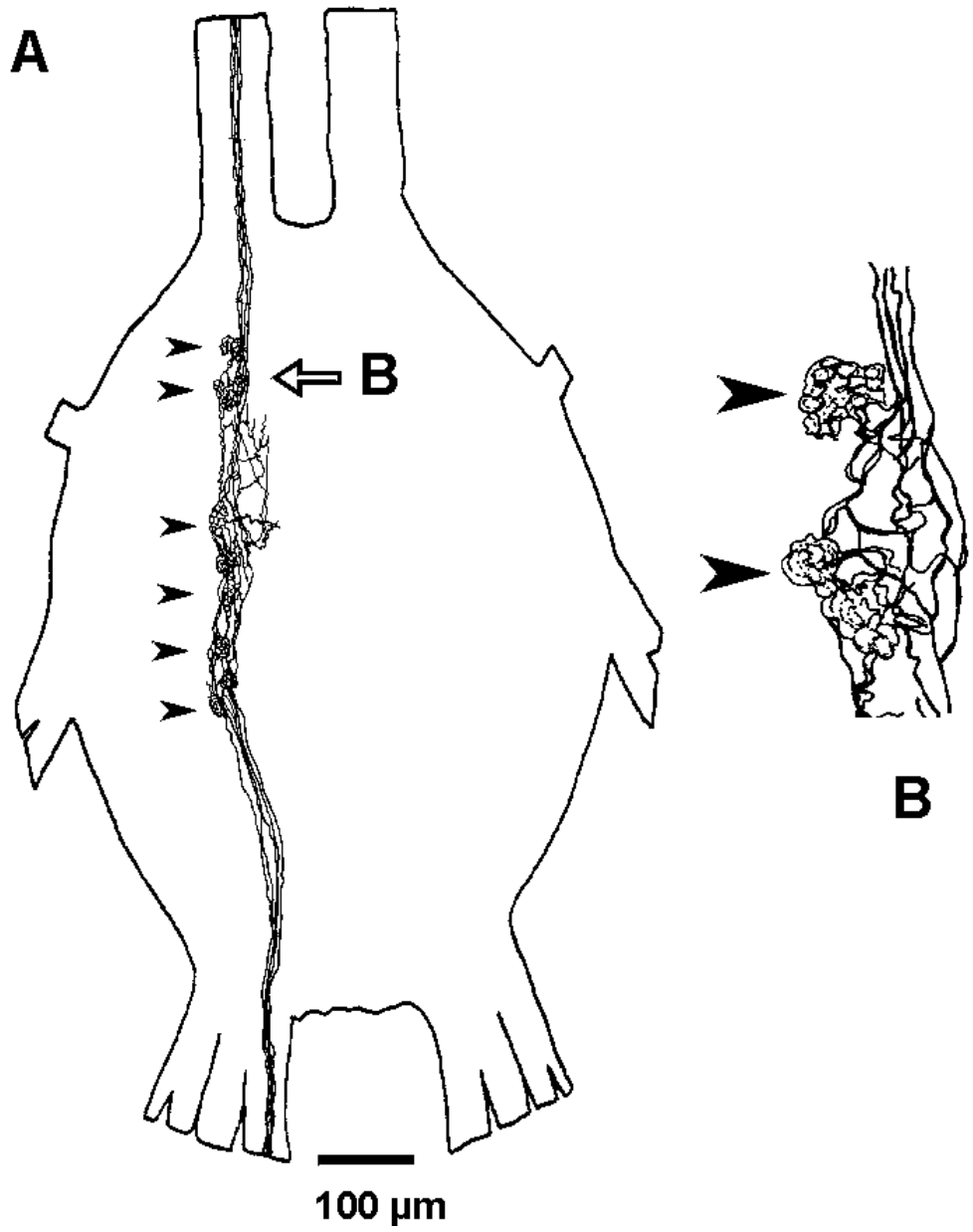


Fig. 17 . A Projections from cercal basiconic sensilla resemble in outline the parproctal, but narrow and dense fields of arborization form in the ninth and the eighth neuromere, some of them shaped like glomeruli (arrowheads). B. Expanded view of glomerular-like structures, usually involving three different afferents.

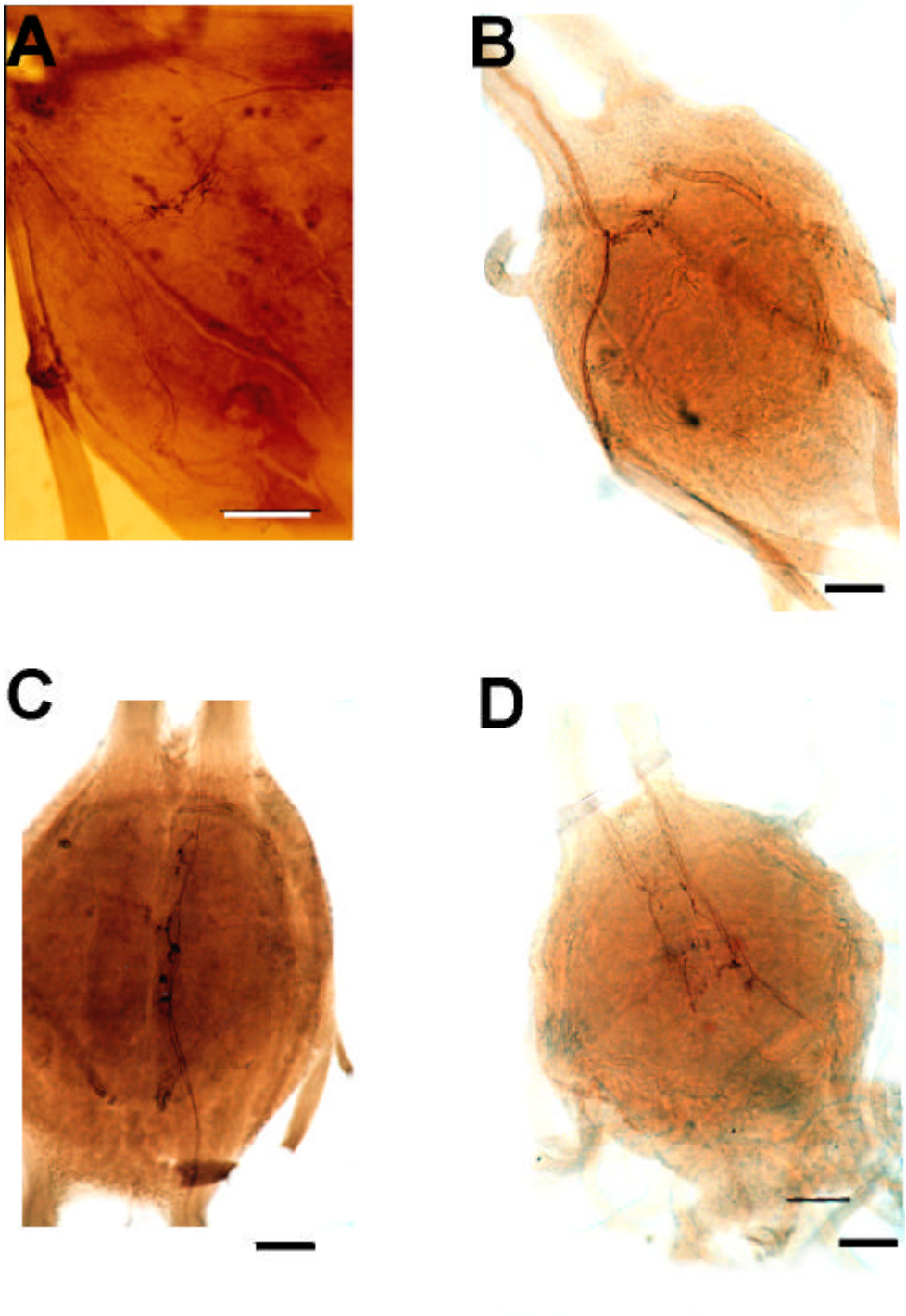


Fig. 18 micrograph of chemosensory hair projections from the ventral ovipositor valve (A), the subgenital plate (eight sternite, B), the cerci (C) and the epiproct (D). A. projection from a single basiconic hair on the ventral ovipositor shows the five axon entering via nerve 8V and branching only ipsilaterally in the VAC of the eighth neuromere at a media-ventral level. B. projection from single sternal basiconic sensillum shows the five

afferents project laterally in the eighth neuromere and a collateral proceeds into the ipsilateral ganglion. C. A Projections from cercal basiconic sensilla resemble in outline the parproctal, but narrow and dense fields of arborization form in the ninth and the eighth neuromere, some of them shaped like glomeruli. D. projection from a single basiconic hair on the epiproct shows the five axon entering via epiproct nerve; scale=100µm.

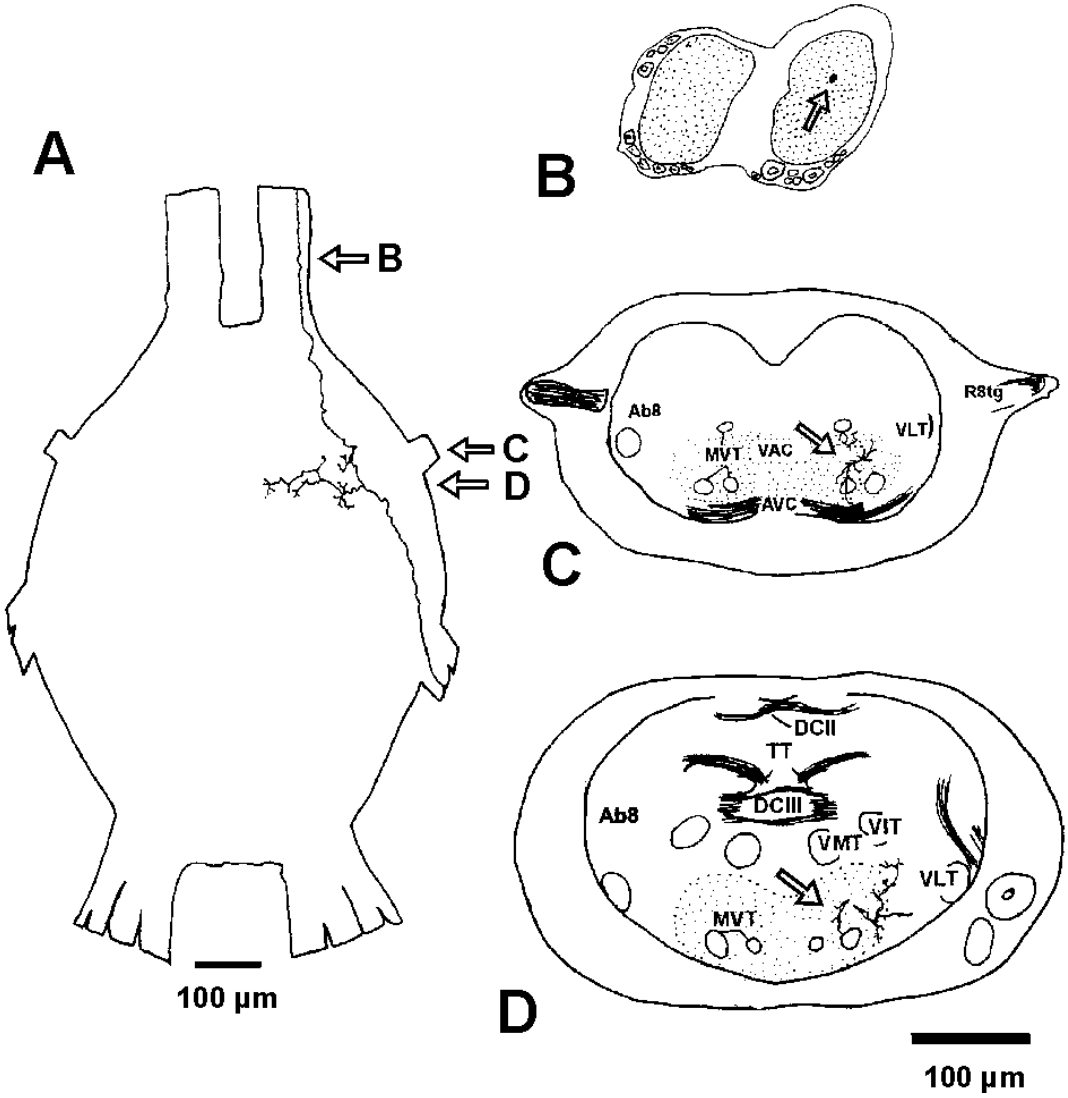


Fig. 19 Projection from a single filiform hair on the ventral surface of the ventral oviposior. A. overview of the wholemount with the axon entering via nerve 8V and branching only ipsilaterally in the VAC of the eighth neuromere at a medio-ventral level. B-D Transverse sections with areas of projections indicated (arrows).

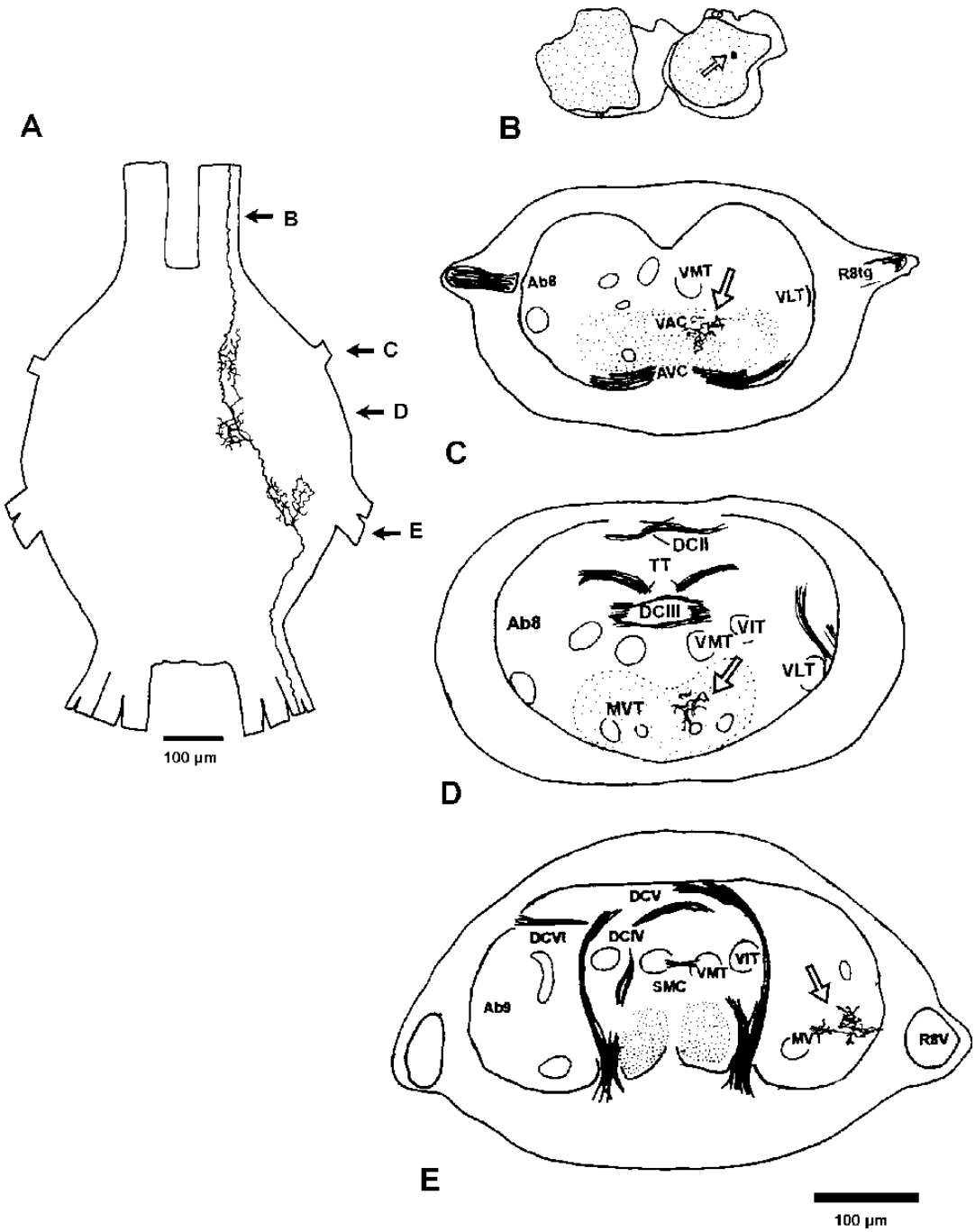


Fig. 20 Projections from a single filiform hair on the dorsal surface of the dorsal ovipositor. A. Overview of the wholemount with the axon entering via nerve 9V, travelling laterally to the ninth neuromere with primary branching medio-ventrally, proceeding medially with more branching at an intermediate level between ninth and eighth neuromere and then more ipsilateral and restricted medial branching in the eighth neuromere proper. The intersegmental collateral enters the ipsilateral connective. B-D Transcerse

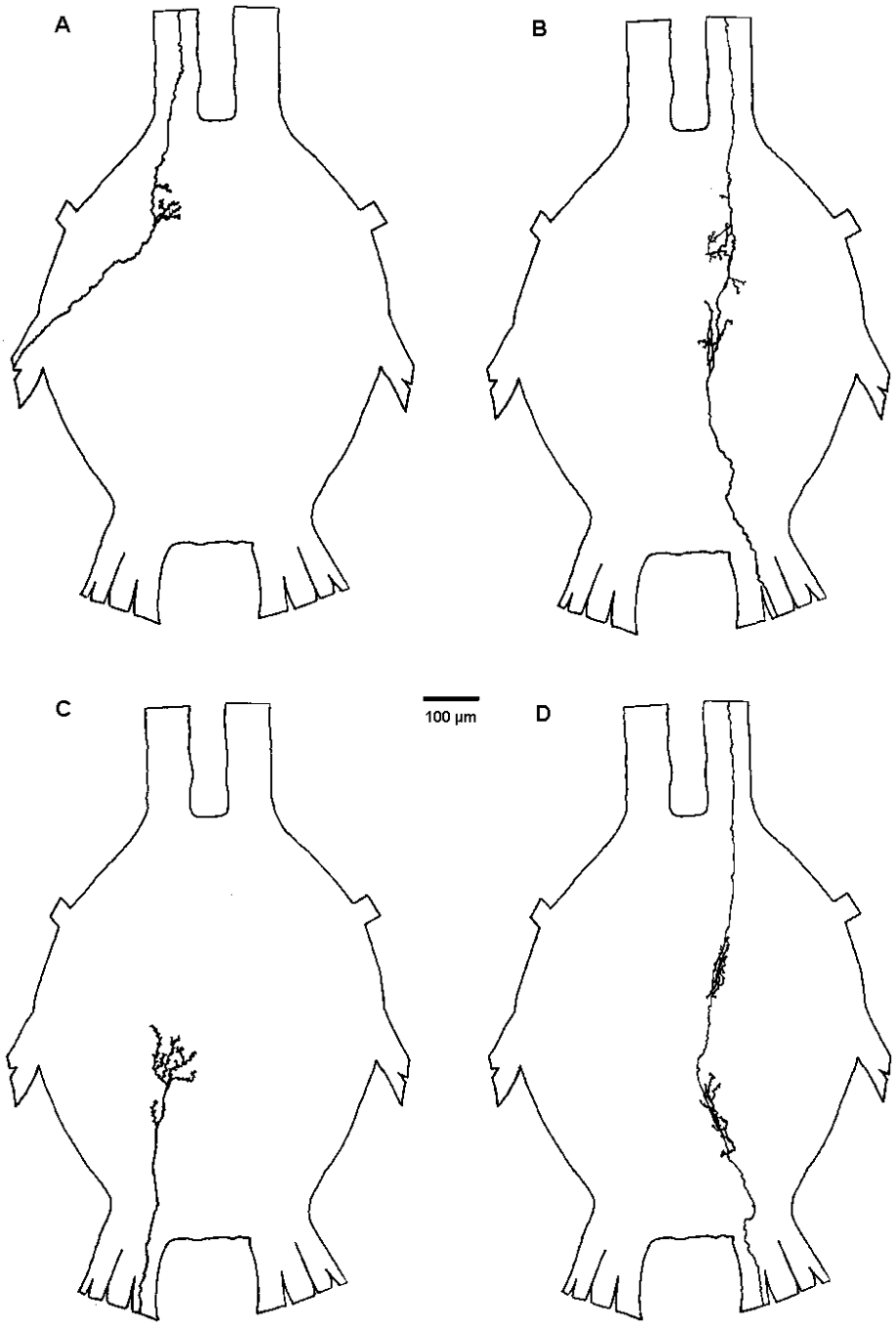


Fig. 21 Mechanosensory hair projections from the subgenital plate (eighth sternite, A), the paraproct (B), the cercus (C), and the epiproct (D). A. From one sternal trichoid sensillum the afferent projects laterally in the eighth neuromere and a collateral proceeds into the ipsilateral ganglion. B. The afferent of a paraproctal hair sensillum progresses through the terminal ganglion ventro-medially with branches in the anterior VAC of the ninth neuromere and the posterior eighth neuromere before it reaches the ipsilateral connective. C. The cercal filiform hair afferent terminates locally in the ‘cercal neuromere’. D. The epiproctal afferent proceeds ventro-medially through the terminal ganglion, first branching near the ‘cercal

neuromere' and near the posterior eighth neuromere before it enters the ipsilateral connective.sections with areas of projections indicated by arrows.

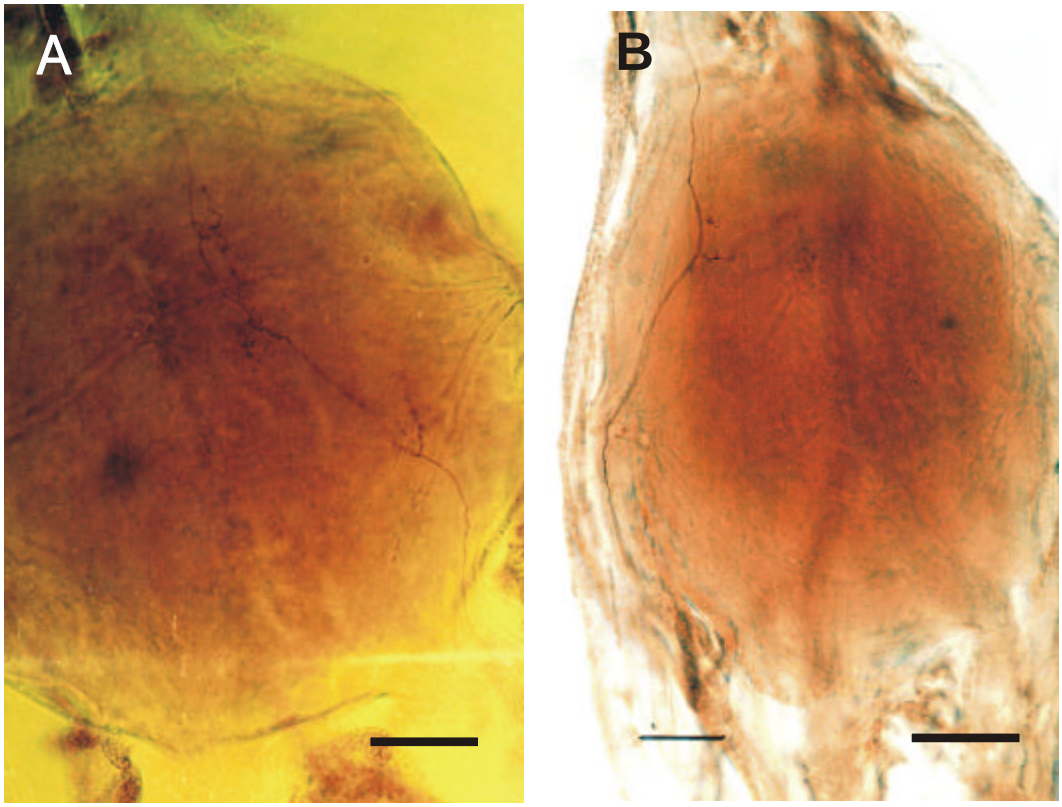


Fig. 22 micrograph of mechanosensory hair projection from the dorsal ovipositor valve (A) and the subgenital plate (B). A. projections from single filiform hair on the dorsal surface of the dorsal ovipositor shows the axon entering via nerve 9V, travelling laterally to the ninth neuromere with primary branching medio-ventrally, proceeding medially with more branching at an intermediate level between ninth and eighth neuromere and then more ipsilateral and restricted medial branching in the eighth neuromere proper. B. projection from single sternal trichoid sensillum shows the afferent projects laterally in the eighth neuromere and a collateral proceeds into the ipsilateral ganglion.; scale= 100µm.

3.5 Extracellular stimulation of single chemosensory cells:

The afferent responses from gustatory receptors of the locust female ovipositors had to be tested with classical tip recording, that is, all solutions applied as a test stimnulus contained a minimum content of salt (10mM NaCl) required for conduction in water between the inner surface of the receptor and the electrode applied to the tip for recording. So at least the two potential stimulants water and salt are present and coded by two different receptor neurons of a single basiconic sensillum at contact with the electrode solution. Therefore, the tip recording method is not suitable for testing pure water or moisture and also not for possible gaseous stimuli.

Responses to water and potential gaseous stimuli were tested only at the level of ascending interneurones after the afferent responses have been integrated within the terminal ganglion.

Identified taste receptors from a well described region of the ventral ovipositor were tested for their responses to different chemical concentrations at the receptor and interneuronal integration level.

3.5.1 Receptor level:

Contact with different chemicals applied with blunt glass electrodes placed with their end over the tip of a sensillum on the locust ventral ovipositor valve evoked spikes in the sensory neurones innervating that sensillum. These extracellular recordings were carried out to study the afferent responses to NaCl, glucose, citric acid, NHT, veratrole, acetophenone, quinine and salicine. The investigation showed that the basiconic sensilla of the ovipositor were sensitive to all mentioned stimuli. Some chemicals evoked spikes of more than one amplitude suggesting that more than one sensory neurone was activated by single chemicals.

The results indicated that both the frequency and the amplitude of afferents from ovipositor basiconic sensilla differed according to the types of chemical stimulation. Moreover, different chemicals evoked spikes in different combinations of sensory neurones. For example, the pattern of spike activity evoked by 0.1% salicine in 10 mM NaCl was very different from that evoked by 0.1M citric acid in 0.01M NaCl or another chemical substances. These results are typical also for of contact chemoreceptors found on the mouthparts of most insect species (Blaney 1974, 1975), on the locust middle legs (Gaboub and Hustert, 1998) and on the locust hind legs (Newland, 1998).

A characteristic feature of the responses of the chemosensory neurones to each of the chemicals tested was a rapid reduction (adaptation) of their spike frequency during maintained chemical stimulation. This rapid adaptation resulted in an almost complete abolition of their response to a chemical stimulant within 200-650 ms of its application (Fig. 23, 24 , 25). Two different response types occurred. In most cases the chemically sensitive neurone began to fire immediately upon stimulation, followed by a period of adaptation. 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).

The response to salt in single basiconic sensilla of the ventral ovipositor is phasic with two main units for salt and possibly water at a concentration of 10mM sodium chloride (Fig. 23). Sometimes initially, as a third class of afferent units very large spikes arise from the mechanosensory neuron at the moment of contact.

The response to glucose solutions in single basiconic sensilla of the ventral ovipositor is phasic. The two large units could respond to suger and the electrolyte salt (0.01M sodium chloride) and a small third unit possibly responds to the water (Fig. 23). Intitally, as a third class of afferent units very large spikes arise from the mechanosensory neuron at the moment of contact.

The response to citric acid in single basiconic sensilla of the ventral ovipositor is phasic with two main units for citric acid and salt (0.01M sodium chloride serving as electrolyte) and a smaller unit possibly responds to the water. initially, as a fourth class of afferent units very large spikes arise from the mechanosensory neuron at the moment of contact.

In response to the aggregation pheromones veratrole and acetophenone in solution (Fig. 24), only a few spikes were elicited in the basiconic sensillum after the mechanosensory unit has responded first near the contact artefact of stimulus application. Responses to application of diluted alkaloids as salicine and quinine hydrochloride to a basiconic sensillum are rather strong and specific (Fig. 25).

3.5.2 Exteracellular recording of chemosensitive interneurones:

The investigation showed that basiconica sensilla on the ovipositor are sensitive to all used chemical substances at different degrees. The spiking responses increased with higher concentrations but with bursting tendency (except after application of sugars) at higher concentration. The adaptation was rapid with lower concentration for ovipositor basiconica sensilla. When a new sensillum was stimulated with low concentration a clear response was observed. However, when a sensillum was examined after applying high concentrations, low concentration elicited less response.

3.5.2.1 Responses to tap water:

At the receptor level recording of responses to pure water are not possible but several ascending interneurons seem to be highly responsive to plain water (Fig 26), but none of these could be identified intracellularly .

3.5.2.2 Responses to sodium chloride:

Ascending interneuron responses to sodium chloride were tested at molraities of 0.01M to 3.0M (Fig. 27). Typically, at least three interneurons responded, recognized from their three different unit amplitudes. It cannot be distinguished to which sensory cue contained in the stimules the responses are specific.

The spikes frequency increases with stimulus concentration until 1.0M sodium chloride a maximum spike frequency was reached. Beyond this concentration, the response of salt sensitive interneurones remains at a constant level. Figure 28 shows that the adaptation was rapid after application of low concentrations and very slowly in high concentration. Three seconds was sufficient to complete adaptation in 0.01M and six seconds for 1.5M sodium chloride.

3.5.2.3 Responses to glucose:

Responses of ascending interneurons to glucose were tested at molarities of 0.01M to 3.0M. The reaction to glucose (Fig. 29) indicates that several units of ascending interneurons respond two of which can only be responses to sugar due to their concentration-related increase in spiking frequency.

Spike activity increases with stimulus concentration until 1.0M glucose a maximum spike frequency was reached. Beyond this concentration, the response of sugars and water sensitive cells remains at a constant level (Fig. 30).

Figure 29b shown that a typical insect chemoreceptor response consists of phasic and tonic responses. The adaptation was rapid at low concentration (0.01M glucose) within four seconds and slower at high concentration as in 0.1M and 1.0M taking five seconds to complete the adaptation response (Fig. 30).

3.5.2.4 Responses to citric acid:

Responses of ascending interneurons to citric acid were tested at molarities of 0.01M, 0.1M and 1.0M (Fig. 31). Typical responses were occurring from concentration of 0.1M indicate that only slowly adapting ascending interneurons is activated.

The spike frequency was reached the maximum at 0.1M citric acid and adapted after two seconds. At 0.01M the adaptation was complete after three seconds, while at 1.0M it required five seconds to complete adaptation as in figure 32.

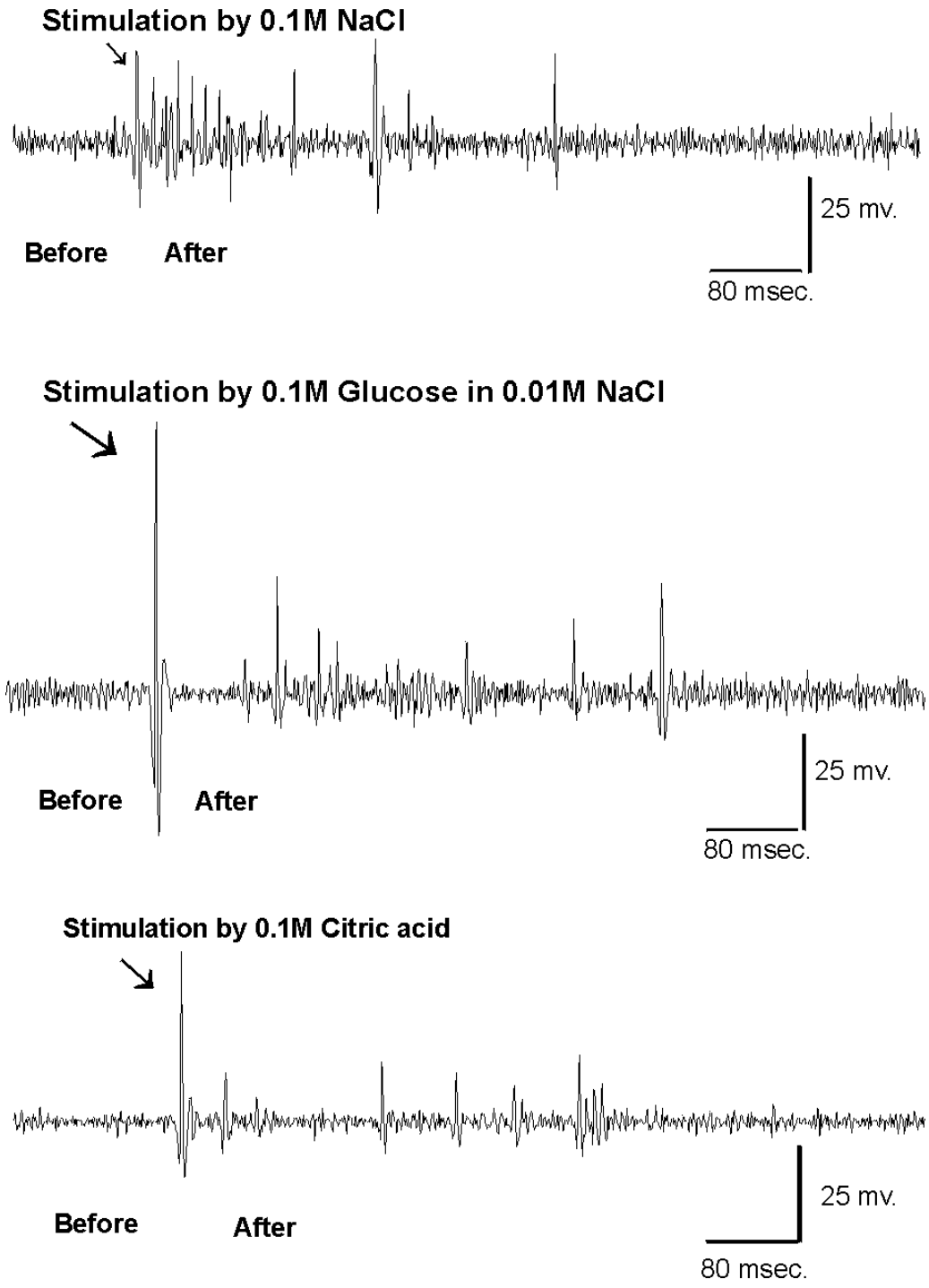


Fig. 23. Sensory responses of basiconic sensilla to contact with chemical stimulants. Spikes responses of a basiconic sensillum evoked by 0.1M NaCl, 0.1M glucose in 0.01M NaCl and 0.1M citric acid in 0.01M NaCl.

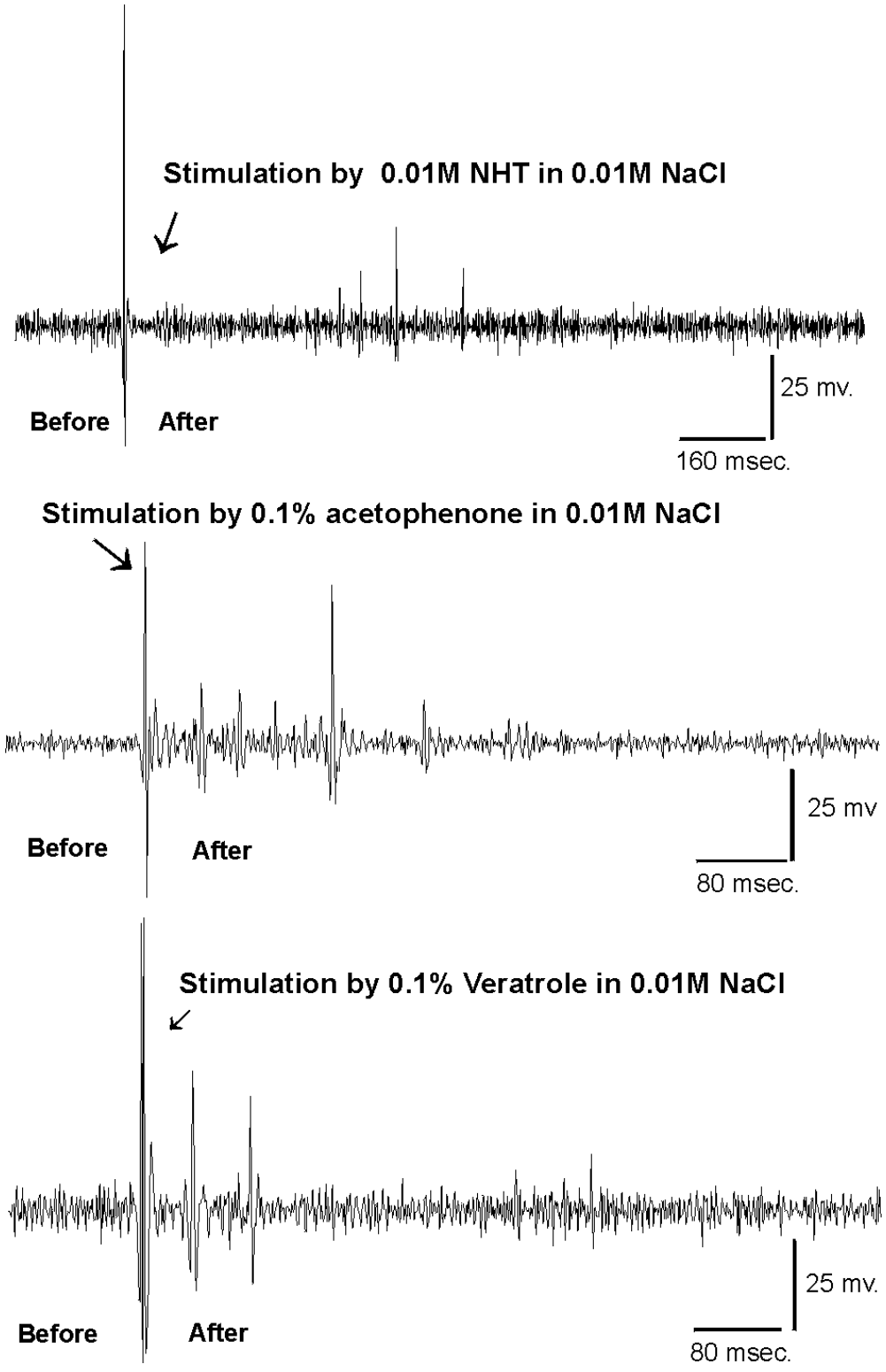


Fig. 24. Sensory responses of basiconic sensilla to contact with chemical stimulants. Spike responses of a basiconic sensillum evoked by 0.01M NHT in 0.01M NaCl, 0.1% acetophenone in 0.01M NaCl and0.1% veratrole in 0.01M NaCl.

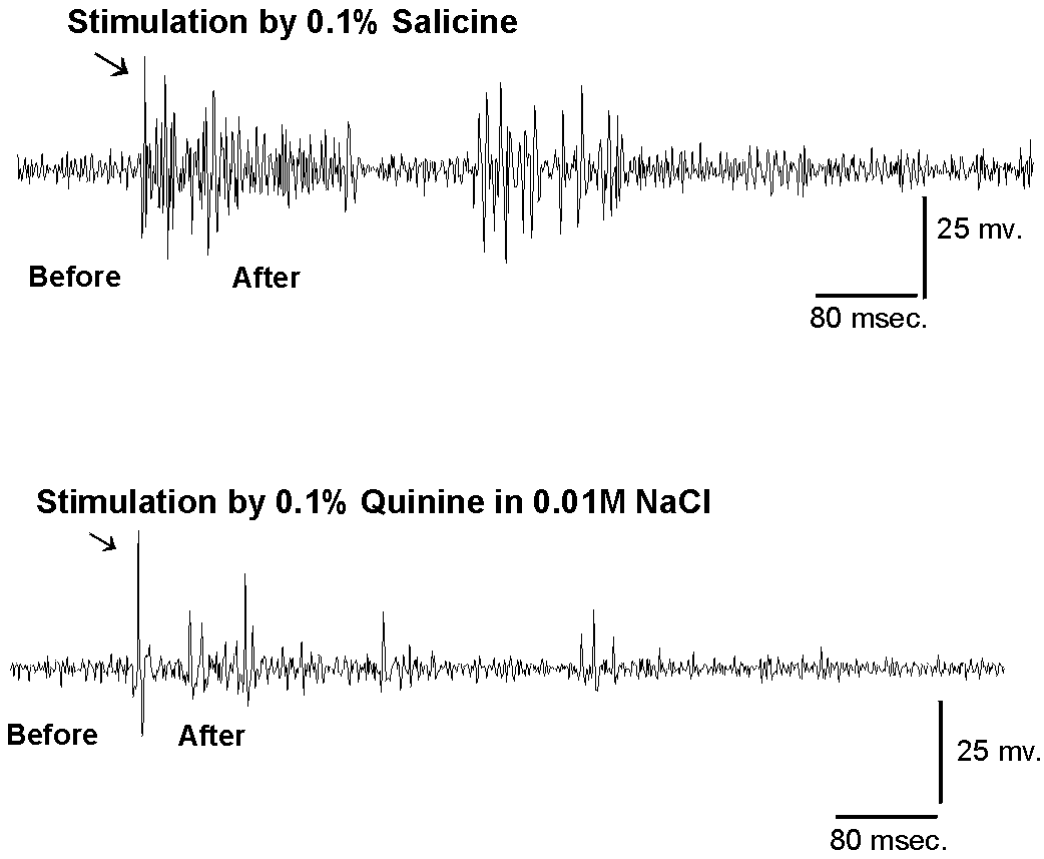


Fig. 25. Sensory responses of basiconic sensilla to contact with chemical stimulants. Spike responses of a basiconic sensillum evoked by 0.1% salicine in 0.01M NaCl and 0.1% quinine hydrochloride in 0.01M NaCl.

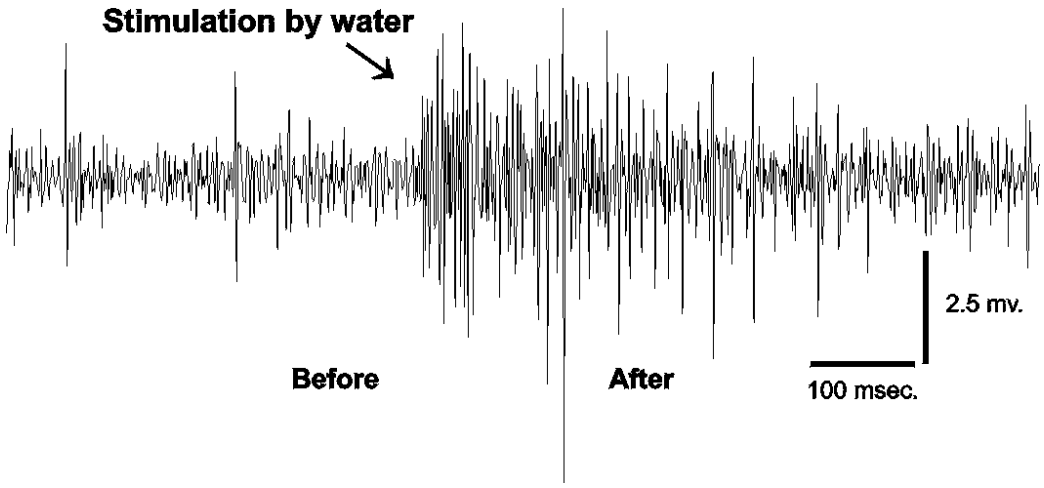


Fig. 26. Projecting interneurone(s), responding to stimulation of one ventral valve basiconic sensillum stimulated with tap water. Multiunit spikes recorded extracellularly from the connective ipsilateral to the stimulus site.

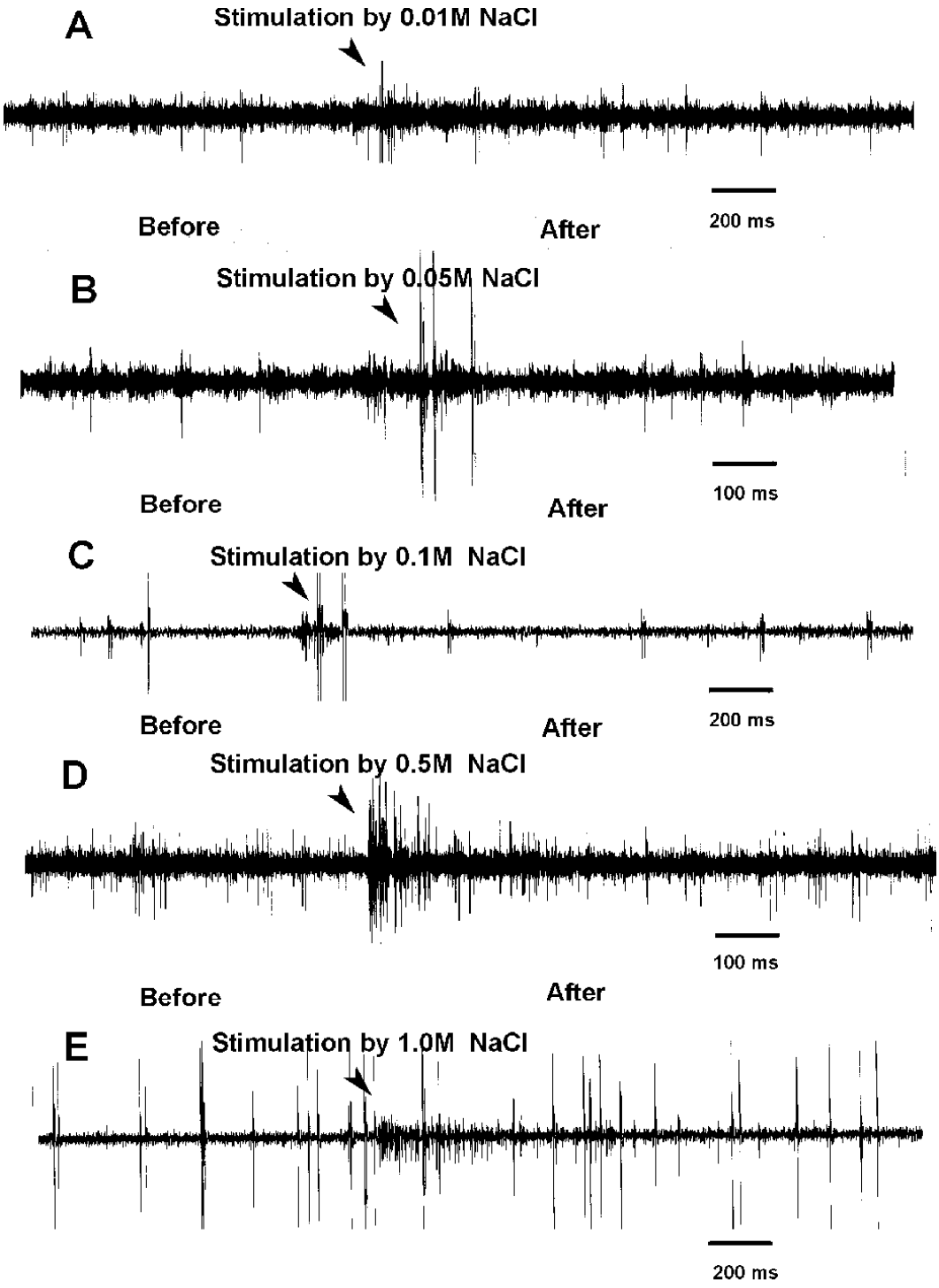


Fig. 27. Projecting interneurones, responding to stimulation of one basiconic sensillum on the ventral valve stimulated with different concentrations of NaCl in water. Multiunit spikes recorded extracellularly from the connective ipsilateral to the stimulus site (Same recording in A/B/D other recording in C/E).

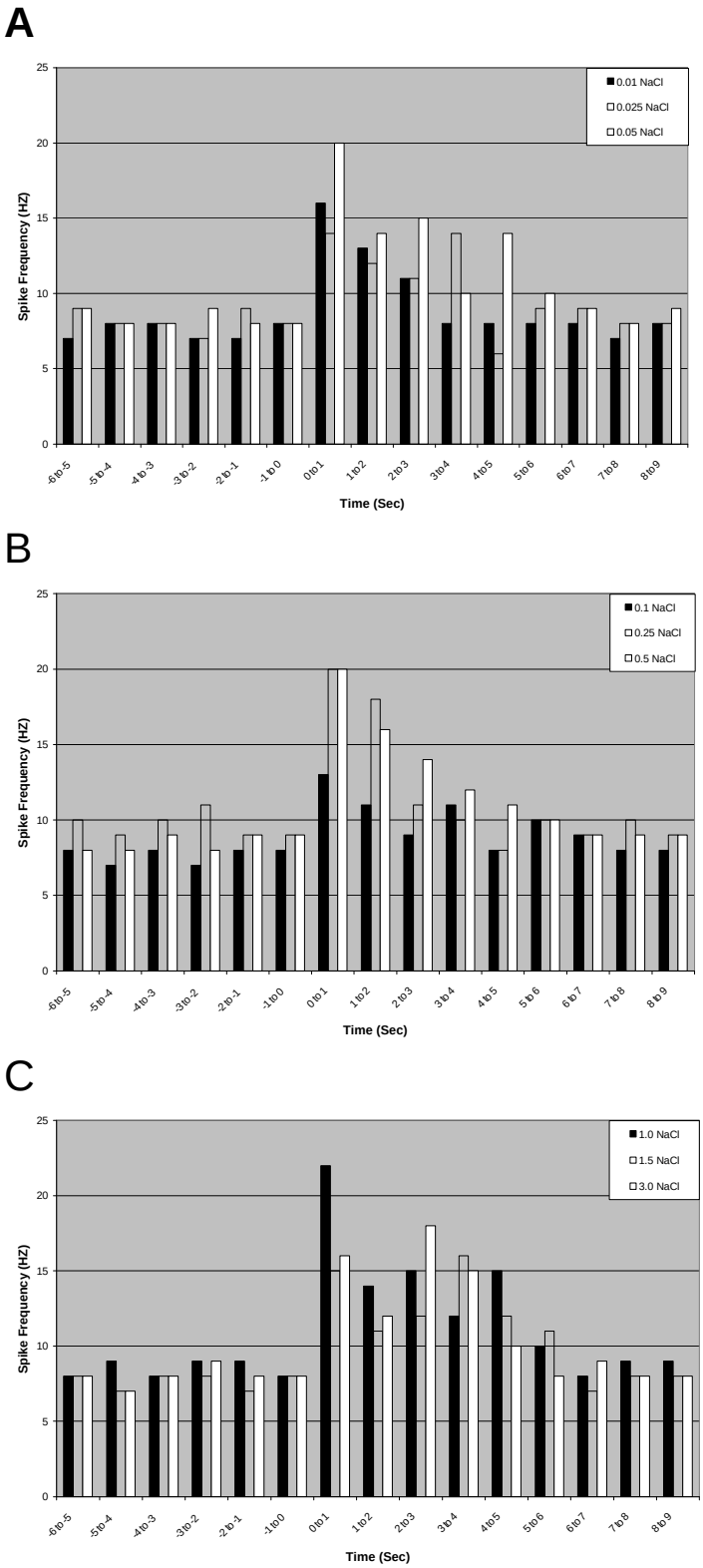


Fig. 28. Sequence of spike frequencies from interneurones (counting intervals: 1 second) before and after stimulation by a basiconic sensillum with different concentrations of sodium chloride. A. Stimulation with concentrations of 0.01 M, 0.025 M and 0.05 M. B. Stimulation of basiconic

sensilla with concentrations of 0.1 M, 0.25 M and 0.5 M. C. Stimulation with concentrations of 1.0 M, 1.5 M and 3.0 M.

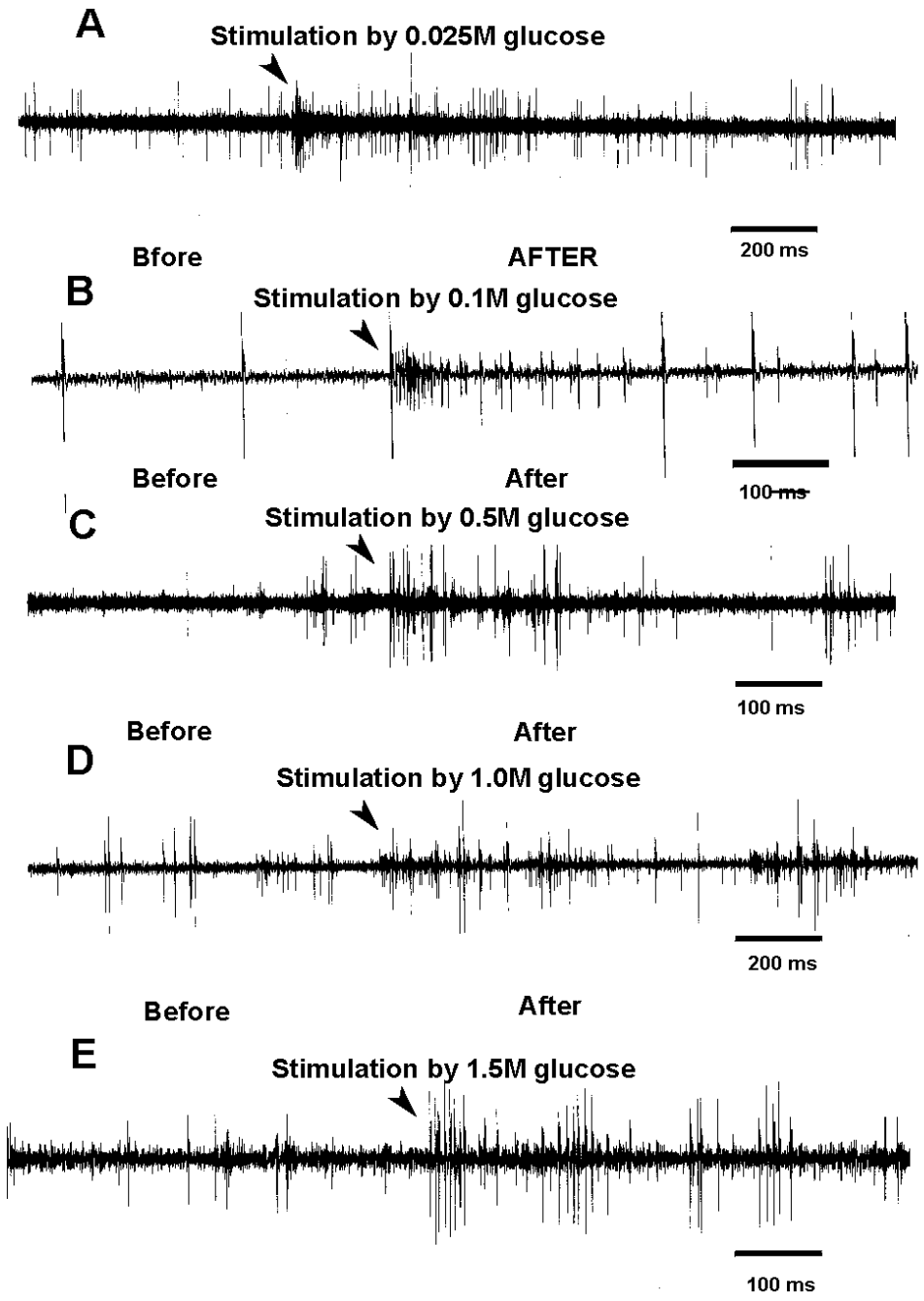


Fig. 29. Projecting interneurones, responding to stimulation of one basiconic sensillum on the ventral valve stimulated with different concentrations of glucose in water. Multiunit spikes recorded extracellularly from the connective ipsilateral to the stimulus site (Same recording B-E).

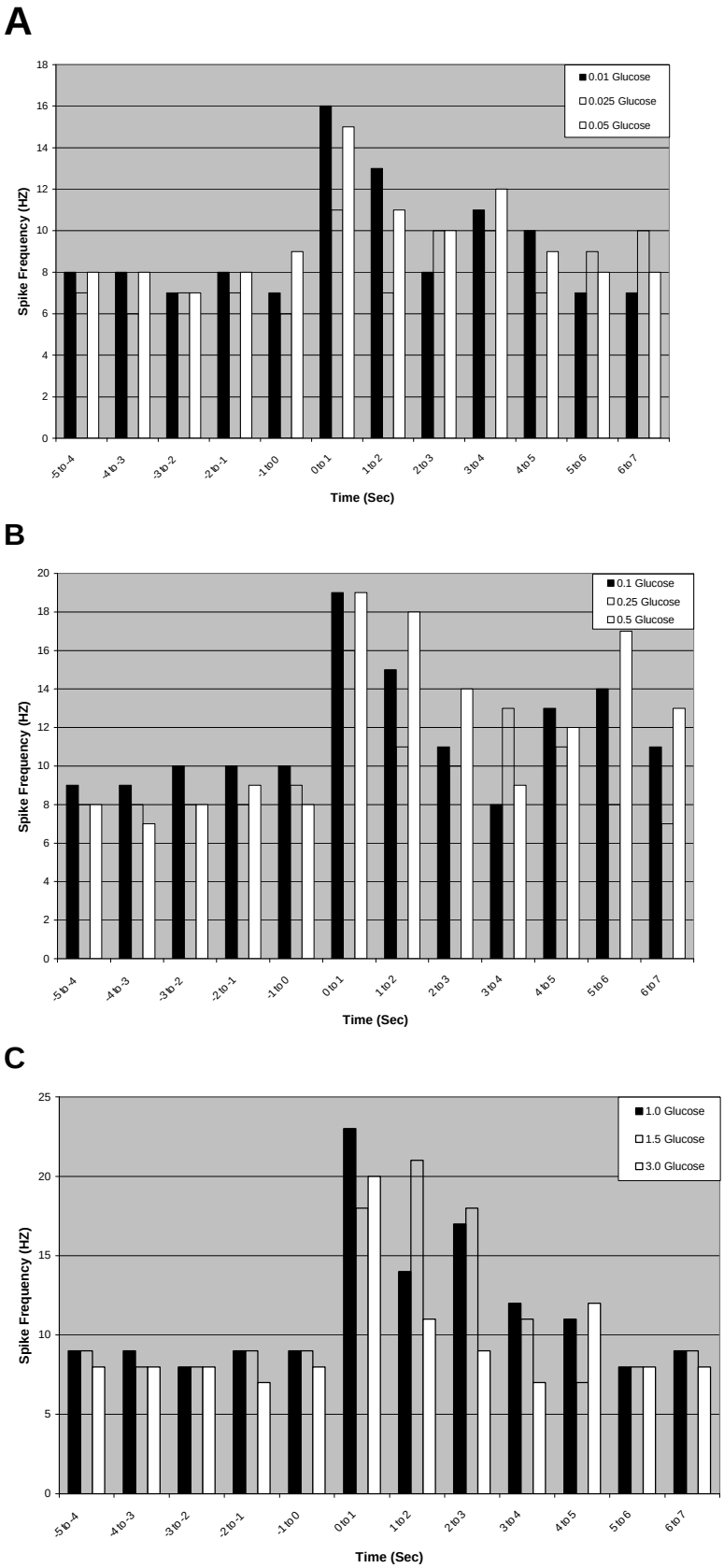


Fig. 30. Sequence of spike frequencies from interneurones (counting intervals: 1 second) before and after stimulation by a basiconic sensillum

with different concentrations of glucose. A. Stimulation with concentrations of 0.01 M, 0.025 M and 0.05 M. B. Stimulation of basiconic sensilla with concentrations of 0.1 M, 0.25 M and 0.5 M. C. Stimulation with concentrations of 1.0 M, 1.5 M and 3.0 M.

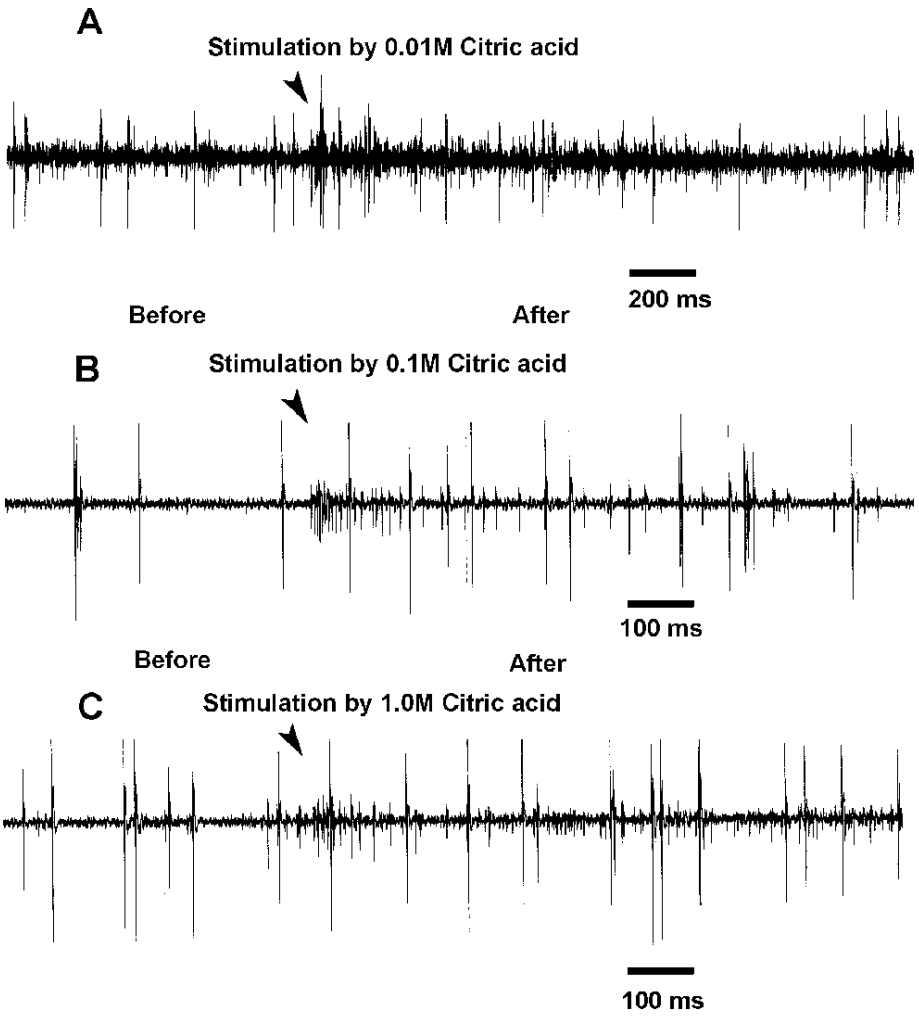


Fig. 31. Projecting interneurones, responding to stimulation of one basiconic sensillum on the ventral valve stimulated with different concentrations of citric acid. Multiunit spikes recorded extracellularly from the connective ipsilateral to the stimulus site (Same recording in B/C).

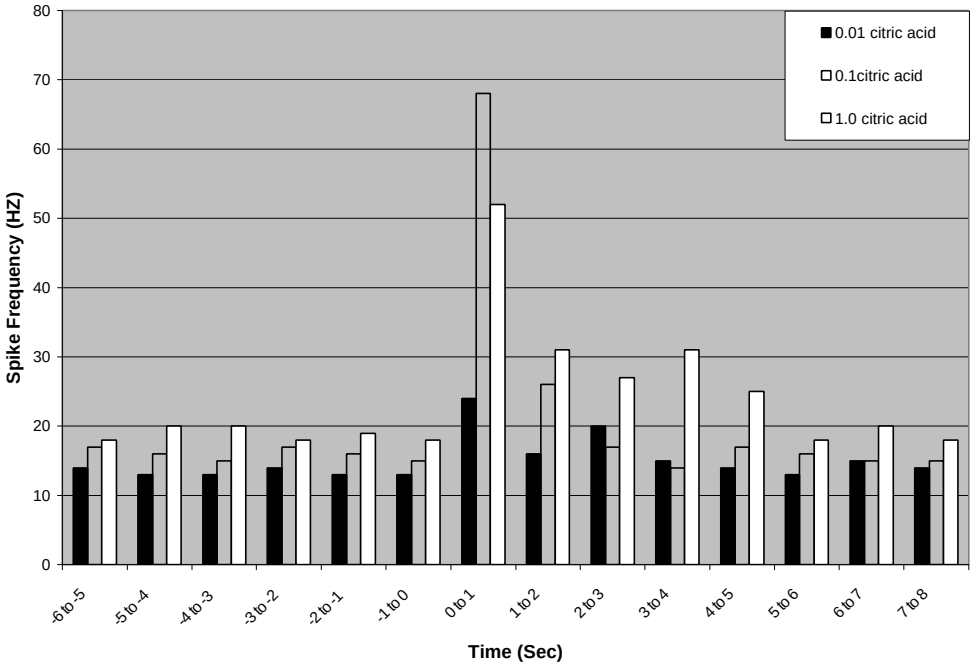


Fig. 32. Sequence of spike frequencies from interneurones (counting intervals: 1 second) before and after stimulation with different of citric acid.

3.5.2.5 Responses to oviposition aggregation pheromones:

Responses of ascending interneurons to veratrole and acetophenone were tested, typical responses was shown in figure 33, the reactions to veratrole and acetophenone indicates that at least three chemosensitive interneurones were excited. Figure 34 shows that the adaptations after application of veratrole or acetophenone was complete after five or six seconds and it was more rapid for acetophenone than for veratrole. Here only qualitative responses were recorded since only two concentration of each stimulus were used.

3.5.2.6 Responses to alkaloides and phenolic compounds:

Responses of ascending interneurons to aqueous solutions of tomatine and quinine hydrochloride were tested. Typical responses are shown in figure 35 (A & C), the reactions to 0.1% of tomatine and quinine hydrochloride indicate that at least two chemosensitive interneurones were activated. Responses to Tomatine were more intense than to

quinine hydrochloride. Here only qualitative responses were recorded since only one concentration of each stimulus was used. Figure 36 shows that the adaptations to tomatine or to quinine hydrochloride were rapid and complete after three seconds of stimulation.

The interneuron responses tostimulation of a ovipositor basiconic sensillum with salicine (phenolic compound) was tested. The typical responses is shown in figure 35b, the reactions to 0.1% of salicine indicates that at least three or four chemosensitive interneurones were excited. Figure 36 shows that the adaptation after salicine application was very rapid within two seconds.

3.5.2.7 Responses to neuromodulators and neurotransmitters:

Ascending interneurones responses to 0.1% of serotonin and octopamine (neuromodulator) are shown in figure 37 (A&B). Only two chemosensitive interneurones were excited. Interneuron responses of ovipositor basiconic sensillum to neurotransmitters as GABA was shown in figure 38, the reactions to 0.1% of GABA indicates that at least three chemosensitive interneurons were excited. Figure 38, shows that, the adaptation to serotonin and GABA were very rapid after one second and after two seconds in octopamin.

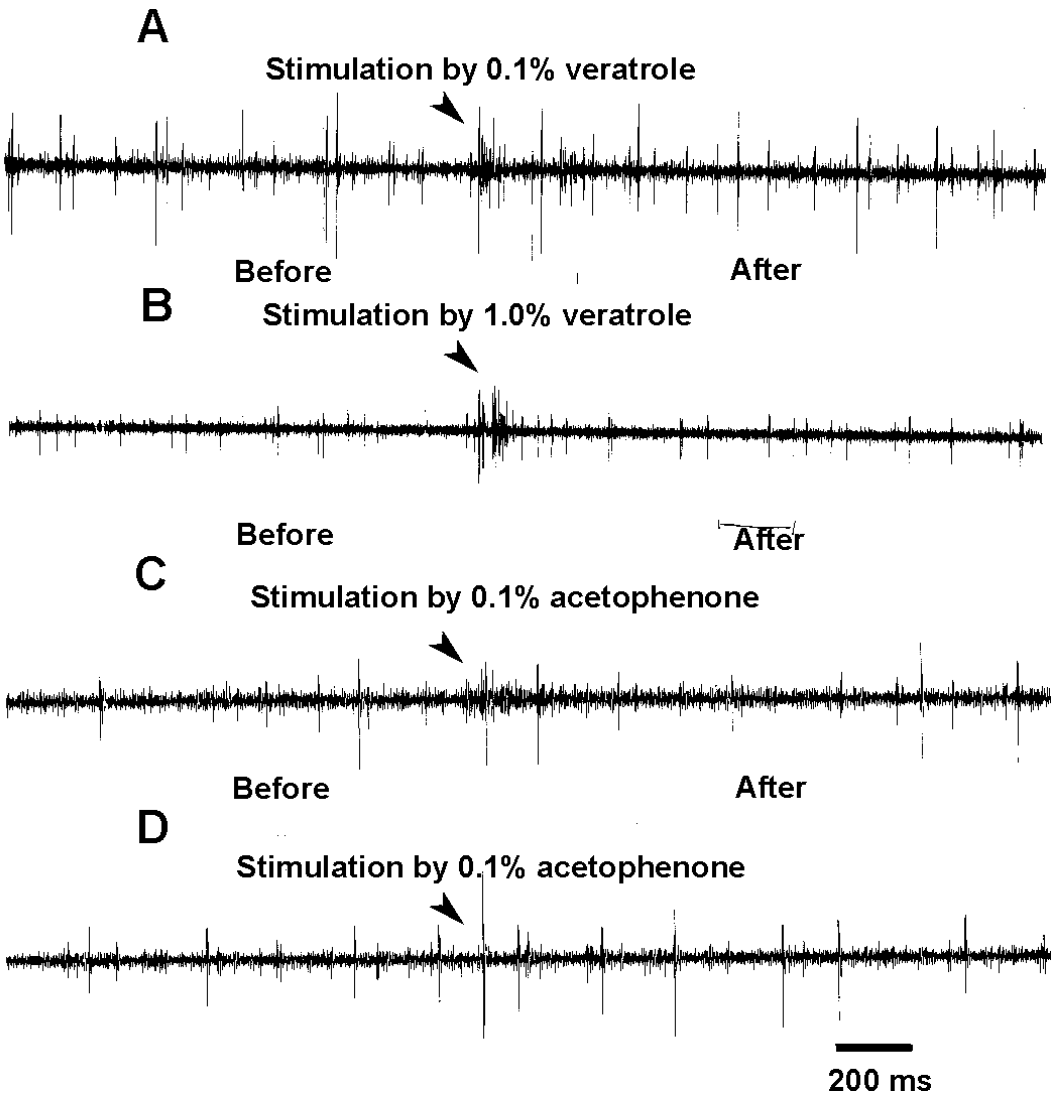
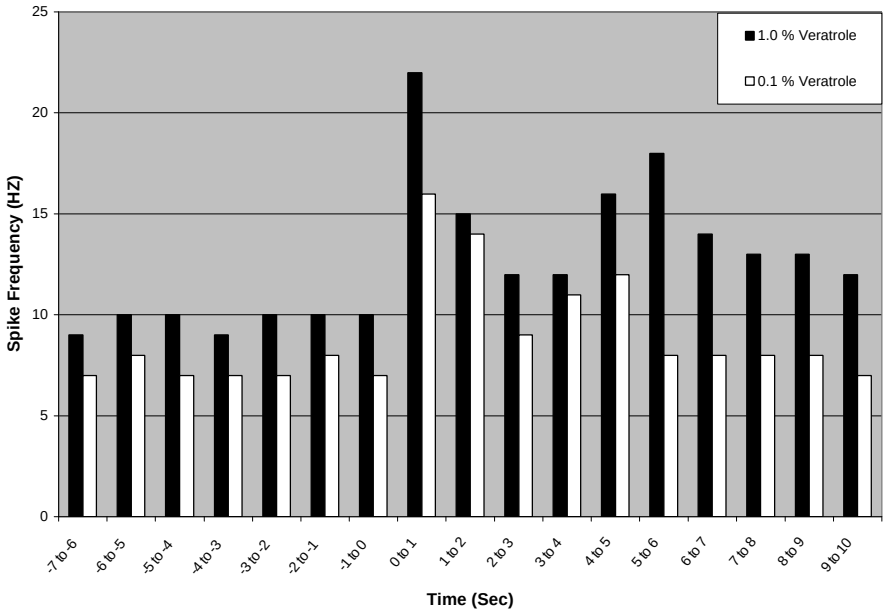


Fig. 33. Projecting interneurones responding to stimulation of one basiconic sensillum on the ventral valve stimulated with different concentrations of veratrole and acetophenone. Multiunit spikes recorded extracellularly from the connective ipsilateral to the stimulus site (Same recording in B/C).

A



B

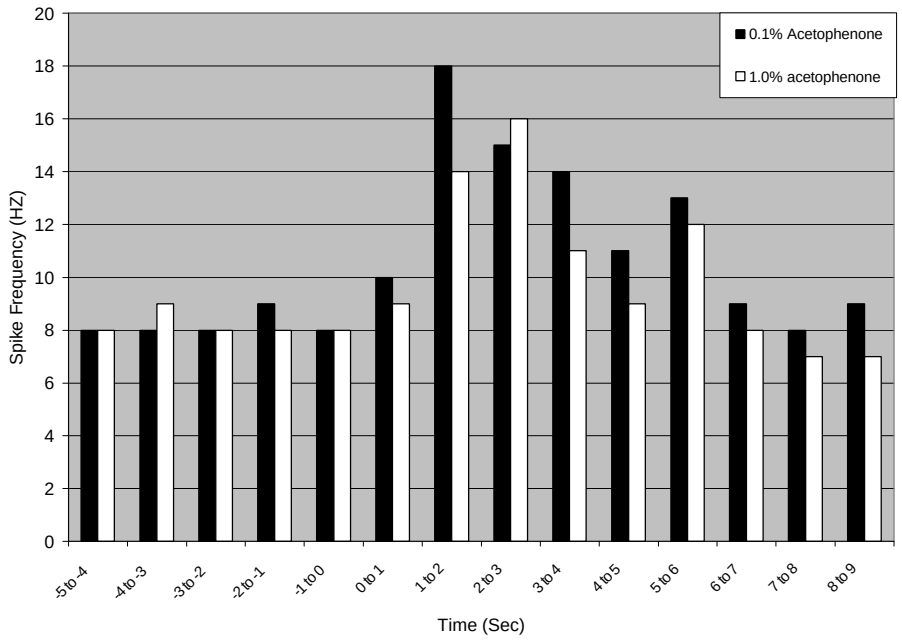


Fig. 34. Sequence of spike frequencies from interneurons (counting intervals: 1 second) before and after stimulation of a basiconic sensillum with different phenolic attractants for locust oviposition: veratrole and acetophenone. Different concentrations were tested in A and B.

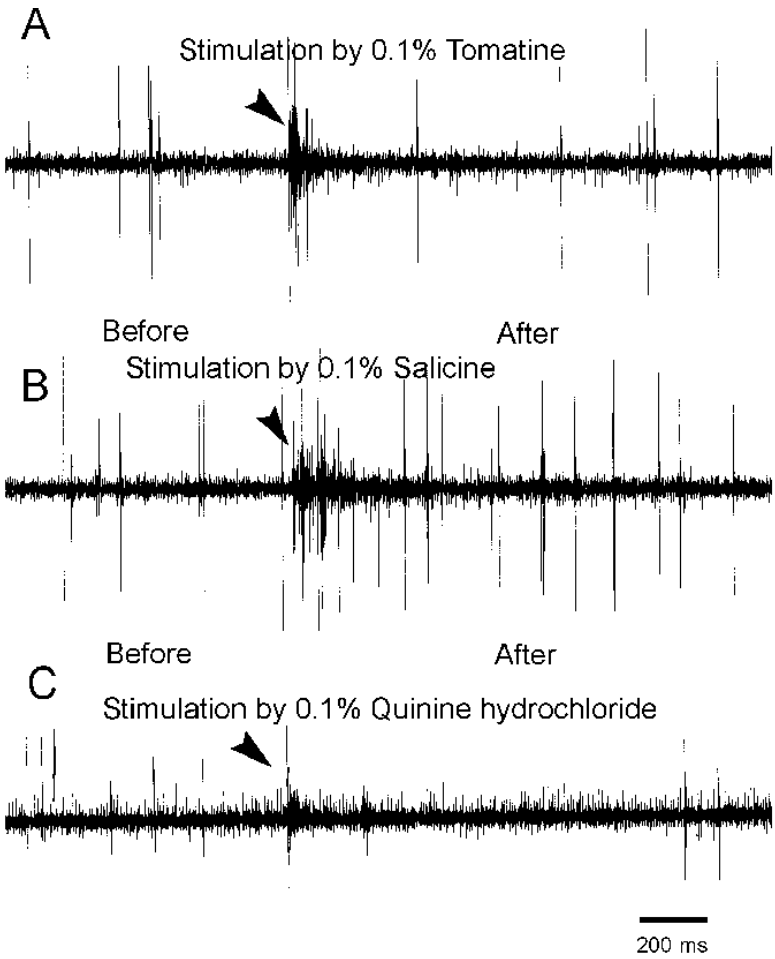


Fig. 35. Projecting interneurones, responding to stimulation of one basiconic sensillum on the ventral valve stimulsted with 0.1 M concentrations of tomatine (A), salicine (B) and quinine hydrochloride (C). Multiunit spikes recorded extracellularly from the connective ipsilateral to the stimulus site (Same recording in B/C).

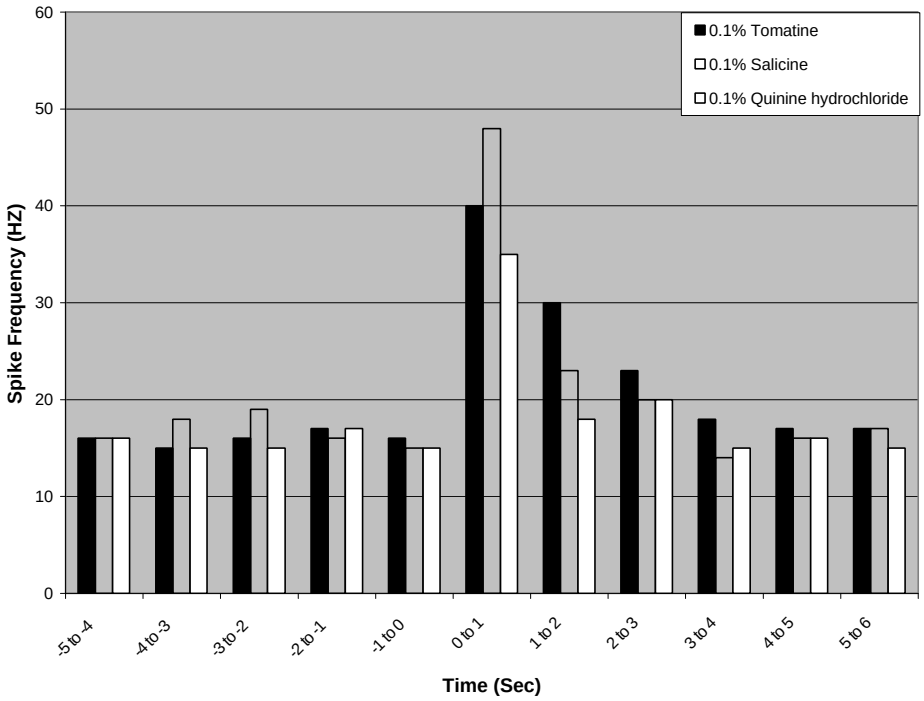


Fig. 36. Sequence of spike frequencies from interneurones (counting intervals: 1 second) before and after stimulation of a basiconic sensillum with phenolic compound as salicin and with alkaloids as quinine hydrochloride and tomatine.

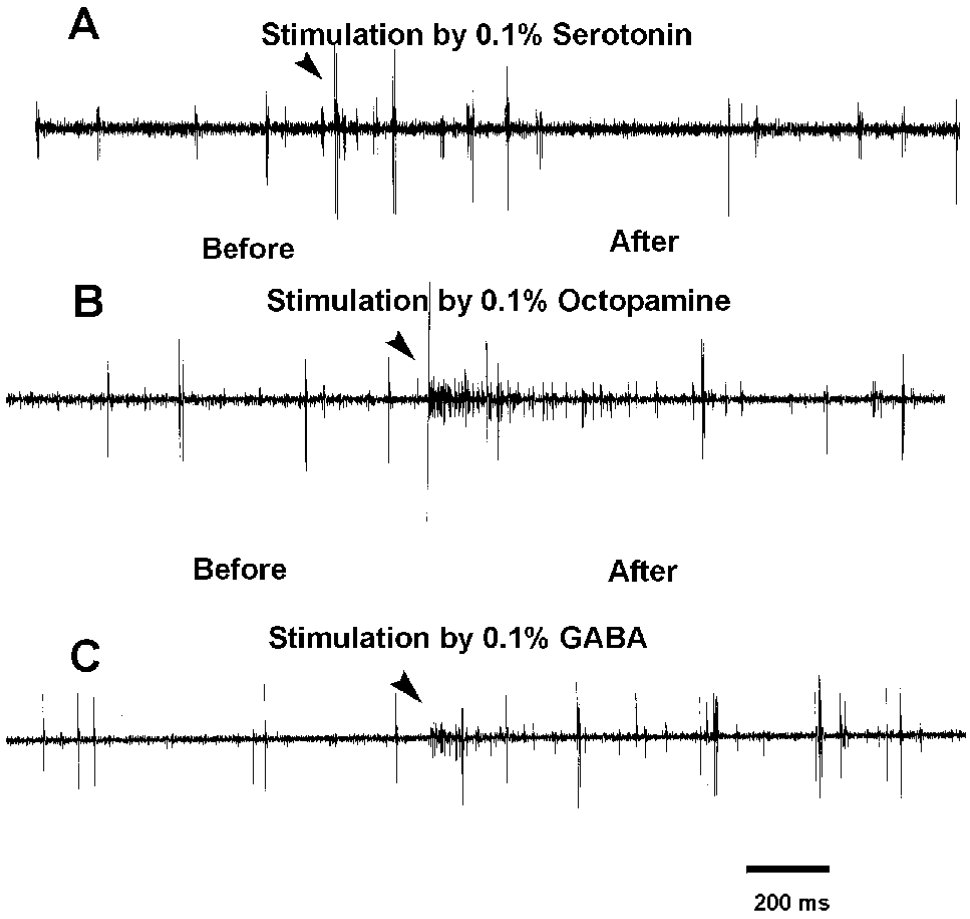


Fig. 37. Projecting interneurones, responding to stimulation of one basiconic sensillum on the ventral valve stimulated with 0.1 M concentrations of serotonin (A), octopamine (B) and GABA (C). Multiunit spikes recorded extracellularly from the connective ipsilateral to the stimulus site (Same recording in B/C).

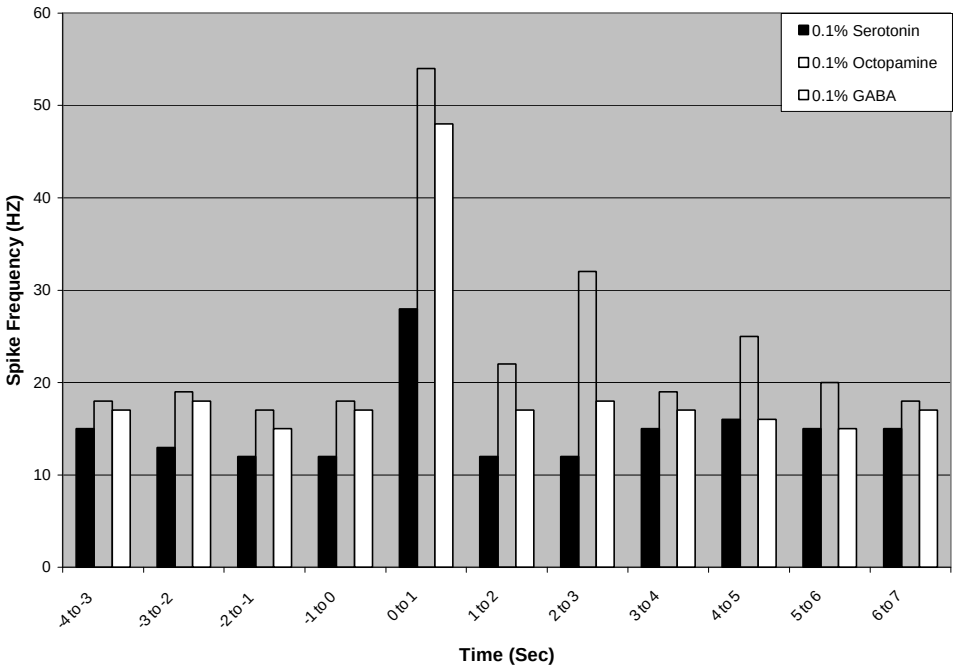


Fig. 38. Sequence of spike frequencies from interneurones (counting intervals: 1 second) before and after stimulation of a basiconic sensillum with neurotransmitters as GABA and neuromodulators as 5-HAT (serotonin) and octopamine.

3.6 Intracellular results:

3.6.1 Morphological properties of interneurones:

Stimulation of the ovipositor contact chemoreceptors by different chemical aqueous solutions evokes excitatory or / and inhibitory responses in the interneurones. Each identified interneuron has a characteristic morphology defined by its array of branches in the regions of neuropil that provide its inputs and the output to other neurones. In the course of this study, we recorded and identified 6 interneurones were identified (2 local and 4 intersegmental interneurones) in the terminal abdominal ganglion that responded to chemical stimulation of basiconic sensilla on the ventral ovipositor valve of *Locusta migratoria*.

The soma and the neurites of each interneuron are located within the terminal ganglion. Some are restricted to one neuromere (as in ChSIN 3 and ChSIN 6^{th} to 8^{th} abdominal neuromere) and others innervate several neuromeres (as ChSIN 2, ChSIN 4 and ChSIN 5 in the neuromeres and ChSIN 1 in the 8^{th} to 10^{th} neuromere). The somata of all identified interneurones lie near the ventral surface of the anterior half of 8^{th} abdominal neuromere in the terminal ganglion.

3.6.1.1 Local interneurones:

These cells lacked an axon projecting out of the terminal ganglion, there fore they are local interneurones. Here we identified two local interneurones (ChSIN 1 and ChSIN 2) based on intracellular staining with Lucifer Yellow in the terminal ganglion (Fig. 39, 41). The soma (approximately 30-35 µm in diameter) of ChSIN 1 lies near the ventral surface of the anterior half of 8^{th} neuromere while the ChSIN 2 soma lies in the posterior half of 8^{th} neuromere. These interneurons are characteristic by their extremely dense pattern of arborisation in the 8^{th} abdominal neuromere and extensions into the 9^{th} and 10^{th} neuromere.

ChSIN 1 has a characterisics ipsilateral dendritic arborisation, confined to the 8th and the 9th neuromeres while only one branch in the 9th neuromere extends across midline towards the anterior-medial edge of the contralateral neuropil. ChSIN 2 extends ipsilaterally in two distinct fields in the 8th and the 9th abdominal neuromere and has two additional branches in the contralateral neuropil. Ipsilaterally, many secondary neurites developed dense and fine branches invading both the ventral neuropil of the eighth and ninth abdominal neuromeres. The neurites continue into the contralateral neuropil where they exhibit fewer but extensive fine branches.

3.6.1.2 Intersegmental interneurones:

Four interneurones have their cell body (soma) and dendrites in the terminal abdominal ganglion and an axon ascending in a connective contralaterally (ChSIN3, ChSIN 4 and ChSIN 6) or ipsilaterally (ChSIN 5) (Fig. 40, 41). The axon diameter of all identified interneurones are under 10 µm and the somata (less than 30 µm in diameter) and lie near the ventral surface of the anterior half of 8th abdominal neuromere in the terminal ganglion.

The axons of interneuron ChSIN 5 ascends through the DIT to the ipsilateral connective to the 7th abdominal ganglion while interneurones ChSIN 3, ChSIN 4 and ChSIN 6 have axons ascending through the DIT into the contralateral connective and reach the 7th abdominal ganglion. Interneurones 7 and 13 have an extremely dense pattern of arborisation in the midline of the 8th abdominal neuromere while the Interneurones ChSIN 4 and ChSIN 5 are characteristed by their dendritic arborisations which are confind to the 8th neuromere with two small branches extending posterioly to the 9th neuromere.

3.6.2 Physiological properties of interneurones :

Stimulating contact chemoreceptors of the ovipositor by different chemicals in aqueous solutions evokes excitation or inhibition in integrating interneurones (Table 2).

Excitatory responses to salt stimulation (100mM NaCl) was seen in two ascending interneurons (ChSIN 4 and ChSIN 5) located in the 8th neuromere of the terminal ganglion with near-midline somata (approximately 25 µm in diameter), one ipsi- and one contralateral to the ascending axon and neuropile branching, and only a few posterior branches extending into the 9th neuromere (Fig 40). The response to stimulating just one basiconic sensillum was short and phasic (Fig 42). A third salt-responsive interneurone (ChSIN 2) responded with inhibition of ongoing activity (Fig. 42). As a local interneuron it exhibits a completely different branching pattern extending from a very lateral soma: extensive ipsilateral branching in the eighth and ninth neuromere and two separate contralateral neurites reaching far laterally into the ninth and eighth neuromere.

The salt-responsive interneuron ChSIN 4 can also respond with increased pasic-tonic excitation to citric acid (100mM) applied to a basiconic sensillum (Fig 42). A morphologically different interneuron (ChSIN 3) responds to the same stimulus concentration with prolonged excitation after a short phasic response. It is also an ascending interneuron with a contralateral ascending axon and an extensive branching area in the 8th neuromere, but some sparse branches extend also in the 8th neuromere ipsilateral to the soma. A third ascending interneuron (ChSIN 6) responds with inhibition or lowered excitation to citric acid (100mM) at the basiconic sensillum (Fig. 43). Its soma is located very lateral and from its long primary neurite the only branching area extends ipsilaterally in the 8th neuromere.

Excitation to glucose stimulation (100mM) of a ventral ovipositor taste receptor was seen in ChSIN 5, which responds to salts as well. The response is short and phasic in this multimodal interneuron (Fig. 44). Pronounced inhibitory responses were seen in ChSIN 1, which is a local and mostly ipsilateral interneuron that extends from the eighth to the tenth neuromere. Its response is very similar to that of ChSIN 6 to glucose, which is inhibited by citric acid as well and has a completely different and intersegmental morphology.

Acetophenone responses (Fig. 45) were seen to excite tonically in ChSIN 5, which is also responsive to salts and sugar.

Intracellularly, quinine-responsiveness is seen as inhibition in ChSIN 6 (Fig. 45), which is also inhibited by citric acid.

Excitation to glucose stimulation (100mM) of a ventral ovipositor taste receptor was seen in ChSIN 5, which responds to salts as well. The response is short and phasic in this multimodal interneuron (Fig. 44). Pronounced inhibitory responses were seen in ChSIN 1, which is a local and mostly ipsilateral interneuron that extends from the eighth to the tenth neuromere. Its response is very similar to that of ChSIN 6 to glucose, which is inhibited by citric acid as well and has a completely different and intersegmental morphology. Acetophenone responses (Fig. 45) were seen to excite tonically in ChSIN 5, which is also responsive to salts and sugar. Intracellularly, quinine-responsiveness is seen as inhibition in ChSIN 6 (Fig. 45), which is also inhibited by citric acid.

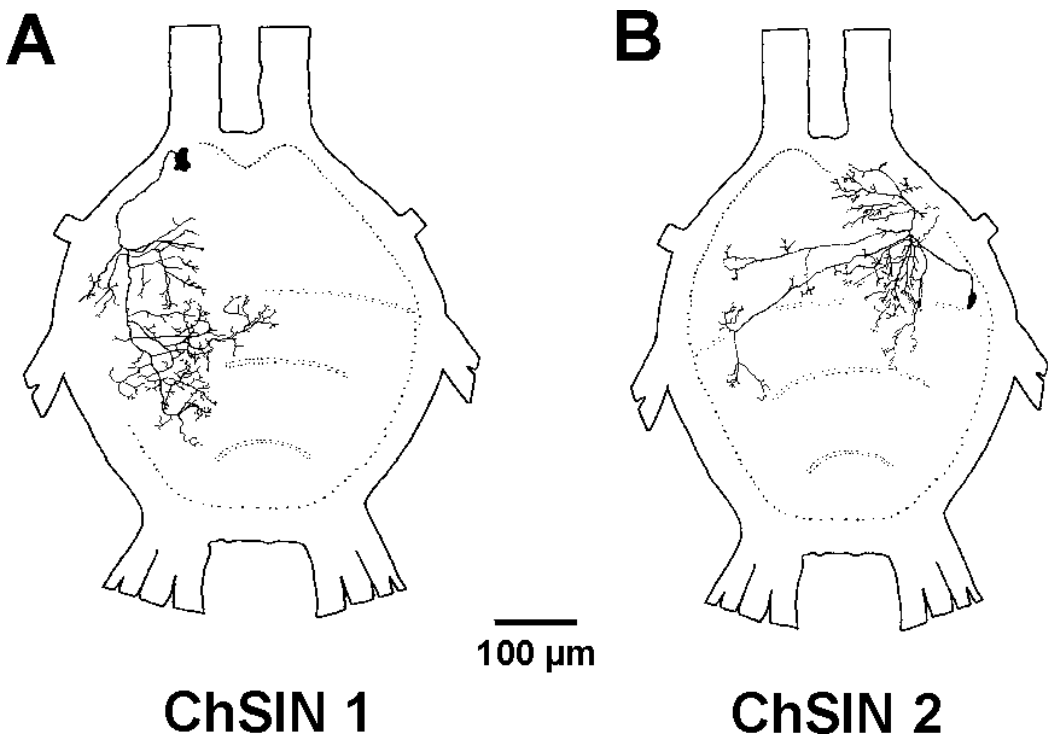


Fig. 39. Drawing of 2 types of chemosensitive local interneurones in the terminal ganglion. Neuronrs were stained intracellulary with Lucifer Yellow and reconstructed from photographs. Ganglia are viewed dorsally. ChSIN = Chemosensitive interneuron. Scale bar 100µm.

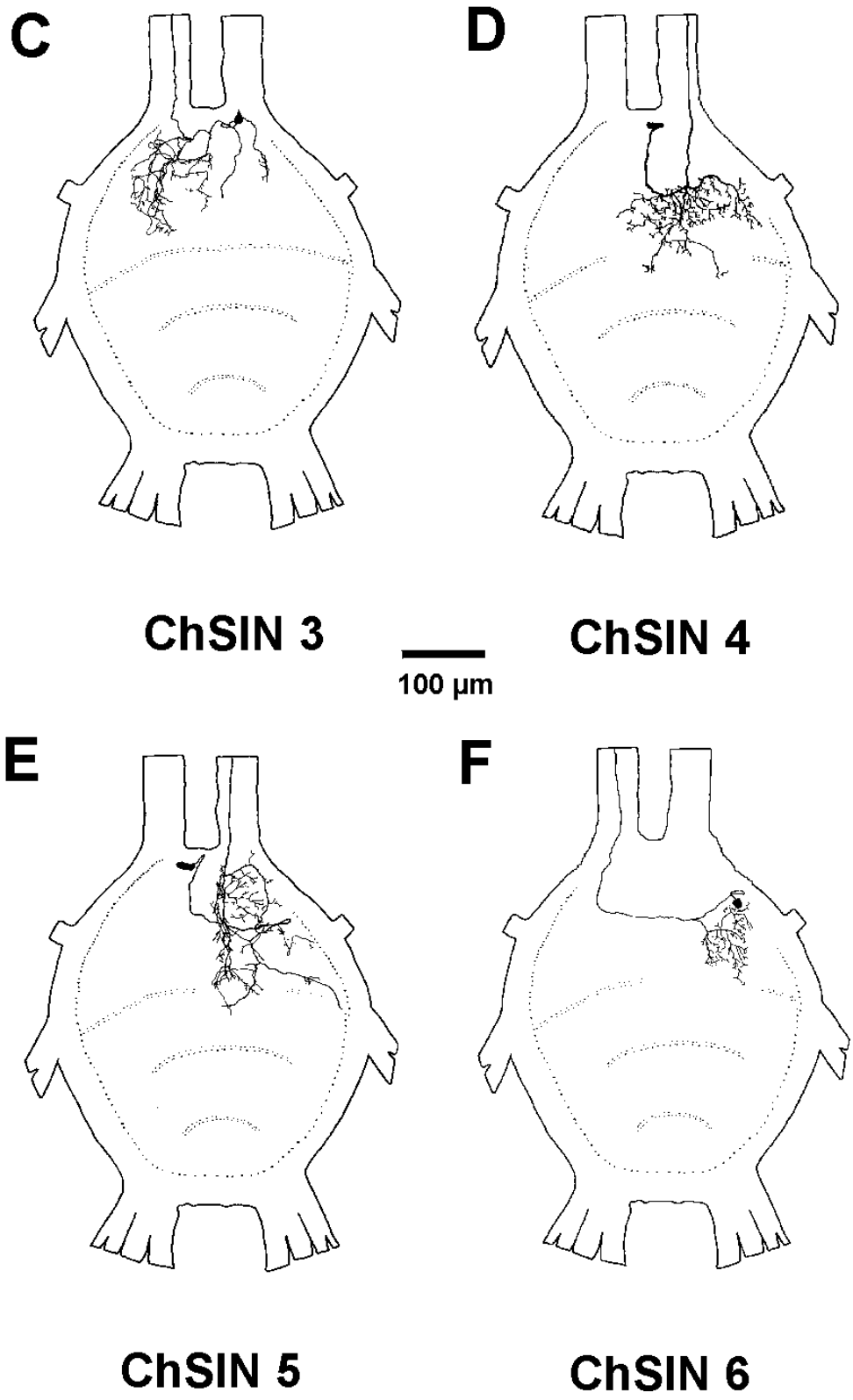


Fig. 40. Drawing of 4 types of chemosensitive intersegmental interneurones in the terminal ganglion. Neurons were stained interacellulary with Lucifer Yellow and reconstructed from photographs.

Ganglia are viewed dorsally. ChSIN = Chemosensitive interneuron. Scale bar 100µm.

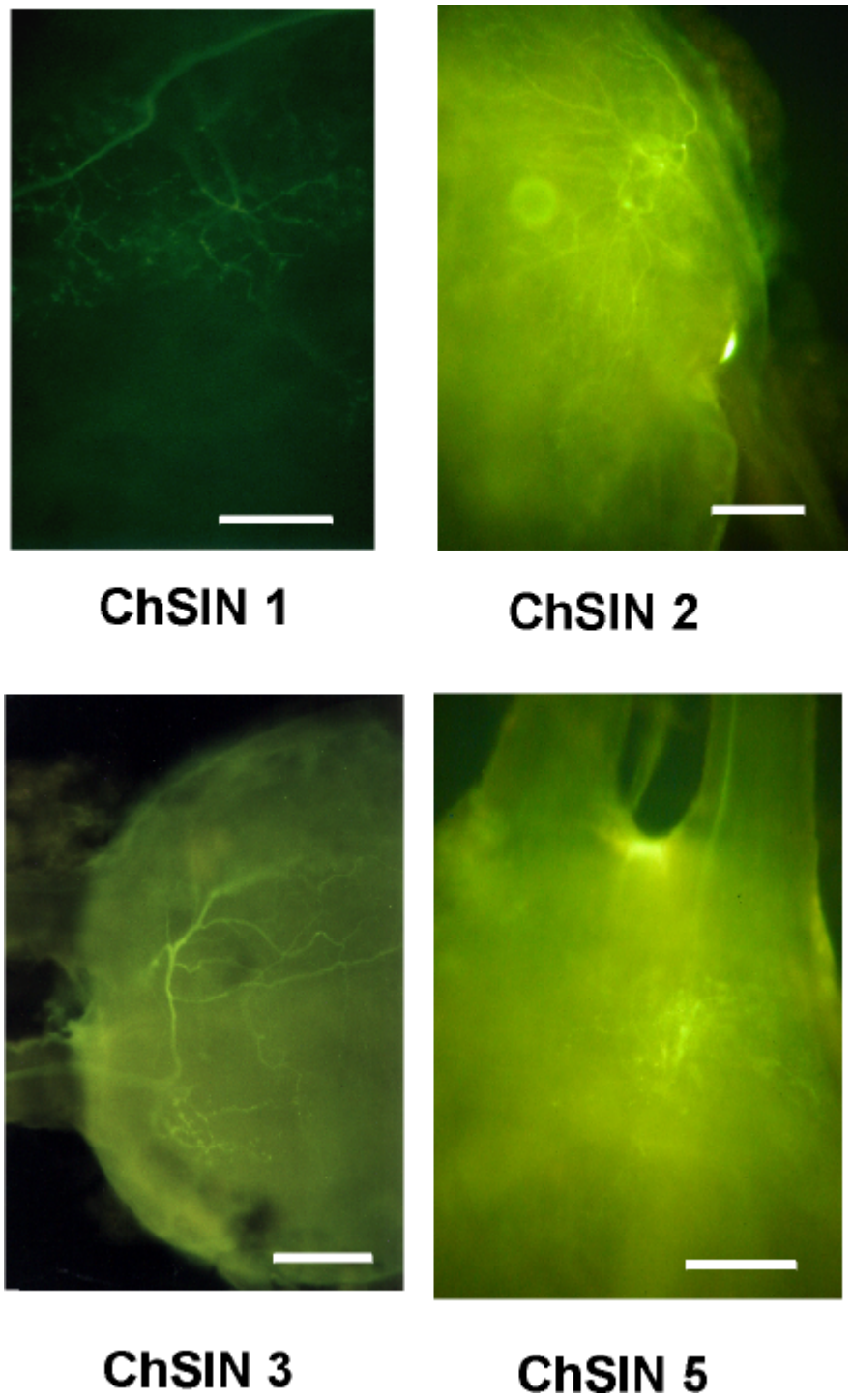


Fig. 41. Four chemosensitive interneurones in the terminal ganglion stained interacellulary with Lucifer Yellow and photographed from a whole-mount. Scale bar 100µm.

	ChSIN 1	**ChSIN 2**	**ChSIN 3**	**ChSIN 4**	**ChSIN 5**	**ChSIN 6**
NaCl	0	-	0	+	+	0
Citric acid	0	0	+	+	0	-
Glucose	-	0	0	0	+	-
Veratrole	0	0	0	0	0	0
Acetophenone	0	0	0	0	+	0
Quinine	0	0	0	0	0	-
Salicine	0	0	0	0	0	0
Tomatine	0	0	0	0	0	0

ChSIN chemosensitive interneuron
- Inhibtion + Excitation 0 no effect

Table 2. Effect of chemical solutions on the chemosensitive interneurones.

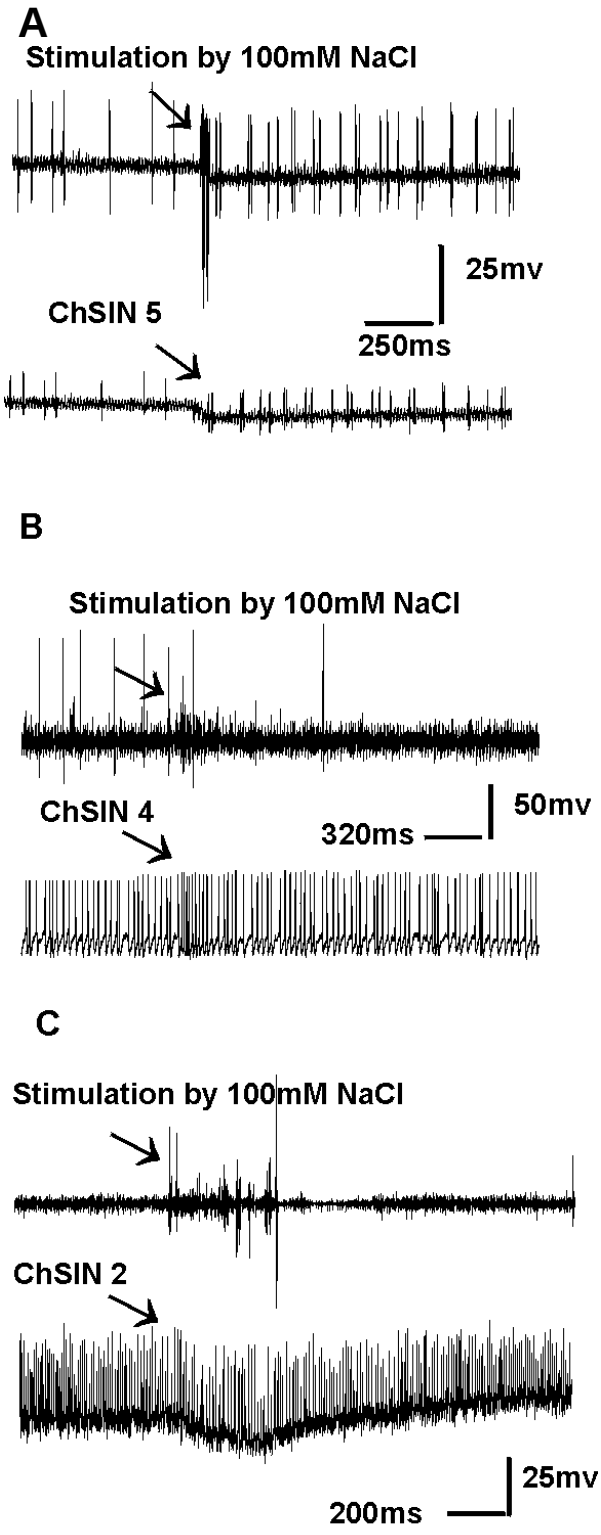


Fig. 42. Projecting interneurones, responding to stimulation of single basiconic sensillum in the ventral valve of locust ovipositor with 100 mM NaCl in water (arrows and contact artefact). A&B. Multiunit spikes recorded extracellulary (upper trace) from the connective ipsilateral to the stimulus site and simultaneous intracellular recording of an interganglionic

intrneurones (ChSIN 4, 5). C. Multiunit spikes recorded exteracellulary (upper trace) from the tip of sensillum and stimultaneous interacellular recording of local interneuron (ChSIN2).

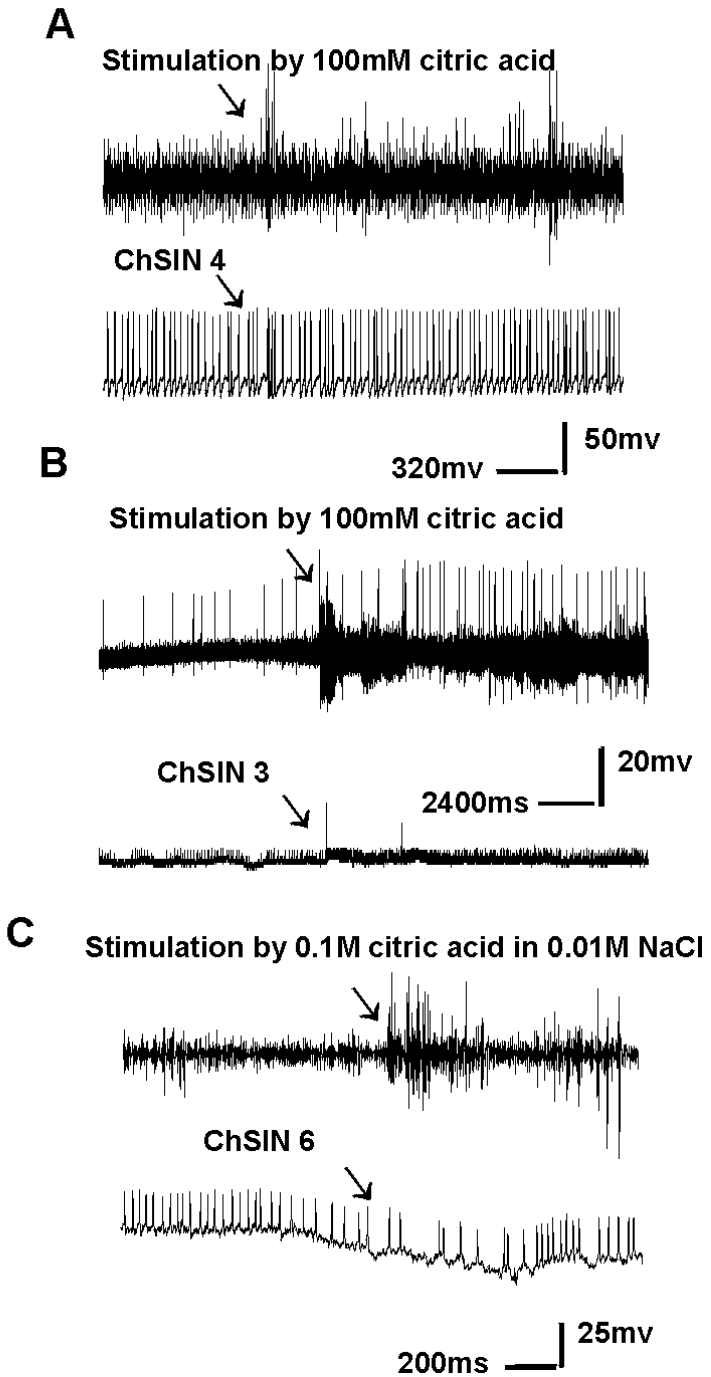


Fig. 43. Projecting interneurones, responding to stimulation of a single basiconic sensillum of the ventral valve of the locust ovipositor with 100 mM citric acid (arrows and contact artefact). A&B. Multiunit spikes recorded exteracellulary (upper trace) from the connective ipsilateral to the

stimulus site (ChSIN 3, 4) or C. from the tip of sensillum (ChSIN 6) and stimultaneous interacellular recording of an interganglionic interneurones.

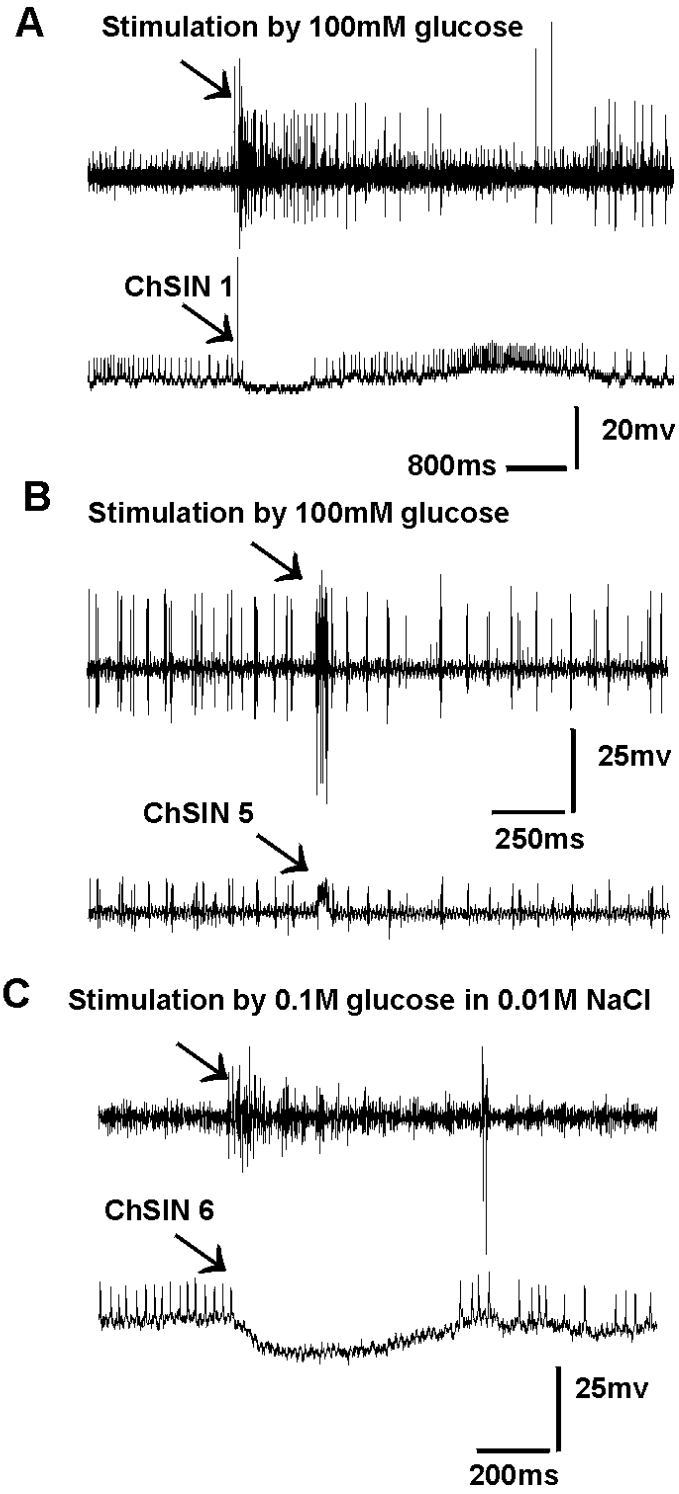


Fig. 44. Projecting intrneurones, responding to stimulation of a single basiconic sensillum in the ventral valve of the locust ovipositor with 100 mM glucose (arrows and contact artefact). A&B. Multiunit spikes recording

extracellulary (upper trace) from the connective ipsilateral to the stimulus site (ChSIN 1, 5) or C. from the tip of sensillum (ChSIN 6) and stimultaneous intracellular recording of interneurones.

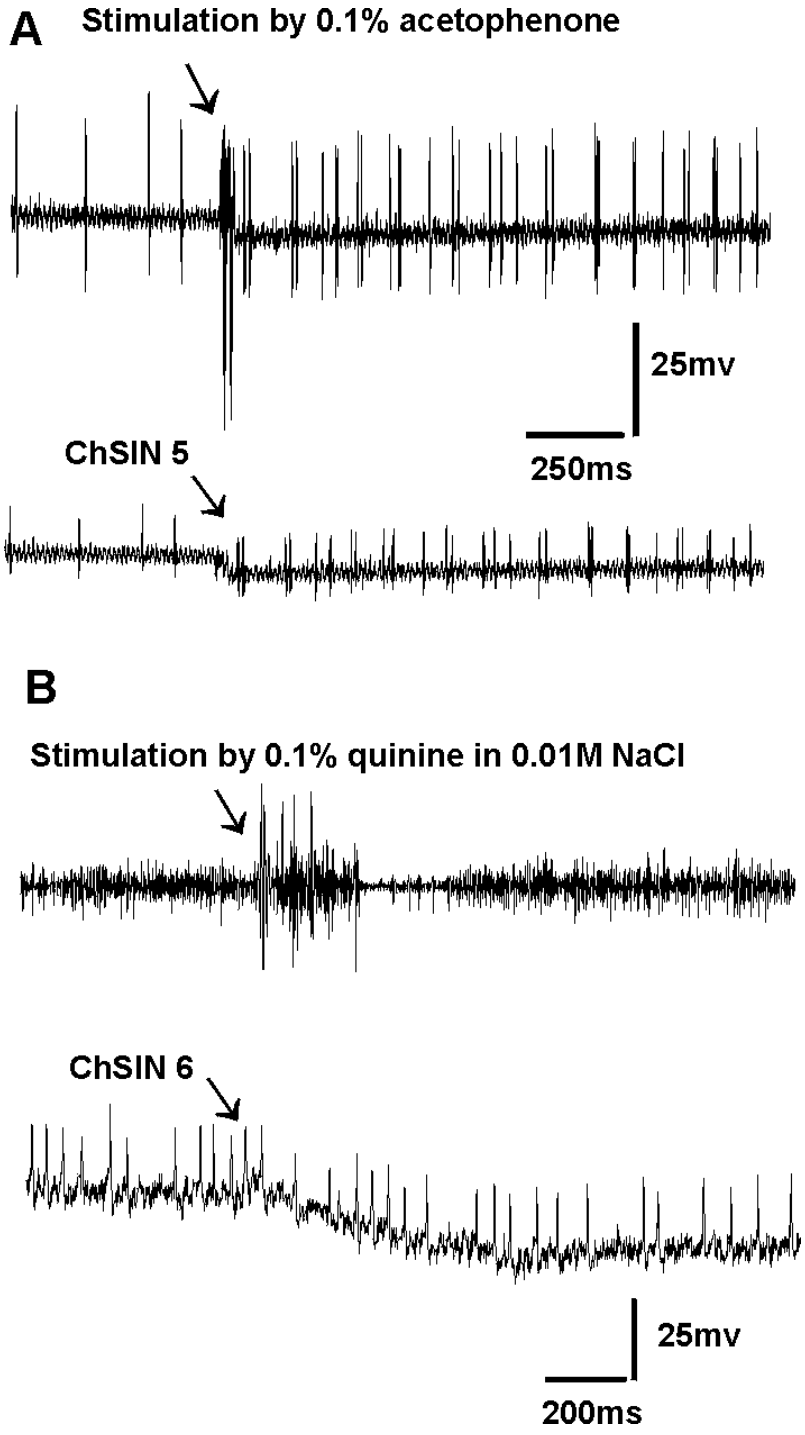


Fig. 45. Projecting interneurones, responding to stimulation of a single basiconic sensillum in the ventral valve of the locust ovipositor A. with 0.1% acetophenone and B. 0.1% quinine hydrochloride in 0.01M NaCl

(arrows and contact artefact). A. Multiunit spike recording extracellulary (upper trace) from the connective ipsilateral to the stimulus site (ChSIN 5) or from the tip of sensillum (ChSIN 6) and B. stimultaneous intracellular recording of an interganglionic interneurones.

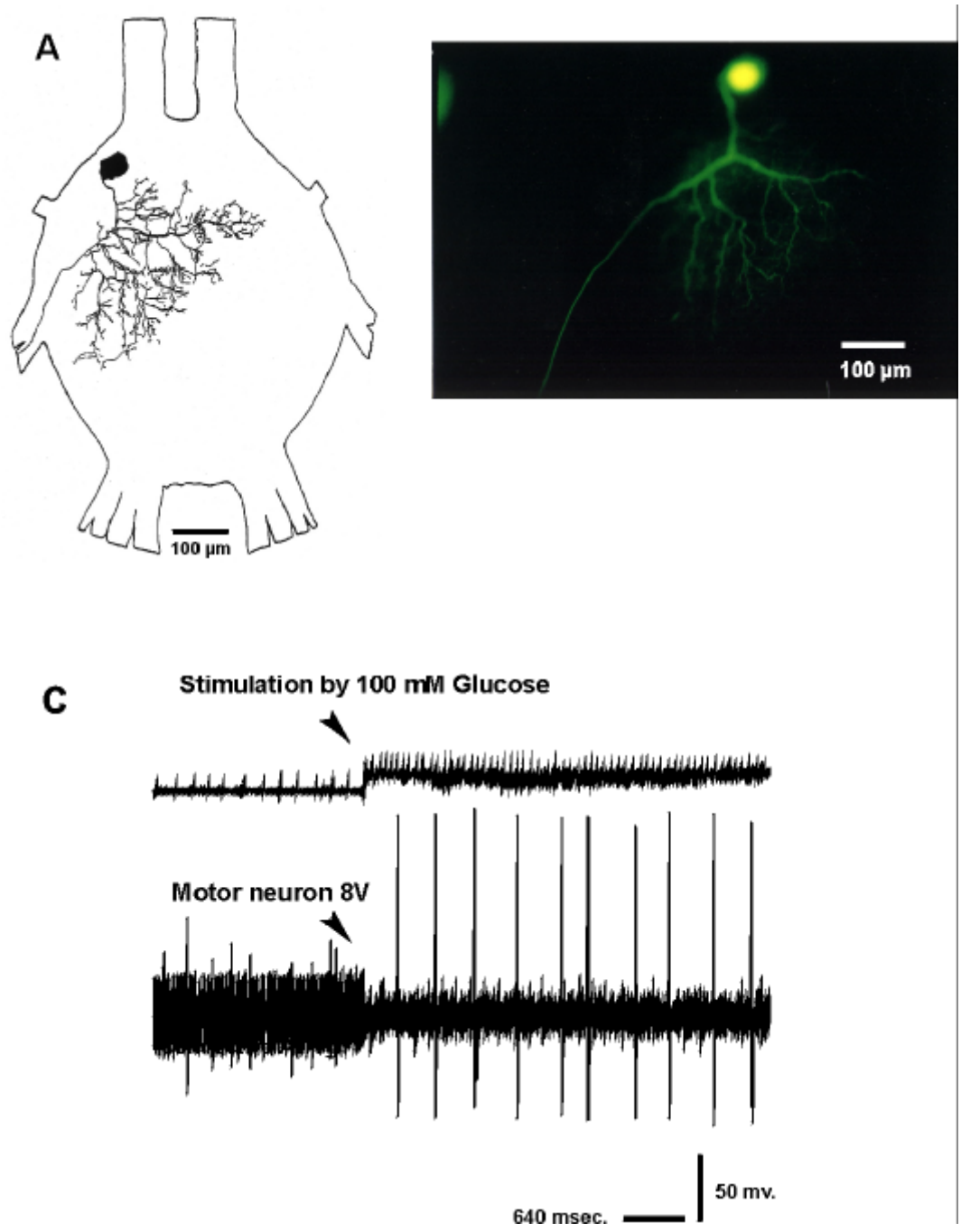


Fig**.** 46 Functional and morphological features of an identified motor neuron responsive to contact chemosensory input from a single ovipositor

basiconic sensillum**.** The motoneuron, entering nerve 8V (its morphology in A and B) C. Intrcellular respondse by excitation (upper trace) and multiunit interneuron spikes (lower trace) recorded extracellularly (lower trace) from the connective ipsilateral to the stimulus site by inhibition: inhibition of one unit and excition of a large unit is elicited by the same stimulus on one basiconic sensillum with 100 mM glucose in water (arrows and contact artefact).

3.6.3 Chemosensitive motoneurones:

For comparison a motoneuron in the terminal ganglion was recorded when it responded to chemosensory input from an ovipositor basiconic sensillum (Fig. 46). The cell body (40µm) of this motoneuron is located dorso-lateraly in the 8th abdominal neuromere and is characterised by its ipsi- and contralateral dendritic arborisation which is confined to the 8th and 9th abdominal neuromere. Stimulation of one ovipositor basiconic sensillum with 0.1M glucose resulted always in excition of this motor neuron and antagonstic responses of several units ascending in the connective. No response to other chemicals (NaCl, Citric acid, Veratrole, Acetophenone and Quinine hydrochloride) could be elicited in the motoneurone.

3.6.4 Morphology of non-chemosensitive interneurones:

In the terminal abdominal ganglion of the migratory locust six other interneurones [two are local and four are intersegmental interneurones (non giant interneurones)] with cell bodies in the eighth abdominal neuromere have been identified morphologically on the basis of intracellular stains with Lucifer Yellow. These intreneurones showed occasional no clear response to the chemosensory contact input on the ventral ovipositor valve (Fig. 47, 48).

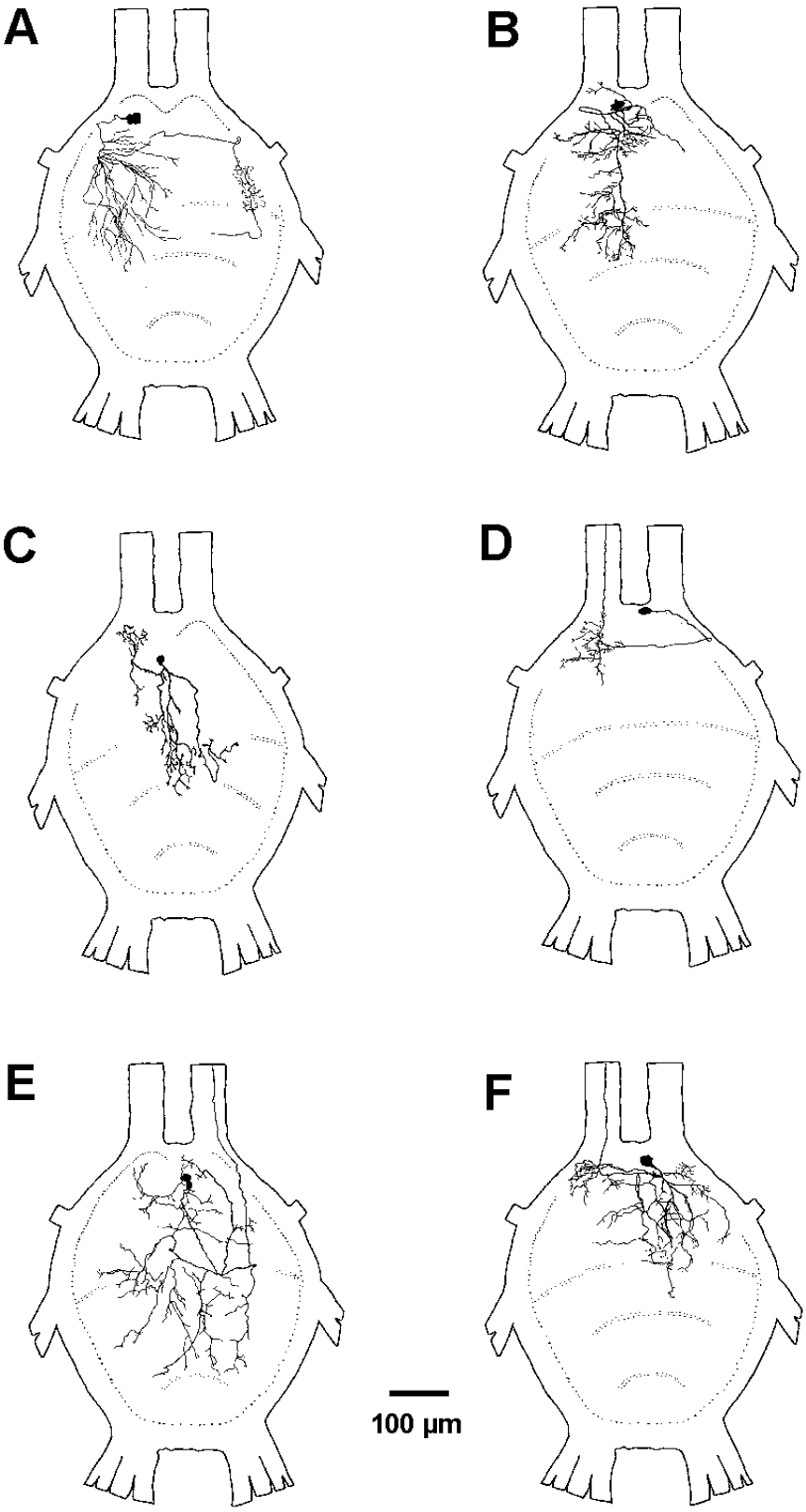


Fig. 47 Drawing of 6 types of interneurones (4 local and 2 intersegmental) in the terminal ganglionof migratory locust with occasional responses contact chemosensory input from one single basiconic sensillum on the

ventral ovipositor valve. Neurons were stained interacellulary with Lucifer Yellow and reconstructed from photographs. Ganglia are viewed dorsally. interneuron. Scale bar 100µm.

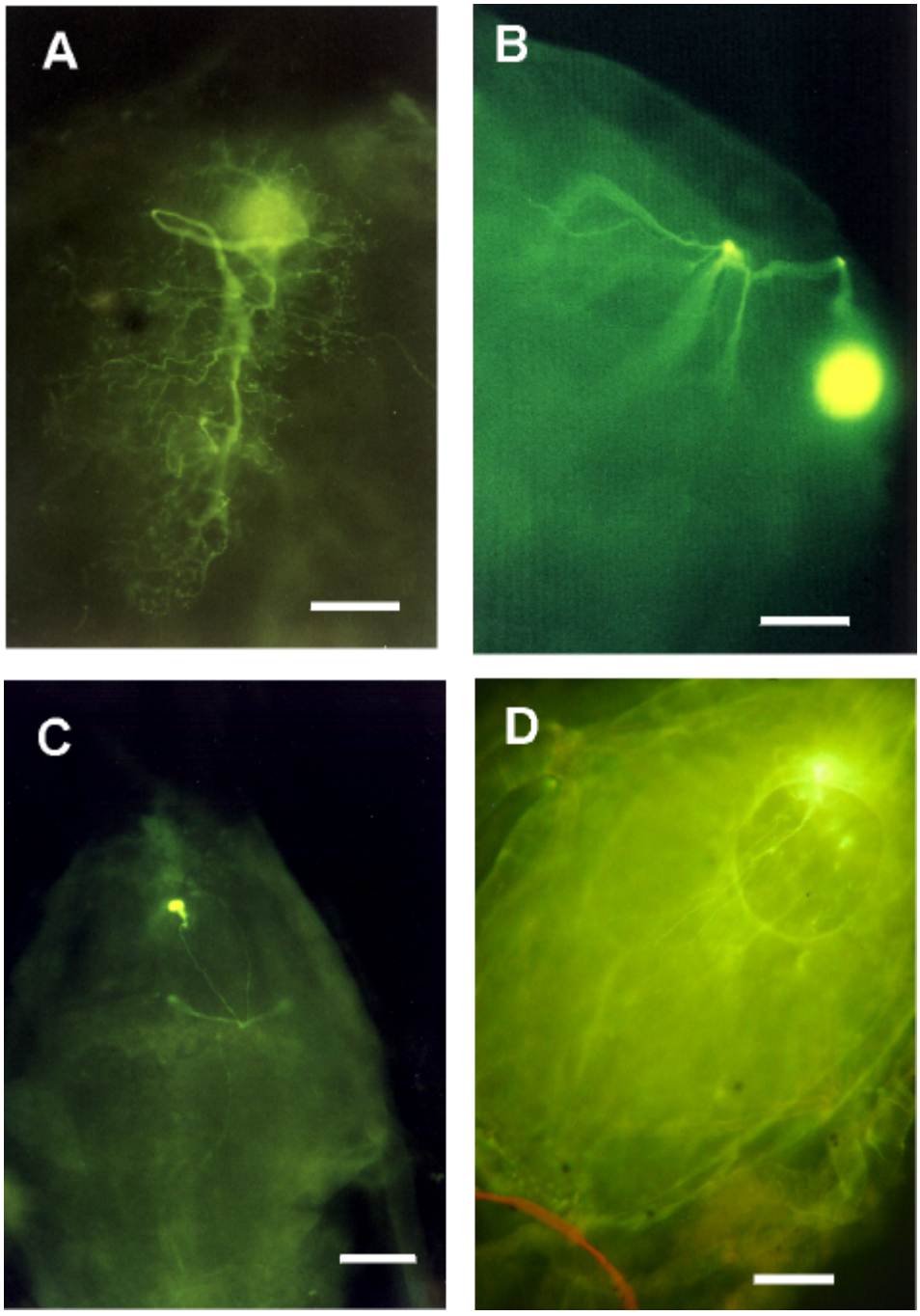


Fig. 48. Photographs of Lucifer Yellow shown the morphological features of interneurones in the terminal ganglion, not responsive to contact chemosensory input from single basiconic sensillum in ventral ovipositor valve. Scale bar 100 µm.

Discussion:

The ovipositor of *Locusta migratoria* has been subject of several morphological, developmental and functional studies, and were used as a model system for the sensory feedback control of oviposition. This work is the first investigation of the typical central projections of afferents from contact chemoreceptors and mechanoreceptors of the ovipositor valves and surrounding structures as they branch in the locust terminal ganglion. The latter was possible only after developing the technique of selectively staining the very small afferent neurons of basiconic sensilla (preliminary account in Tousson and Hustert, 1998). It focussed also on the periphery and sensory innervation of both ventral and dorsal ovipositor valves. The fine structure and distribution of various types of ovipositor sensilla in the desert locust were investigated with cobalt chloride backfilling and scanning electron microscope. Another focus was on behavioural as well as physiological responses and central nervous integration of ventral ovipositor basiconic sensilla to different chemicals.

Ovipositor structure and innervation

The locust ovipositor is primarily a digging organ that works by a forcible separation of the short recurved valves. The eggs are laid some distance below the surface of the soil, arranged in pods. The ovipositor must therefore be able to execute digging movements as well as to help in the arrangement of the eggs. The exposed part of the ovipositor consists of lower and an upper pair of strong, sclerotic, prong-like processes with curved tips turned ventrally and dorsally. These processes are the ventral and the dorsal ovipositor valves. The third pair (intermediate valves) are small, and are ordinarily concealed between the others, but they are not rudimentary in the sense of being functionless structures. The present study shows that, the locust ovipositor valves are innervatied by the terminal abdominal ganglion. The eighth ventral abdominal nerve receives sensory axons from the ventral valves while the ninth ventral abdominal nerve supplies sensory innervation for the dorsal ovipositor valve. This view is showed by Albrecht (1953) and by Seabrook (1968). The innervation pattern of the female ovipositor corroborates Qadri`s (1940) and Bharadwaj and Banerjee (1971) views on

the tergal and sternal origin of different valves. The innervation shows that ventral, dorsal and intermediate ovipositor valves are supplied by the nerves originating from the ventral nerves. Thus probably all the valves are of sternal origin. Since the ventral valve of the ovipositor is supplied by the nerve, which gives off a branch to the eighth abdominal ventral muscles, it is inevitable that the ventral valve originates from the eighth abdominal sternum. As the dorsal and intermediate valves are innervated by a separate nerve originating just ventral to the ninth abdominal tergal nerve, it is clear that these two valves are derivatives of the ninth sternite. This view is also showed by Albrecht (1953).

Ovipositor as a sensory system:

It is difficult to imagine that the female migratory locust could perform complex oviposition behaviour with only a central motor pattern, in the absence of tuning by a peripheral sensory loop. Thus, it is not surprising to find a great number of receptors on ovipositor structures. The present study shows that the locust ovipositor is well endowed with sense organs that could be the source of information about position, movement and the chemical character of oviposition substrate. Four different specific sensilla were identified and classified as basiconic sensilla, trichoid sensilla, campaniform sensilla and pits. These sensilla are defined by their dendritic innervations, sizes, shapes and distributions on the ovipositor valves as contact chemoreceptors or mechanoreceptors.

The external surface of locust ovipositor is covered by approximately 1205 receptors (about 579 sensilla in ventral valve and about 626 sensilla in dorsal valve) which are distributed unequally over the ovipositor valves. This results also shows about 33% of ovipositor receptors to be basiconic chemoreceptors that are typically gustatory or contact chemosensory sensilla of the thick-walled type of Slifer (1970) and the uniporous with simple pit pore chemosensillum type of Zacharuck (1980). The contact chemoreceptors of the locust ovipositor 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). About 45% of receptors on the ventral surface of ventral valve are contact chemoreceptors, this explains that the ventral surface of ventral ovipositor valve is more sensitive than other ovipositor sides and indicate that , the ventral surface of ventral ovipositor valve plays an important role in the search for suitable substrate before starting oviposition and about 22% of receptors are contact chemoreceptors in the internal sides of ventral and dorsal valves where the chemoreceptors in the internal sides have functionless while we found a great number of mechanoreceptors on the internal sides which may play an important role in the progression of eggs during oviposition. Thomas (1965) indicates large numbers of sensilla on the ovipositor valves of *Schistocerca gregaria*. It is not certain which of these are chemoreceptors, but probably her types F, I and K have a chemoreceptor function. There are about 80, 300 and 200 of these types on the dorsal and ventral valves of each side. Rice and McRae (1976) record about 50 papillae, equivalent to Thomas`s type I, on the ovipositor of *Locusta migratoria* while we record about 309 contact chemoreceptors on the dorsal and ventral ovipositor valves of *Locusta migratoria*.

The central projection technique:

We have shown for the first time (Tousson and Hustert 1998) how neurons of single insect contact chemoreceptors (morphologically called basiconic sensilla) project in the CNS. Previously, cobalt staining of single sensory neurons was performed successfully mainly in insect mechanoreceptors (Hustert 1978, review: Burrows 1996) but it did not work reliably for axon diameters of less than 1 µm that prevail for insect contact chemoreceptors. Our modification of existing neurobiotin staining methods (Bayer and Wilcheck 1980, Consoulas et al. 1993) made backfilling from the destroyed dendrites of single contact chemoreceptors reliable. When cutting the hair cuticle to injure the dendrites within the hair for access of the dye, apparently not all five dendrites of a basiconic sensillum are always injured equally. The dendrite of the mechanosensory neuron that reaches just the base of the sensillum may be left intact and remain unstained, while the chemosensory neurons that reach the tip of the sensillum near the terminal pore are crushed

routinely and diluted neurobiotin can enter. So occasionally just four stained afferents are seen reaching the CNS from one sensillum in the periphery. Nevertheless, this does not discriminate reliably possible differences in the projection characteristics of mechanosensory vs. chemosensory neurons in the neuropiles of the CNS. Occasionally one stained afferent fibre from a basiconic sensillum is larger in diameter than its accompanying afferents. This may be attributed to the mechanosensitive afferent which in physiological recordings from the pore at the tip usually has larger amplitude spikes than the chemosensory units.

Projection patterns:

Several major rules apply for central nervous projections from the contact chemoreceptors on genital segments of the female locust abdomen which are all basiconic sensilla: i) Usually all chemosensory projections from one basiconic sensillum branch in the same neuropile regions (see below for the exception in intersegmental projections). A segregation into projection targets as the glomeruli of olfactory centers in the insect brain (Vickers et al. 1998) was not found. ii) We could not distinguish between specific termination regions of the mechanoreceptor and the chemoreceptor neurons, in contrast to what was postulated for projections from long contact chemoreceptors of legs in flies (Murphey et al. 1989). Therefore, interneurons that may respond selectively to the different chemical informations from contact chemoreceptors should find their way between intermingling afferent fiber types in order to find ‘their’ specific afferent projections in a meshwork of receptor neurons with different chemosensitivity mixed with mechanosensory afferent projections.

Intersegmental and inter-neuromere projections:

In the connectives between abdominal ganglia of insects a high number of sensory afferents project between the ganglia. There are proportionally more intersegmental primary afferents than between thoracic ganglia of the CNS (Zawarzin, 1924; Hustert, 1978; Kalogianni, 1995, 1996). Intersegmental cooperation for sensorimotor control of locust abdominal movements and positions must be strongly supported by this intersegmental divergence of

sensory afferents, which is also seen for abdominal proprioceptors (Ferber and Hustert 1996). Intersegmental projection of afferent neurons is also the rule for most mechano- and chemoreceptors on the genital segments (except cercal hair projections). Divergence of afferent information to different segmental centers of the CNS implies that the original information is required there without previous filtering *via* secondary neurons – the local and intersegmental interneurons of the abdominal CNS. On the other side higher order neurons located in one of the ganglia or neuromeres could integrate convergent information of chemosensory and mechanosensory input from different segments and surfaces of the genital region (Kalogianni 1996).

In many projections from a single basiconic sensillum one of the afferents just branches in the primary, segmental neuropile area and does not send a collateral in further neuromeres or ganglia as all companion afferents do. We still do not know the reason for this. It is not likely to be the mechanosensory fiber that remains local, considering that the single mechanosensory projections from neighboring tactile hairs usually project to several neuromeres and between ganglia.

Projections of tergal and sternal origin:

A basic difference in projection patterns emerges from the results in contact chemoreceptors as well as mechanoreceptors of the locust female genital segments. From dorsal sclerites and appendages afferent branches in the terminal ganglion tend to project in the medial neuropile and intersegmentally, similar to the mechanosensory hair projections from dorsal thoracic regions of locusts (Bräunig et al. 1983) and thoracic and abdominal regions of crickets (Johnson and Murphey 1985, Hustert 1985, Murphey 1985). For afferents of the ventral and sternal cuticle sensilla more lateral pathways in the terminal ganglion are typical. A position-related topography as for leg afferents (Hustert et al 1981, Johnson and Murphey 1985, Newland and Burrows 1994) was not studied for the ovipositors although similarities may be seen since developmentally the valves are limb-derived structures. Technically it is rather difficult to stain afferents from all (anterior, posterior, ventral and dorsal) sites on the circumference of one ovipositor valve.

Chemosensory Stimulation:

The ovipositor basiconic chemoreceptive sensilla 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.

Five sensory neurons innervating each ovipositor basiconic sensilla of Locusta migratoria can be identified and discriminated electrophysiologically by the impulses they generate. Four of these units are chemosensory as judged by their responses to all tested chemical solutions applied directly to the sensillum tip. One neuron in each sensillum is a mechanoreceptor with optimal responsiveness to phasic deflection of the sensillum. These findings are the first physiological confirmation of chemosensory functions for the ovipositor basiconic sensilla and they support the morphological and the neuroanatomical evidence that the ovipositor basiconic sensillia are contact chemoreceptors (Tousson and Hustert, 1998, 2000).

Extracellular recording of the sensory stimulation of the ovipositor basiconic sensilla by different types of chemicals elicits a response from at least three neurones in these sensilla and the adaption was rapid with the lower concentrations and delayed with higher concentrations (Tousson et al., 1999). The afferent and 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 burst-like activity. This irregular impulse patterns or bursting activity in sensory neurons are generally considered to reflect injury effects on sensory neurones.

Interpretation of the compound response of hairs to any chemical requires, therefore, that the stimulus be studied over a fairly wide concentration range. When a comparative study of compounds is undertaken, it is preferable that each hair serve as its own control. Analyses made with these constraints permit the following generalizations about response to low concentrations of NaCl and glucose respectively. At the low end of a concentration range beginning at 0.01 M only two cells are stimulated. These cells are the water and salt cell in the case of NaCl and water and sugar cells in the case of glucose respectively. Over the middle range of concentration three cells respond, one of these cells is water cell, the 2nd is salt cell and the 3rd is sugar cell in the case of NaCl but in the case of glucose, the 1st is water cell, the 2nd is sugar cell and the 3rd is salt cell. The 3rd cell in both cases are adapted faster than the another two cells. Dethier (1974) found four chemoreceptors of the labellar setae of the blowfly *Phormia regina*. These have been termed sugar, water, salt (cation) and salt (anion) respectively. For the tarsi of insects, four types of chemosensory neurones for water, sugar, salt and alkaloids (White and Chapman, 1990) have been identified. Insects, like other animals have evolved chemical sensing devices for detecting stimuli that have adaptive value, so that most of their receptor are sensitive to a specific selection of stimuli available

Our observation shows that the stimulation with veratrole or acetophenone vapours did not evoke a response in the chemosensory neurons suggesting that the ovipositor basiconic sensilla do not have olfactory capabilities. Olfactory water-vapour sensillia have in fact been demonstrated electrophysiologically on the tarsi of the brown dog tick Rhipicephalus sanguineus (Haggart and Davis 1980).

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.

It is generally considered (Haskell and Schoonhoven, 1969; MuCutchan, 1969; Rees, 1969; Städler, 1978; Gaaboub and Hustert, 1998 ; Tousson et al., 1999) that the response of contact chemoreceptor to salt solution involves different neurones for each of the modalities (the salt and water neurons of most authers and the Type 1 and Type 3 neurons of Rees).In the present study it is clear that salt and water invoke activity from more than two neurones in many cases. Similary, van der Starre (1972) on *Phormia* has shown that water alone can elicit activity in more than one neuron in terminal sensilla on the maxillary palp where it was previously thought that only one water-sensitive neuron existed. Other reports of compounds stimulating more than one cell have been given by Schoonhoven (1969), Dethier and Kuch (1971), Blaney (1974, 1975), Newland (1998), Tousson et al.,(1999) and Newland et al., (2000). Chapman and Ascoli-Christensen (1999) suggested that all the grasshoppers that have been examined electrophysiologically appear to have deterrent-sensitive neurons comparable with those present in some other phytophagous insects, and they conclude that the gustatory sensilla of gasshoppers contain neurons that provide qualitatively different information to the central nervous system and in this respect they are comparable with those of other insects.

On the ovipositor of *Lucilia cuprina* there are five uniporous sensilla (Rice, 1976) apparently sensitive to salts, acids, blood and osmotic pressure but water was not tested (Rice, 1976). Wallis (1962a, b) described olfactory pegs on the ovipositor of *Phormia regina* that were electrically stimulated by contact with distilled water, but he questioned whether or not dissolved impurities may have been responsible for the response. Hood (1981) had found water-vapour sensilla on the ovipositor of *Metasyrphus venableis* and

*Eupeodes volucr*is that were stimulated by water although these types of insects do not depend upon water in oviposition.

Chemosensitive interneurones:

When a chemical stimulus elicits a response in interneurons via chemosensory receptors it can be considered as perceived by the CNS. Generally, recordings of the direct responses of insect taste receptors are only possible with stimulating and recording from the only and terminal pore of a gustatory hair at the same time since extracellular recording from their afferent axons of their very small neurons is impossible. Therefore, a system was selected in which are could also study the integrating higher order interneurones as to the chemicals perceived by the contact chemoreceptor of their receptive field: the sensilla can be stimulated by just one diluted chemical or possibly even high concentration smells (acids: Lefebvre 1981, Newland 1998) that transgress the pore and reach the sensory dendrites without electrolytes that must be added in the classical tip recording method developed by Hodgson et al. (1955).

Locust contact chemoreceptors are distributed “randomly” on the body and extremities and have central projections that do not sort out or converge in specific glomeruli in the CNS according to sensory classes of taste (e.g. salts, acids, sugar, water and others) but seem to project more or less position specific like mechanosensory afferents even if they occur crowded in areas of increased body contact with the substrate as on the tarsi or genital segments of females (Gaaboub and Hustert 1998, Newland et al 2000, Tousson and Hustert 2000). When higher order interneurons integrate one or several taste classes their postsynaptic input terminals must collect information from widespread presynaptic sites of afferent terminals by means of distributed branches. So interneurons integrating taste information selectively should have wide branching areas for chemosensory input (Kalogianni 1995, 1996; Kalogianni and Burrows 1996). Intracallular staining showed this for all interneurons even if they responded to just one taste class. The occurence of several local interneurons responsive to tastes

indicates local processing of chemical cues mostly before and during oviposition but possibly also during mating.

Unfortunately the interganglionic projections could not be traced with Lucifer yellow further rostrally. So it was not possible to pursue possible long ascending axons into areas of decision making (brain or thoracic ganglia) that might initiate continuation or cancellation of oviposition on the basis of chemical cues perceived on or near the ovipositor.

Overall, the study could not provide insight in specific principles of how chemosensory taste information is connected with neural networks of behavioural decisions in the terminal ganglion, preceding abdominal ganglia or in the thoracic ganglia.

Perceived Stimulants:

Recording from higher order neurons of the chemosensory pathway arising from taste receptors of the ovipositor has demonstrated that the typical chemical qualities sensed by the primary taste receptors are transferred and perceived separately and jointly in the higher order interneurones of the locust terminal ganglion. Also, other unknown stimulants might be perceived by means of isolated taste receptors which have not been included into the testing protocol. All the responses seen here fall into the categories wet (water), salty, acid, sweet, bitter (alkaloid-like) and possibly phenolic, all these being typical taste receptor sensitivies on other locations of the locust (White and Chapman 1990, Newland 1998, Gaaboub and Hustert 1998). So presently, one must assume that the gustatory basis for the locust decision to start, continue or terminate oviposition depends on combinations and concentrations of these basic tastes perceived on or in the substrate, but if remains unknown where in the CNS this decision is made.

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Curriculum Vitae

Name Ehab Mostafa Tousson

Date of Birth January, 3rd., 1965

Locality Damenhour, Egypt.

Nationality Egyptian.

Adresses In Germany:

Inst. Für Zoologie und Anthropologie

Berliner Str. 28, 37073 Göttingen

Tel. 0049(0)551-395420

Fax.0049(0)551-395427

E-mail. etousson@hotmail.com

ehabtousson@maktoob.com

In Egypt:

Zoology Department, Faculty of Science

Tanta Univ., Tanta, Egypt

Tel. 002045342140

Qualifications

B.Sc. in Zoology, May 1986 with general grade Excellent, Faculty of Science, Tanta Univ., Egypt.

M.Sc. in Zoology (Endocrinology and Histology) from Zoology Department, Faculty of Science, Tanta Univ., Egypt. 1991. Thesis title (Studies on host parasite relationship in some experimental animals) with general grade Excellent.

Ph.D. Student 92 till 1996 Tanta Univ., research topic (Parasitological and histopathological studies on experimental animals infected with Hymenolepis nana).

Registered for Ph.D. in Summer semester 1997,Göttingen Univ., research topic (Neural processing of chemosensory information from the locust ovipositor).

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Khalil IS, Massoud AA, Abou shafey A and **Tousson EM** (1991): Effects of crowding on Cynodiplostomum azimi (Trematoda: Digenea) in experimentally infected rats and mice. Sci J Fac Sci Menoufia Univ. Egypt. Vol. V: 129-139.

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Acknowledgements

I first of all thank Allah who blessed me with this opportunity to conduct this study and endowed knowledge upon me. I would like to express my deepest appreciation to my supervisor Prof. Dr. Reinhold Hustert for his effective supervision, continual criticism, guidance and encouragement throughout the period of this work. I also thank all the members of our lab, especially Silvia Gubert, Heba Abuoharga, Henning Lahmann, Hussam Bustami, Frank Funke, Kai Härtel, Hans Georg Post and Ibrahim Gaaboub for their help. I would like to thank my parents for all that they have done for me throughout my education. I would like to thank my wife, Heba, and children (Ahmed, Youmna and Zeyad) for her unfailing interest and encouragement during the period of this research. Finally, I am indebted to the Egyptian Government for granting me financial support.