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Neuroanatomical Relationships between Sensory Afferent Arborizations in the Locust Paraproctal Sensory Systems

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ABSTRACT

Relatively little is still known about the function, types and location of paraproctal sensory systems. This system detects and encodes four different sensory modalities: wind, touch, gustatory and olfactory. The left and right paraproct of the female locusts are located between the dorsal ovipositor, the epiproct and the ninth abdominal sclerite. They are positioned as the most posterior abdominal segments. In the present study, the distribution and the peripheral innervation of the sensory organs on the paraproct has been studied in whole-mount preparations by using the cobalt backfill techniques. The paraproct of the female locust bears hair sensilla of three basic types: a) Mechanosensory hairs (bristle or trichoid) each supplied with one sensory cell, b) Dual innervated mechanosensory hairs with a fine cuticular shaft which are restricted to the region near the posterior edges of the outer faces, c) Basiconic hairs which are multimodal receptors which encode both mechanical and chemical contact cues. The morphology and organization of the central projections of chemoreceptors and mechanoreceptors afferent from the paraproct were examined by neurobiocytin staining individual hair afferents. All afferent fibres project in the tenth neuromere of the terminal abdominal ganglion. Projections from single multiply innervated hair sensilla do not segregate with the exception of one afferent of contact chemosensory hairs which terminate only in its segmental neuromere, as was shown for other contact chemoreceptors of the abdomen. It is concluded that these sensilla at the very tip of the abdomen play a major role for mating, for the selection of oviposition sites and during the different oviposition subroutines.

Keywords: Neurobiology; Sensory neuron; Innervation; Immunocytochemistry; Electrophysiological recording.

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Introduction

Escape from a predator is one of the most dramatic episodes in an animal's life, and successful escape depends in large measure on the efficiency of the synaptic mechanisms and neuronal pathways linking sensory input to motor activity. Both the clarity of its expression and its importance to the animal's survival has ensured that escape behaviour has attracted much attention from neurobiologists (Camhi, 1980; Eaton and Hackett, 1984; Kransne and Wine, 1987; Hoy, 1989; Kohstall, 1996; Hörner and Heblich 2001). Cercal and paraproctal receptor of insects mediate a range of behaviours such as running, jumping and flight (Dumpert and Gnatzy, 1977; Camhi, 1980; Boyan *et al.*, 1989; Boyan and Ball, 1990; Kohstall, 1996), while these behaviours are expressed in a variety of context (Feeding, oviposition, mating, migration), each can be used to escape from a predator.

Description of nervous system in some acridids are given by Albrecht (1953), Thomas (1965) and Bharadwaj and Banerjee (1971). The abdomen of grasshopper consists of eleven segments, the first eight been easily recognizable while the remaining three are much modified in connection with external genitalia and innervated by a terminal abdominal ganglion (Seabrook, 1968). The central projections of insect sensory neurons are anatomically highly organized. Receptors associated with different types of external transducing apparatus project to different regions of the central nervous system (Tousson and Hustert, 1998). Within the appropriate central processing centres, axon is in some cases arrayed in topographic order. Studies of different sensory systems reveal retinotopic (Strausfeld, 1976), somatotopic (Gaaboub and Hustert, 1998; Tousson and Hustert, 1998; 2000a, b, c; Tousson *et al.*, 1999; Gaaboub, 2000; Newland *et al.*, 2000; Tousson, 2001, 2004), and tonotopic organizations (Römer, 1983). 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; Newland and Burrows, 1994; Gaaboub and Hustert, 1998; Gaaboub, 2000; Newland *et al.*, 2000). In contrast, olfactory chemoreceptors of the insect antennae segregate their projections according to functional principles into a few or many glomerular centres of the brain (Hildebrand and Shepherd, 1997), often corresponding to the specificity of the sensory neurons for classes of chemicals or smells. This anatomical organization as correlated with the synaptic connections between sensory fibres and their postsynaptic partners. These findings are extended in the current study to sensory receptors on the paraproctal plate of female locust. Also, in the present study we give an account of the projections of single hair receptors into the terminal abdominal ganglion of locusts is given to reveal their differences in relation to the electrophysiological effects.

Materials and Methods

FoIn the present study, adult females of *Schistocerca gregaria* (Forskal, 1775), were taken 1-2 weeks after their final moult from crowded colony at the University of Goettingen, Germany and at the University of Southampton, United Kingdom. Animals were reared under a 12h light / 12h dark regime, and fed fresh wheat seedlings supplemented. Prior to dissection they were anaesthetized by cooling the preparation to 2-4°C. To identify the sensory receptors on the surface of paraproct, 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).

Neuroanatomical studies:

The distribution and peripheral innervation of sensory receptors on the paraproct were revealed in whole-mount preparations with the cobalt chloride backfilling technique (Pitman *et al*., 1972; Gaaboub, 2000 and Tousson, 2001), and consecutive silver intensification (Bacon and Altman, 1977). Briefly, an intact insect was anaesthetized by chilling on ice and then 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 terminal abdominal ganglia, that innervate the paraproct and cerci, was exposed by an incision along the ventral midline pins. In order to backfill the peripheral nerves and the sensory neurons of the receptors on the paraproct and cerci, the cut ends of cercal nerve (Cer.N) was exposed, cut before their entrance into the paraproct and cerci, and the distal stump was put in a small well of Vaseline containing 3 molar cobalt chloride, while the rest of the preparation was bathed in locust saline. The preparation was kept at 4-6°C for 36-48h and then cobalt chloride in the neuronal structures was precipitated into black cobalt sulphite with 1-3 drops of ammonium sulphide in saline for 10-15 min. After rinsing with pure saline, the preparations were dehydrated and cleared in methyl salicylate. Subsequent silver intensification revealed in more detail the cobalt precipitate in the nerve axons, the sensory somata and the sensory dendrites .Sensory neurons and the peripheral nerve distribution were drawn by using a camera Lucida attachment on a Zeiss compound microscope (Carl Zeiss, Germany).

Immunocytochemical studies:

The central projection of both contact chemoreceptor and mechanoreceptor neurons of paraproct were visualized with neurobiotin (Vector Laboratories Inc.) in backfills (Tousson and Hustert, 1998) from single receptors in the periphery. The chemosensitive and the mechanosensitive sensilla from different areas of the paraproct were stained by surrounding the receptor with a wall of Vaseline. A droplet of distilled water was placed in this well and the sensillum was shaved off with a broken glass microelectrode, exposing the sensory dendrites. The distilled water was replaced with a droplet of 3% aqueous neurobiotin solution. Animals were then incubated for 72-96h at 4°C or at room temperature for 48-72h.

After incubation, the 7th and terminal abdominal ganglia were dissected out in insect saline and fixed in 4% Paraformaldehyde for 1 h, and then dehydrated and cleared in xylene for 30 minutes. Then they were rehydrated and rinsed twice in phosphate-buffered saline (PBS; pH 7.2, 10 minutes each). The labelled ganglia were incubated for 1 h at 37°C in a solution of 1 mg collagenase, 1 mg hyaluronidase in 1 ml PBS and then rinsed in PBS with two changes of 15 min then three changes of 15 min with 0.5% Triton X-100 added. Peroxidase binding to neurobiotin was achieved by using the avidin-biotin complex in buffer, incubated for 5-12 h at room temperature. Then after the preparations were rinsed in two changes of buffer with 0.5% Triton X-100 and finally in PBS (each 15 min). Peroxidase bound to neurobiotin in the central afferent projection was localized with the black chromogen 3,3-diaminobenzidine tetrahydrochloride (DAB) reaction. The preparations were incubated for 5-15 min in a solution of 30 mg DAB and 45 µl hydrogen peroxide (30%) in 100 ml PBS. Then the reaction was stopped by two changes of PBS for 5 min. The preparations were then dehydrated in an ascending alcohol series and cleared in methyl salicylate for whole-mount viewing. The results were drawn by using a camera Lucida attachment on a Zeiss standard compound microscope and photographed by Nikon digital Camera (Nikon, Japan). At least five successful stains of afferents were made generally, and one was selected to be used for every representative figure in this study.

Electrophysiological studies:

The tip-recording technique (Hodgson *et al*., 1955) was used to record from the sensory neurons innervating both the tactile hairs (trichoid sensilla) and contact chemoreceptors (basiconic sensilla) on the paraproct. Before recording, tactile hairs were cut to approximately half their length, but basiconic sensilla were left intact. Blunt glass recording microelectrodes containing 100 mM sodium chloride were then placed directly over the tips of the sensilla. The salt solution in the electrodes evoked spikes in some of the chemosensitive neurons, and movements of the electrode, which deflected the shafts of the sensilla, induced spikes in mechanosensory neurons (Newland and Burrows, 1994).

The same electrode was therefore used to evoke and record simultaneously the spikes of both the mechano- and chemosensory afferents. For odour (olfactory) stimulation, a glass microelectrode with standard locust saline was driven through the soft cuticle at the base of a receptor close to the somata of its sensory neurones. Signals were fed to a standard high impedance D.C. amplifer and then A.C. coupled.

Results

The peripheral innervation of the paraproctal organs were revealed by whole-mount preparations after using the cobalt chloride backfill techniques. The terminal abdominal ganglionic mass in *Schistocerca gregaria* is an ovate structure lying in the 8th abdominal segment and innervates the abdominal segments number 8, 9, 10 and 11; thus comprising the fused ganglia of last four segments (Fig. 2A). At the anterior surface, the ganglionic mass receives the closely apprised ventral connective and laying between them the fine median nerve. On superficial examination, it gives rise to four pairs of nerves. The 8th dorsal abdominal nerve (8th DN) arises from the anterolateral angles of the ganglionic mass, the 8th ventral abdominal nerve (8th VN) arises close behind and below them. The 9th dorsal abdominal nerve (9th DN) joins the ganglion at the posterolateral angles, and immediately posterad and mesad of these a pair of large nerve trunks arise which supply branches to the 9th abdominal sternum and the 10th and 11th abdominal terga and sterna. These nerve trunks contains the separate 9th ventral abdominal nerve (9th VN) to the 9th sternum; the epiproct nerve (Ept.N) and the cercal nerve to the 11th sternum (Fig. 2).

The cercal nerve proceeds posterad from the common root passing lateral of the epiproct nerve. A considerable distance caudad of the ganglion, the large rectal nerve (Rec.N) emerges and passes dorsal dividing to innervate the muscles associated with the rectum. The rectal nerves is similar to that in the male as described in Bharadwaj and Banerjee (1971) except for the nerve to the dorsal dilator muscle of the rectum (286) which emerges close to the posterior ramification of the caudal branch and directly opposite to the branch to the lateral dilator muscle of the rectum (290). The remainder of the caudal branch ramifies to the intrinsic muscles of the rectum. The cephalad branch innervates only the intrinsic muscles of the rectum continuing posterad and crossing above the epiproct nerve, the cercal nerve divides into cercal and paraproct branches. The lateral or cercal branch (Fig. 2, 3B, 3D) gives rise to a branch which turns lateral passing beneath the cercal nerve to innervate the transverse muscle (292) and a small sensory branch (S) is then received from the ventral region of the 10th tergum (Fig. 2A, 2D). The remainder of the cercal branch proceeds posterad and divides to innervate the lateral and mesial surfaces of the circus (S). The mesial or paraproct branch also proceeds posterad and is divided to lateral branch innervates, the ventral muscle of the paraproct (291) and receives sensory fibres (S) from the base of the paraproct while the mesial branch enters the paraproct to innervate the lateral dilator muscle of the anus (296) and receives sensory fibres from the paraproct (Fig. 2A, 2C).

The scanning electron microscopy (Fig. 1) and the staining of the peripheral nerves and sensory neurons of paraproct with cobalt chloride (Fig. 2) confirm the identity mechanosensory hairs (trichoid sensilla) with one neuron (Fig. 4B), dual mechanosensory hairs with two neurons (Fig. 5B) and chemosensory hairs (basiconic sensilla) with five neurons (Fig. 6B). The trichoid sensilla act as mono mechanoreceptors which 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. 1D) over 100 µm in length and about 4.5 µm in diameter at their base and the short sensilla (Fig. 1C) 40-100 µm long and about 3.5 µm in basal diameter.

The innervation of the trichoid sensilla is similar in the two types, each hair has a single sensory neuron with a large ovoid–shaped cell body that is about (4–5 µm) in diameter below the socket (Fig. 2). 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 dual mechanosensory sensilla (Fig. 1C, 1F) act also as mechanoreceptor with a shaft that is typically much longer (30 - 50 µm) than that of the chemosensory sensilla and supplied with two deeply staining neurones (4-5 µm in diameter) below the socket (Fig. 2, 5B). The chemoreceptors (basiconic sensilla) are peg-like structures (Fig. 1C, 1E) with a shaft that is typically much shorter (20 - 30 µm) than that of the trichoid sensilla and which has a pore at its tip (1,19 µm in diameter). The basiconic sensilla are supplied with groups of five deeply staining neurones (4-5 µm in diameter) that lie beneath each basiconic sensillum. Proximal to the somata each sensory neurone extends its axon, which joins those from the other cells in the group to form a small nerve that finally enters larger nerves. As a means of estimating the total number of receptors that were found on paraproct and cerci (Fig. 6B), five successful silver-intensifed cobalt staining whole mounts were used and the average number of different types of receptors on the locust paraproct shows a ratio of 1:3 for chemosensory to mechanosensory hairs (Fig. 3).

Trichoid hairs on the paraproct are each innervated by a single mechanosensory neuron, as indicated by the presence of spikes with a single amplitude only in tip recordings from these sensilla following deflection of the hair-shaft (Fig. 4C). Similarly, only single sensory neurons were stained in the terminal ganglion in backfills from trichoid hairs using neurobiotin (Fig. 4E). These sensory neuron greater in diameter than the basiconic axon and entering the terminal ganglion via cercal nerve and have projection patterns in the 9th and 8th neuromere that is not resemble the neighbouring basiconic afferents (Fig. 4). The ipsilateral collateral ascends into the preceding ganglion (Fig. 4D).

Conversely, spikes with two amplitudes were elicited by placing sucking electrode filled with saline solution on the cut shaft of a dual mechanosensory sensilla, these means that, these dual mechanoreceptor is innervated by double sensory neurons (Fig. 5C). Similarly, two sensory neurons were stained in the terminal ganglion in backfills from dual mechanoreceptors using neurobiotin (Fig. 5) and its projections in the terminal ganglion is similar to those from adjacent trichoid sensilla, with branching in the ventral associatiation centre (VAC) of the 9th and 8th 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. 5D, 5E).

Spikes with several distinct amplitudes were elicited by placing electrodes containing 100 mM sodium chloride over basiconic sensilla (Fig. 6C), indicating that more than one chemosensitive neuron was activated by the salt solution. In the example shown, the spike frequency of the chemosensory neurons decreased rapidly with time. Deflecting the shaft of the basiconic sensillum 3 seconds later evoked a further burst of larger amplitude action potentials from a mechanosensitive neuron (Fig. 6D). All sensilla that responded to contact with salt solutions also responded to particular odours. For example, acetic acid odours delivered to a sensillum positioned on the paraproct evoked bursts of spikes from at least two chemosensory neurones, based on differing spike heights (Fig. 6H).

Whenever a basiconic sensillum from the paraproct was stained iontophoretically, five neurons (one mechanosensory and four presumably chemosensory) were revealed in the whole-mount (Fig. 6F).The axons enter the terminal abdominal ganglion via cercal nerve and proceed medially to the 9th and 8th abdominal neuromere. There, all axonal projections branch ventrally and send neuritis medially to the VAC at a median level within the VAC (Fig. 6F, 6G). From there, several branches extend into the contralateral neuropil and one turns posteriorly and only four collaterals proceed ventrally as a compact bundle through the anterior ipsilateral connective and into the 7th abdominal ganglion (Fig. 6E). These axons then proceed to the medio-ventral contralateral area of the 7th abdominal neuromere where they terminate.

Discussion

This work in its first part is the first investigation of the central projections of paraproct sensory system in locusts or other insects. It is focussed on the different paraproct sensory sensilla of the female locust, using cobalt chloride and neurobiotin backfills techniques.The fine structure and distribution of various types of paraproctal sensilla in the desert locust were investigated with scanning electron microscope. Another focus is on the electrophysiological response of individual mechanoreceptors or chemoreceptors to mechanical or chemical stimulation were analysed. Three different specific sensilla were identified and classified as trichoid sensilla, dual mechanosensory sensilla and basiconic sensilla. These sensilla are defined by their sizes, shapes and distributions on the paraproct as mechanoreceptors or contact chemoreceptors.

It was demonstrated that the sensory neurons from basiconic sensilla, dual mechanosensory sensilla and trichoid sensilla on the paraproct of female locust are organised into parallel and largely overlapping somatosensory maps within the terminal abdominal ganglion and that the position of the sensilla on the paraproct is the major correlate of the destination of its sensory projection. The total area occupied by the arborizations of all the sensory neurons from individual basiconic sensilla on the paraproct were similar to the area occupied by those of the single neurons from the trichoid sensilla or similar to the area occupied by those of the double neurons from the dual mechanoreceptors. Besides, all mechanosensory hairs (trichoid sensilla) are innervated by a single sensory cell and project into the ventral neuropiles regardless of their location on the body and the nerve through which their axons enter the ganglion (Tousson 2004). In this respect the arrangement of tactile hair afferents from the trichoid sensilla closely resembles that of tactile hair afferents on ovipositor valves as described by Tousson *et al*. (1999) , Tousson und Hustert. (2000a, b, c), Tousson (2004) and follows the well- established pattern for abdomen bristle afferent sensory projections described in other insects (Murphey et al., 1989; Thompson et al., 1999; Hegazi *et al*., 2001).

All receptor fibres terminate ventro-medially in the ipsilateral half of terminal abdominal ganglion that like most of the cuticular receptors of distal parts of insect terminal segments (Boyan *et al*., 1989; Murphey *et al*., 1989; Boyan and Ball, 1990; Kalogianni, 1996; Tousson and Hustert, 1998, 2000a,b; Tousson, 2001; 2004). Whenever a basiconic sensillum from the paraproct was stained iontophoretically, five neurons (one mechanosensory and four presumably chemosensory) were revealed in the whole-mount and the branches of all the sensory neurons are project to the ipsilateral neuropil of the terminal abdominal ganglion. Furthermore, because all the sensory neurons from individual basiconic sensilla terminate within the same region of neuropil it appears that not only all mechanosensory neurons are arranged somatotopically, but that the gustatory neurons from these sensilla also follow a closely similar pattern.

It is surprising to find dual mechanosensory sensilla on the paraproct. We have thought that the dual mechanosensory sensilla on paraproct of the female locust have an important role in the transverse of sperms during the mating and these roles can be investigated morphologically in addition to the important role in escape and as tactile receptors (Boyan and Ball, 1990). Conversely it is not surprising to find a great number of contact chemoreceptors on the paraproct structures. The contact chemoreceptors on paraproct of the female locust have an important role in many aspects of the insect's life. They help it in the assessment of food materials, of oviposition site, or mating and these roles can be investigated morphologically and correlated with the insect behaviour (Blaney and Simmonds, 1990). The basiconic sensilla of the paraproct 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. Enough understanding of how different tastes are coded at the level of individual receptors (Blaney, 1974, 1975; Maes and Harms, 1986; Kohstall, 1992, 1996; Gaaboub and Hustert, 1998; Tousson *et al.*, 1999; Newland *et al.*, 2000; Gaaboub, 2000; Tousson and Hustert, 2000a,b; Tousson, 2001, 2004) has been achieved, but little is known of how and where chemosensory information from the contact chemoreceptors of the basiconic sensilla is processed, how different tastes are coded in the CNS, or how chemosensory information is integrated with signals coding other senses. This work has increased our knowledge regarding these points.

The present observation shows that the stimulation with citric acid vapours (Fig. 6H) appears to activate the sensory neurones in advance of contact. It is assumed that these uniporous chemoreceptors (basiconic sensilla) on paraproct detect some chemicals by olfaction. According to the study of Newland (1998) and Gaaboub (2000) 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 results of Städler and Hanson (1975) show that contact chemoreceptors on the maxillae of Manduca also respond to odors 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. Little work has been done on the processing of inputs derived from the paraproct hair receptors. In contrast, there are studies which describe interneurones in the terminal abdominal ganglion that receive inputs from ovipositor sensory system (Kalogianni and Burrows, 1996; Tousson and Hustert 2000a,b,c). Preliminary evidence, however, indicates that paraproct sensory processing also involves local and intersegmental interneurones. Further studies are currently under way to identify the interneurones that are postsynaptic to the paraproct afferents and to investigate the effect of paraproct afferent excitation on the oviposition and escape motor programme.

References

Albrecht, F.O. (1953): The Anatomy of the Migratory Locust. University of London, Athalone Press.

Bacon, J.P. and Altman, J.S. (1977): A silver intensification method for cobalt-filled neurones in whole-mount preparations. Brain Research, 138: 395-363.

Bharadwaj, R.K. and Banerjee, S.K. (1971): The nervous system of the desert locust with a discussion on muscle innervation. J. Nat. Hist., 5: 183-208.

Blaney, W.M. (1974): Electrophysiological responses of the terminal sensilla on the maxillary palps of *Locusta migratoria* to some electrolytes and non-electrolytes. J. Exp. Biology, 60: 275-293.

Blaney, W.M. (1975): Behavioural electrophysiological studies of taste discrimination by the maxillary palps of larvae of *Locusta migratoria*. J. Exp. Biology, 62: 555- 569.

Blaney, W.M. and Simmonds, M.S.J. (1990): The chemoreceptors. in Biology of Grasshoppers, Chapman, R. F. and Joern, A Eds., Willey, New York.

Boyan, G.S. and Ball, E.E. (1990): Neural organization and information processing in the wind sensitive cercal receptor / giant interneurone system of the locust and other orthopteroid insects. Progress in neurobiology, Vol. 35: 217-243.

Boyan, G.S.; Williams, E.E. and Ball, E.E. (1989): The wind sensitive cercal / giant interneurone system of the *Locusta migratoria*. I Anatomy of the system. J. Comp. Physiology, 165: 495-510.

Camhi, J.M (1980): The escape system of cockroach. Sci. Am. 243: 144-157.

Clements, A. N. and May, T. E. (1974): Studies on locust neuromusclar physiology in relation to glutamic acid. J. Exp. Biology, 60: 673-705.

Dethier, V.G. (1972): Sensitivity of the contact chemoreceptors of the blowfly to vapours. Proc. Natl. Acad. Sci. USA, 69: 2189-2192.

Dumpert, K. and Gnatzy, W. (1977): Cricket combined mechanoreceptors and kicking response. J. Comp. Physiology, 122: 9-25.

Eaton, R.C. and Hackett, J.T. (1984): The role of the mouthner cell in fast starts involving escape in teleost fishes. In: Neural mechanisms of startle behaviour, PP. 213-266. Ed. R.C. Eaton, Plenum: New York.

Gaaboub, I. and Hustert, H. (1998): Motor response to chemical stimulation of tarsal sensilla in locusts. Proc. 26th Goettingen Neurobiology conference, Thieme, Stuttgart, New York, Vol. 1: p. 336 (Abstract).

Gaaboub, I. (2000): Neural processing of chemosensory information from the locust legs. Ph.D. Goettingen University, Germany.

Heblich, R. and Hörner, M. (2001): Aminergic modulation of the cricket giant pathway: Electrophysiology and pharmacology. Proc. 28th Goettingen Neurobiology conference 2001, volume 2: 758 (Abstract).

Hegazi, E, Tousson, E.M. and Hustert, R. (2001): The Intersegmental Network of Afferents in the Locust Abdominal Ganglia. Proc. 28th Goettingen Neurobiology conference 2001, volume 2: 694 (Abstract).

Hildebrand, J.G. and Shepherd, G.M. (1997): Mechanism for olfactory discrimination: converging evidence for common principles across phyla. Ann. rev. Neuroscience, 20: 595-631.

Hodgson, E.S.; Lettvin, J.Y. and Roeder, K.D. (1955): Physiology of a primary chemoreceptor unit. Science 122: 417-418

Hoy, R.R. (1989): Startle, categorical response and attention in acoustic behaviour of insects. An. Rev. Neuroscience. 12: 355-375.

Kalogianni, E. (1996): Morphology and physiology of abdominal intersegmental interneurones in the locust with mechanosensory inputs from ovipositor hair receptors. J. Comp. Neurology, 366: 656-673.

Kalogianni, E. and Burrows, M. (1996): Parallel processing of mechanosensory inputs from the locust ovipositor by intersegmental and local interneurones. J. Comp. Physiology, 178 : 735-748.

Kohstall, D. (1992): Beziehungen zwischen Laufverhalten und Aktivität von Neuronen das Terminalganglions bei *Gryllus bimaculatus*. Diplomarbeit Dissertation, Goettingen Univ., Germany.

Kohstall, D. (1996): Verarbeitung Cercaler Eingänge durch Lokale und Aszendierende Interneurone im Terminalganglion Laufender Grillen. Ph.D. Dissertation, Goettingen Univ., Germany.

Kransne, F.B. and Wine, J.J. (1987): Evasion responses of the crayfish. In: Aims and methods in neuroethology, PP. 10-45. Ed. D. M. Guthrie Manchester University press: Manchester, England.

Laurent, G.J. and Naraghi, M. (1994): Odorant-induced oscillation in the mushroom bodies of the locust. J. Neurosci., 14: 2993-3004.

Maes, F.W. and Harms, G. (1986): Neural coding of salt taste quality in the blowfly *Calliphora vicina*. I. Temporal coding. J. Comp. Physiol., 159: 75-88.

Murphey, R.K.; Possidente, D.; Pollack, G. and Merritt, D.J. (1989): Modality-specific axonal projections in the CNS of the flies *Phormia* and *Drosophila*. J. Comp. Neurol. , 290: 185-200.

Newland, P.L. (1998): Avoidance reflexes mediated by contact chemoreceptors on the legs of locust. J. Comp. Physiology, 183: 313-324.

Newland, P.L. and Burrows, M. (1994): Processing of mechanosensory information from gustatory receptors on a hind leg of the locust. J. Comp.Phys., 174: 399-410.

Newland, P.L.; Rogers, S.; Gaaboub, I. and Matheson, T. (2000): Parallel Somatotopic Maps of Gustatory and

Mechanosensory Neurons in the Central Nervous System of an Insect. J. Comp. Neurology, 425 : 82-96.

Pflüger, H.J.; Bräuning, P. and Hustert, R. (1981): Distribution and specific central projections of mechanoreceptors in the thorax and proximal leg joints of locusts. II. The external mechanoreceptors: Cell Tissue Res., 216: 79-96.

Pitman, R.M.; Tweedle, C.D. and Cohen, K. (1972): Branching of central neurons: Intracellular cobalt injection for light and electron microscopy. Science, 176: 412 –414.

Römer, H. (1983): Tonotopic organization of the auditory neuropil in the bushcricket. Nature 306: 60-62.

Seabrook, W.D. (1968): The innervation of the terminal abdominal segments (VIII-XI) of the desert locust, *Schistocerca gregaria*. Canadian Entomology, 100, 693-715.

Städler, E. and Hanson, W.D. (1975): Olfactory capabilities of the gustatory chemoreceptors of the tobacco hornworm larvae. J. Comp. Physiology, 104: 97.

Strausfeld, N.J. (1976): Atlas of An Insect Brain. New York: Springer Verlag.

Thomas, J.G. (1965): The abdomen of female *Schistocerca gregaria* with special reference to the sense organs. Anti-Locust Bull., 43: 1-20.

Thompson, K.J., Sivanesan, S.P. and Campbell, H.R. (1999): Efferent neurons and specialization of abdominal segments in grasshoppers. J. Comp. Neurology, 415: 65-79.

Tousson, E.M. (2001): Neural processing of chemosensory information from the locust ovipositor. Ph.D. Dissertation, Goettingen University, Germany.

Tousson, E.M. (2004): Neuroanatomical and electrophysiological studies of identified contact chemoreceptors on the ventral ovipositor valve of 3rd instar larvae of lubber grasshoppers (*Taeniopoda eques*). Zoology, 107: 65 –73.

Tousson, E.M. and Hustert H. (1998): Contact chemoreceptors from different sites have different projection patterns in the locust terminal ganglion. Proc. 26th Goettingen Neurobiology conference, volume2. p. 594 (Abstract).

Tousson, E.M. and Hustert, H (2000a): Central projections from contact chemoreceptors of the locust ovipositor and adjacent cuticle. Cell Tissue Res., 302 (2): 285-294.

Tousson, E.M. and Hustert, H. (2000b): sensory responses to chemical stimulation of ovipositor contact chemoreceptors in *Locusta migratoria*. Zoology, 103: Supplement III p. 75 (Abstract).

Tousson, E.M. and Hustert, H. (2000c): Contact chemoreceptors from the locust ovipositor and their integrating interneurones. European J. of Neuroscience, 12: Supplement II. p. 267

Tousson, E.M.; Gaaboub, I. and Hustert, H. (1999): Response characteristics and specificity of contact chemoreceptors from different sites in *Locusta migratoria*. In Elsner, N. & Eysel, U. (Eds) Proc. 27th Goettingen Neurobiology conference 1999, volume II, P 348.

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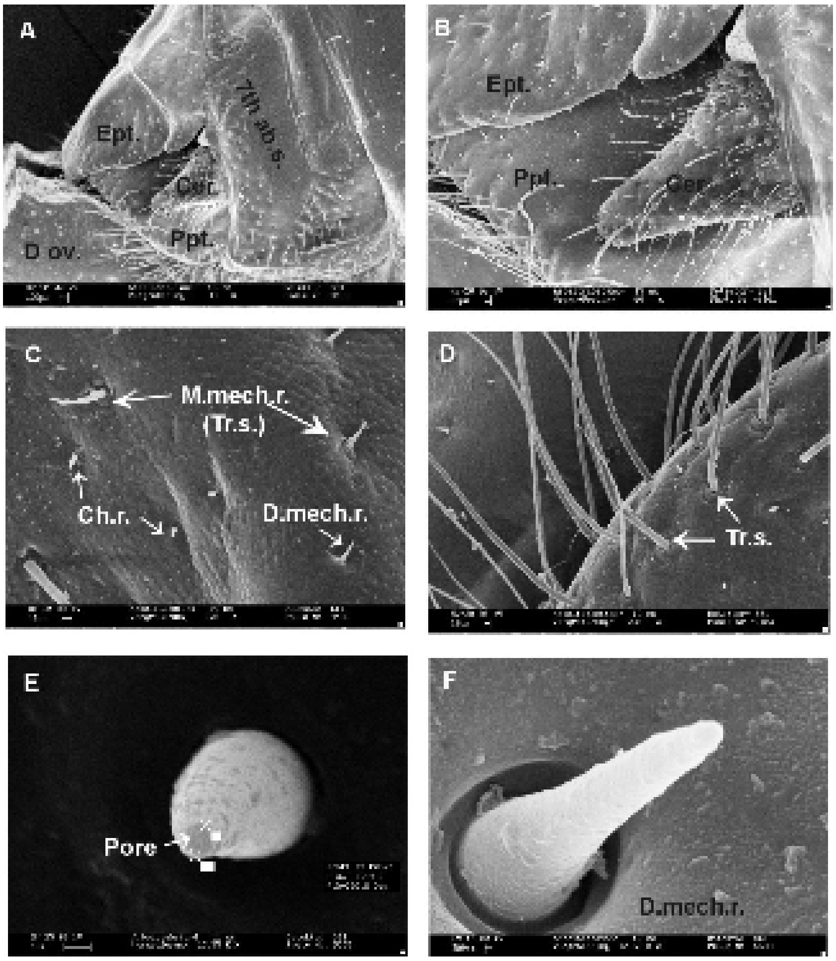

Fig.1. Scanning electron micrographs (SEM) of the terminal abdominal segments in female locust. (A): Lateral view of the terminal abdominal segment showing the external dorsal ovipositor valve (D ov.) and the neighbouring sclerites, the paraproct (Ppt), the cercus (Cer)and the epiproct (Ept). (B): high magnification of A. (C): Cuticle with different sensory sensilla chemoreceptor (Ch.r.), mono- mechanoreceptors or trichoid sensilla (m.mech.r.) or (Tr.s.) and dual mechanoreceptors (D.mech.r.)). (D): Lateral margin of paraproct fill with mono mechanoreceptors or trichoid sensilla (Tr.s.). (E): High magnification of an individual basiconic sensillum with a pore of approximately 1.19 m diameter (arrows). (F): High magnification of an individual dual mechanoreceptor.

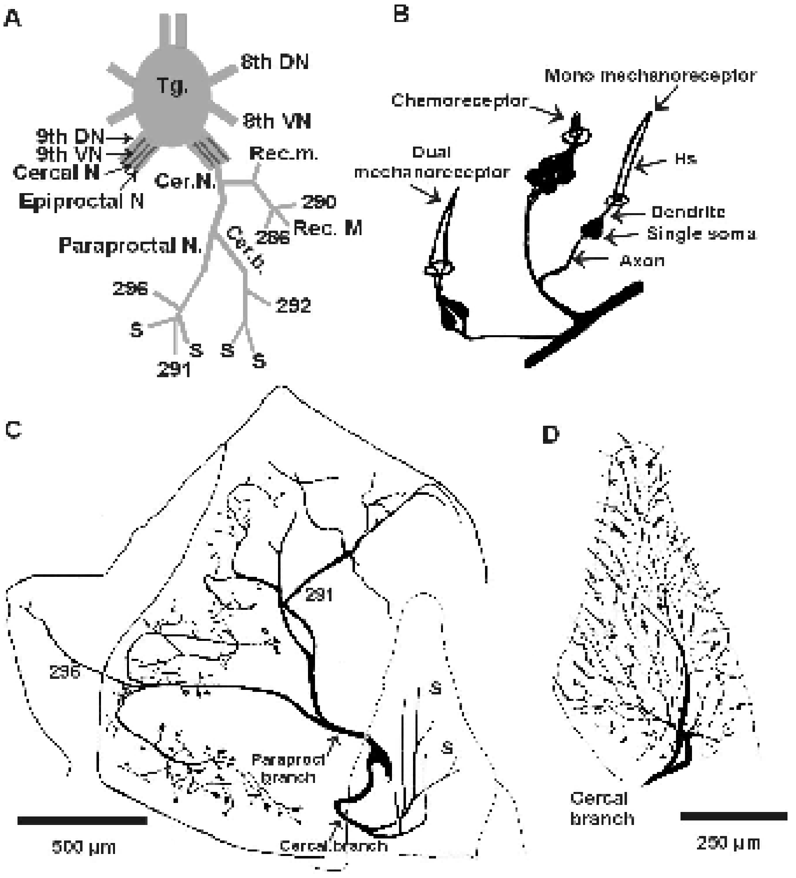

Fig.2. (A): Camera Lucida drawing of the terminal abdominal ganglion mass and the peripheral innervation of the ninth abdominal segments. (B): Inset from a proximal site of the paraproct shows terminal branching of the common nerve (C&D): Camera Lucida drawing of the peripheral innervation of female locust shown the cerci and the paraproct sensilla and their innervation.

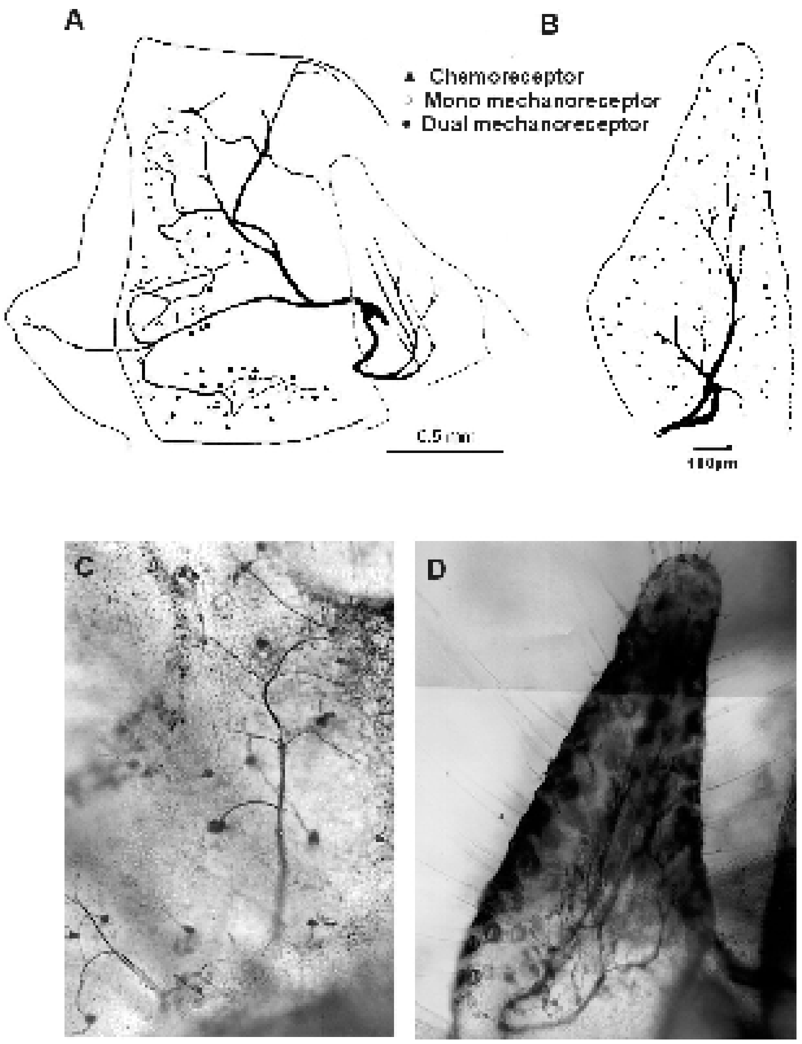

Fig. 3. (A&B): Distribution of contact chemoreceptors (filled triangles), mono mechanosensory hair receptors (open circle) and dual mechanosensory hair receptors (filled circle) on the paraproct and cerci of the female locust. (C): Light micrograph of a whole mount stained with silver-intensified cobalt staining, showing the paraproct sensilla and their innervation. (D): Light micrograph of a whole mount stained with silver-intensified cobalt staining, showing the cerci sensilla and their innervation.

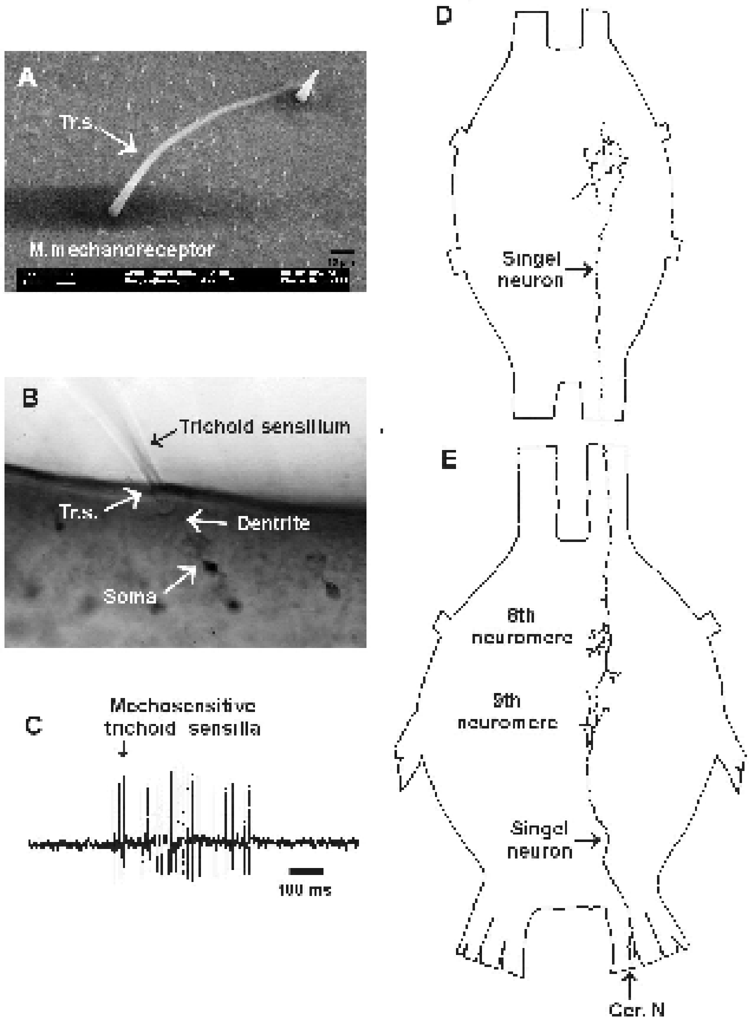

Fig.4. (A): SEM of individual trichoid sensilla in the locust paraproct. (B): Light micrograph for individual trichoid sensilla in the locust paraproct innervated by single sensory neurons. (C): Response of individual cut shaft trichoid sensilla in the locust paraproct to deflecting evokes a burst of action potentials of single amplitude, indicating that the trichoid sensillum is innervated by a single neuron. (D&E): Central projections morphology in the terminal ganglion (Tg) and the seventh abdominal ganglion (7th abg) from individual trichoid sensilla located on the paraproct of the female locust.

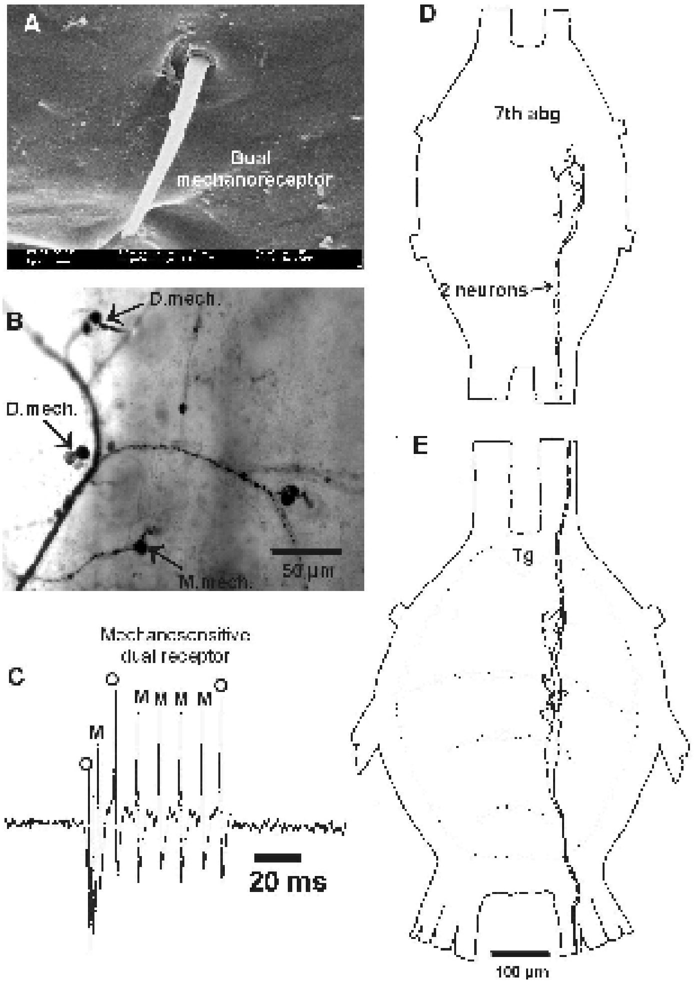

Fig.5. (A): SEM of individual dual mechanosensory sensilla in the locust paraproct. (B): light micrograph for individual dual mechanosensory sensilla in the locust paraproct innervated by two sensory neurons. (C): Response of individual cut shaft dual mechanosensory sensilla in the locust paraproct to deflecting evokes a burst of action potentials of two amplitude, indicating that the dual mechanosensory sensilla is innervated by two neuron. (D&E): central projections morphology in the terminal ganglion (Tg) and the seventh abdominal ganglion (7th abg) from individual dual mechanosensory sensilla located on the paraproct of the female locust.

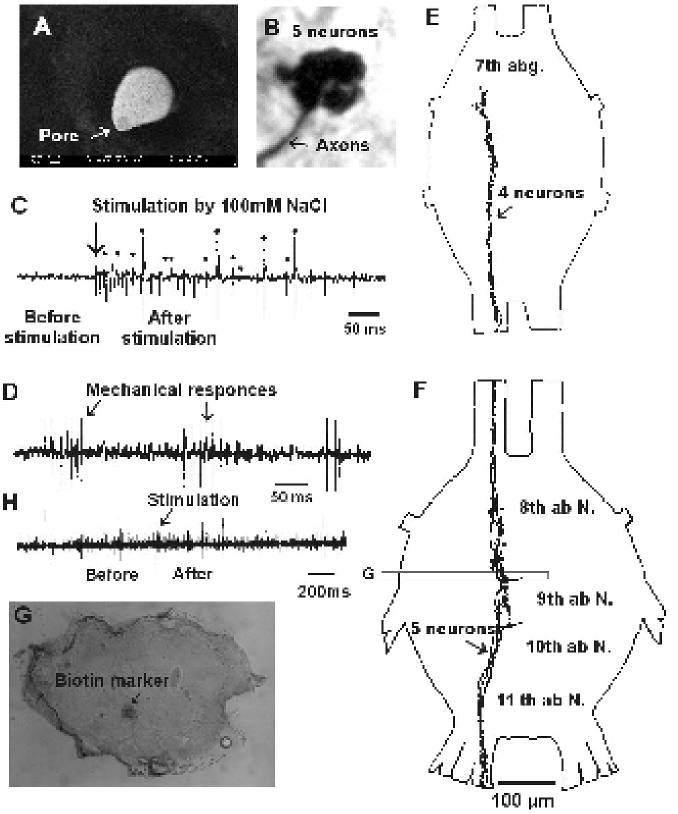

Fig.6. (A): SEM of individual basiconic sensilla in the locust paraproct. (B): Light micrograph for individual basiconic sensilla in the locust paraproct innervated by five sensory neurons. (C): Response of individual basiconic sensilla in the locust paraproct to 100 mM NaCl. (D): Deflecting the cut shaft of a basiconic sensillum (arrows) evokes a burst of action potentials of a single amplitude, indicating that the basiconic sensillum is innervated by a single mechanosensory neuron. (E&F): Central projections morphology in the terminal ganglion (Tg) and the seventh abdominal ganglion (7th abg) from individual basiconic sensilla located on the paraproct of the female locust, Five axons enter the terminal ganglion via the cercal nerve and proceed medially to the ninth and eighth abdominal neuromeres. Note that only four lateral afferent collaterals proceed through the anterior ipsilateral connective on a ventral level into the seventh abdominal ganglion, where they terminate medio ventrally. (G): Cross section through the ninth abdominal neuromere shown that the neuritis project ventrally. (H): Response of individual basiconic sensilla in the locust paraproct to acetic acid odour.