Local and Intersegmental Interneurons with Chemosensory Inputs from the Locust Ovipositor

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ABSTRACT

Sensory afferents from the ovipositors influence the behaviour of locusts before and during egg-laying. Contact chemoreceptors, known as basiconic sensilla in insects, occur dispersed and crowded in fields between mechanosensory receptors on the ovipositor of the female desert locust *Schistocerca gregaria* and serve to control the chemical features of the substrate before and during oviposition. Responses of contact chemoreceptors to aqueous solutions of salts (NaCl), sugars (glucose), acids (citric acid), oviposition aggregation pheromones (veratrole and acetophenone), alkaloids (quinine and tomatine), and phenolic compounds (salicin) were seen. Higher order processing occurs in local and ascending interneurones of the terminal abdominal ganglion. We focussed on a cluster of interneurons extending in the anterolateral region of the eighth abdominal neuromere. Several have ascending collaterals to more anterior abdominal ganglia. The physiological and morphological differences between the chemosensory interneurons suggest that there is no specific centre for processing taste information in the locust terminal ganglion.

Key words: Contact chemoreceptors, locust ovipositor, local and ascending interneurons, chemical stimulants.

INTRODUCTION

In insects, chemoreception plays a major role in a number of behavioural reactions including avoidance (Blaney, 1974, 1975; White and Chapman, 1990; Newland *et al.,* 2000), the detection and the selection of food (Dethier, 1976; Newland *et al.*, 2000; Gaaboub *et al.*, 2005; Gaaboub and Tousson, 2004) and the selection of egg-laying sites (Ma and Schoonhoven, 1973; Städler *et al.,* 1995; Dougherty *et al.,* 1995; Tousson and Hustert, 2000; 2004; Tousson and Gaaboub, 2004).

Chemoreceptors are primarily olfactory or gustatory, and these two categories have well defined and quite different structures. In locusts, suitable substrates for starting oviposition are detected first by the tarsal contact chemoreceptors of fore- and middle legs (White and Chapman, 1990; Gaaboub and Hustert, 1998; Tousson *et al.,* 1999). Consecutively, further chemical cues are given for starting and maintenance of digging as well as egg laying by the direct contact of chemoreceptors of the subgenital plate and ovipositor valves of the abdomen (Tousson and Hustert, 2000).

However, 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. Neural responses from the tips of insect taste sensilla were first recorded with the technique of Hodgson *et al*. (1955) that allows studying chemosensory specificity of the different afferents from single basiconic contact chemoreceptors. Many studies showed that the chemosensory neurons of the gustatory sensilla adapted very rapidly to contact with chemical stimulants (White and Chapman, 1990; Newland, 1998; Tousson *et al*., 1999; Gaaboub, 2000; Newland *et al*., 2000; Rogers and Newland, 2002, 2003; Tousson, 2004; Gaaboub *et al*., 2005).

Projections of mechanosensitive interneurons originating from the terminal abdominal ganglion and ascending to the anterior ganglia are well known in crickets (Kohstall, 1992 and 1996), cockroaches (Daley *et al*., 1981; Ritzman and Pollack, 1981) and grasshoppers (Boyan *et al*., 1989; Kalogianni, 1996; Tousson and Hustert, 2000). These receptors respond to different types of mechanical stimuli such as wind and sound applied to the cercal hair receptors. Recently, it seems likely that taste receptors have a large array of interneurons that integrate neural aspects of contacted chemicals and transfer their information to other ganglia. Intracellular recording and staining of several chemosensory integrating interneurons from the locust terminal ganglion gave an insight into the integrative properties, the morphology types and the distribution of ascending taste sensitive interneurons. The aim of this study was to investigate the identification of chemosensory interneurons integrating chemosensory information of ovipositor was also investigated.

Material and Methods:

All experiments were performed on sexually mature females of *Schistocerca gregaria* taken from our crowded laboratory culture reared at 25 °C under a 12h light / 12h dark regime and fed mainly with fresh wheat. Prior to the dissection locusts were anaesthetised by cooling to 2-4°C and experiments were performed at 22-25 °C.

In order to record from the anterior connectives of the terminal ganglion the 7th and the terminal abdominal ganglia were first exposed by dorsal dissection removing internal genitals, fat and viscera. All nerves from the terminal abdominal ganglion (TG) were cut except for the terminal branch of the eighth ventral abdominal nerve (8V) that innervates the ventral ovipositor valve. Locust saline at 22-25°C was exchanged regularly throughout an experiment in the terminal abdominal segments.

Electrophysiological recording of the activity of chemosensory neurones were obtained from the terminal pore of basiconic sensilla using a modified version of the tip recording technique (Tousson; 2001). For simultaneous stimulation and recording, contact was made with the meniscus of the salt solution at the end of the fine tapering plastic tip of a suction electrode. The recording and stimulating electrodes for the basiconic sensilla contained different concentrations of salts as NaCl (0.01 M to 3.0 M), sugar as glucose (0.01 M to 3.0 M), acids as citric acid (0.01M, 0.1M and 1.0 M), oviposition aggregation pheromones as veratrole and acetophenone (1.0% and 0.1%), alkaloids as quinine and tomatine (0.1%) and phenolic compounds as salicin (0.1%).

For specific stimulation, different chemicals were applied to a distinct single basiconic sensillum (contact chemosensitive sensillum) in the ventral region of the ipsilateral ventral ovipositor valve. Each stimulus was repeated 8-10 times for each stimulant chemical. For testing the specific response of stimulants all basic classes of stimulating chemicals (salts, acids, sugar, alkaloids) diluted in water with electrolyte (0.01M NaCl) were applied consecutively with interspersed pauses of several minutes in each experiment. In contrast to chemical stimulation and recording simultaneously at the terminal pore of the gustatory sensilla, stimulation with just the specific chemical in water for recording from interneurons had the great advantage of being unbiased by an additional electrolyte.

For intracellular recordings from neurons of the terminal ganglion while stimulating a basiconic sensillum on the ventral ovipositor the last abdominal ganglia were isolated except for their connection with the ventral ovipositor valves and fixed dorsal side down in a Petri dish on non-toxic plasticine. The ovipositor apodemes were pinned down. On a wax-covered stainless steel platform the terminal ganglion was mounted and its sheath was treated with a solution of about 1% (Wt / Vl) of protease (Sigma XIV) to facilitate intracellular recording from the interneurons. Intracellular microelectrodes were filled at their tips with a solution of about 4% Lucifer yellow CH (Molecular Probes, Inc.) in 1 M lithium chloride. The main shaft of the electrode was back-filled with 1.0 M lithium chloride. Electrode resistances ranged from 60 to 80 MV. Chemosensitive interneurons could be classified according to their specific responses to chemical stimuli and further identified morphologically with Lucifer Yellow dye injected into each recorded cell by passing depolarising current pulses 500 ms at 1 Hz for up to 20 minutes. The ganglia were then left in saline for 1 hour to allow the dye to diffuse into the arborizations and collaterals of the cell. Then the caudal ganglia (7th and terminal abdominal ganglia) were isolated from the preparation and fixed for 30 minutes in a buffered (pH 7.4) 4% formaldehyde solution, dehydrated, and cleared in methyl salicylate. Ganglia containing stained interneurones were viewed first as whole-mounts under an epifluorescence microscope (Leitz Aristoplan), photographed (35 mm or digital camera, Nikon Coolpix 950) and the interneuron was then either drawn directly by using a camera Lucida attachment on the compound microscope or reconstructed from negatives or computer printouts.

For testing the specific response of the stimulants all basic classes of stimulating chemicals were applied consecutively with interspersed pauses of several minutes in each experiment. Before each stimulation by diluted substances, stimulation with plain water solution served as a test for presence or absence of water responsiveness of an interneuron.

RESULTS

Afferent responses from gustatory receptors of locust female ovipositors were tested with the stimulation/recording electrode containing a minimum content of salt (0.1mM NaCl) for the conduction in water between the inner surfaces of the receptor. So at least the two potential stimulants water and salt are present and that can be coded by different receptor neurons of a single basiconic sensillum at contact with the electrode solution. Therefore, we could not test directly the afferent responses to pure water but rather at the postsynaptic level of afferents: from higher order interneurons of the terminal ganglion. Generally, identified taste receptors from a well described region of the ventral ovipositor were tested, both for their responses to different chemical concentrations at the receptor level and at the interneuron integration level.

The response to salt in single basiconic sensilla of the ventral ovipositor is phasic with two main units, possibly one for salt and one for water at the concentration of 0,1M sodium chloride (Fig. 1A). Sometimes initially as a third class of afferent units, very large spikes arise (shortly after a contact artefact) 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 for water (Fig 1B). Initially, as a fourth class of afferent unit's 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 larger units could respond to sugar and the electrolyte salt (0.01M sodium chloride) and a small third unit possibly responds to the water (Fig 1C). Initially, as a third 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 (0.1M) solution (Figs. 1D, 1E), 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 quinine (0.1M) and the phenolic compound salicine (0.1M) to a basiconic sensillum are rather strong and specific (Figs.1F, 1G).

Responses to application of pure water to basiconic sensilla of the ventral ovipositor were only observed in several larger ascending interneurons (Fig. 2). Their summated extracellular responses are mainly phasic, but cannot be counted or separated as identifiable units. In spite of their intensity none of these could be identified with intracellular methods. It would be interesting to see whether they respond to chemically inert substrates containing different levels of moisture.

In the interneurons, either excitation or inhibition occurred in response to stimulation of the ovipositor contact chemoreceptors. The responses could be mono- or polysynaptic in the case of excitation and di- or polysynaptic for inhibited interneurons. The specific interneurons were identified by specific morphological features, mainly their soma positions (all near the ventral surface of the 8th abdominal neuromere); the arrays of their neurite branching patterns, and the path of their axons. Six interneurones were identified in the terminal abdominal ganglion [two local (ChSIN 1, 2) and four intersegmental (ChSIN 3-6) interneurons] due to their selective responses to chemical stimulation of gustatory sensilla (Fig.3). Several other identified interneurons were found perceiving chemosensory as well as other sensory input, but are not included here.

Excitatory responses to salt stimulation (0.1M NaCl) was seen in two ascending interneurons (ChSIN 4/5) located in the 8th neuromere of the terminal ganglion with near-midline somata, one ipsi- and one contralateral to the ascending axon and neuropile branching, and only a few posterior branches extending into the ninth neuromere (Fig.1 D/E). The response to stimulating just one basiconic sensillum was phasic with a long tonic after-discharge in ChSIN5 and just phasic in ChSIN4. A third salt-responsive interneuron (ChSIN 2) responded with some inhibition of ongoing activity. This local interneuron (Fig.1B) it exhibits a completely different branching pattern extending from a very lateral soma: wide ipsilateral branching in the eighth and ninth neuromere and two separate contralateral neurites reaching far laterally into the ninth and eighth neuromere.

Discussion:

The central projections and intersegmental interneurons with chemosensory inputs from the contact chemoreceptors in *Schistocerca gregaria* have never been reported before. Tousson and Hustert (1998) have shown, for the first time, how neurons of a single insect contact chemoreceptors project in the CNS. Previously, cobalt staining of single sensory neuron was performed successfully, mainly in insect mechanoreceptors (Hustert, 1983; Hustert *et al*., 1981; Pflüger *et al*., 1981) but it did not work reliably for axon diameters of less than 1 µm that prevail for insect contact chemoreceptors. In the current study, we used the neurobiotin backfill technique (Tousson and Hustert, 1998) to identify the central projections of a single contact chemoreceptor. The present study also focused on the periphery and sensory innervation of the subgenital plate, in addition to the fine structure and distribution of various types of sensory sensilla investigated with cobalt chloride backfilling and scanning electron microscope. Another focus was on physiological responses and central nervous integration of basiconic sensilla to different concentration of sodium chloride solution.

We could study both the type of chemicals recorded by the contact chemoreceptors and their perception due to integration by higher order interneurones extra- or intracellularly from the anterior connectives or the neuropile of the terminal ganglion. In this way, taste sensilla can be stimulated by just one chemical diluted in pure water (without the salts added for electrical conduction) or possibly even gaseous chemicals: smells (for acids: Lefebvre 1981, Newland 1998; Tousson and Hustert, 2006; Newland and. Yates, 2007; Ômura, *et al*., 2008).

When higher order interneurons integrate one or several taste classes their synaptic input sites must collect information from widespread presynaptic terminals of afferents in the neuromeres of the terminal ganglion. Correspondingly, interneurons integrating single classes of taste selectively from non-glomerular structures in the neuropile should have wide branching areas for chemosensory input similar to the interneurons integrating mechanosensory input (Kalogianni 1996; Newland, 1998; Tousson, 2001). Our intracellular staining showed this for all taste sensitive interneurons especially when they responded to just one taste class. The occurrence of several local (but inter-neuromere) interneurons responsive to tastes indicates local processing of chemical cues for bilateral and inter-neuromere comparison of information which is required before and during oviposition (but possibly also during mating).

The interganglionic projections could not be traced with Lucifer yellow to their full extent

rostrally. If we had been able to pursue the long ascending axons we might have encountered the location of the most anterior CNS areas of decision making (brain or thoracic ganglia) that initiate continuation or cancellation of oviposition on the basis of chemical cues perceived on or near the ovipositor. We have no clue to where in the CNS of locusts commands for initiation, continuation or abandoning of egg-laying originate, while it is clear that most of the motor programs for the muscles are organised in the abdominal ganglia (Belanger and Orchard 1992, Thompson 1986; Gaaboub, 2000; Tousson, 2001; Chapman, 2003; Tousson and Gaaboub, 2004; Tousson and Hustert, 2006; Tousson and Youssef, 2006; Newland and. Yates, 2007). Therefore, this study could only indicate basal anatomical and physiological features of primary sensory integration from contact chemosensory information of the ovipositor.

Recording from higher order neurons of the chemosensory pathway originating 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 stimulants unknown to us might be perceived by means of isolated taste receptors which have not been included into our testing protocol. The responses seen here fall into the categories wet (water), salty, acid, sweet, bitter (alkaloid-like) and possibly phenolic, all these being typical for taste receptor sensitivity on other locations of the locust (Gaaboub and Hustert 1998; Gaaboub and Tousson, 2005; Gaaboub, et al., 2005). So presently, we 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.

We also are curious to know whether functionally the taste sensitive interneurons subserve local and restricted abdominal reflexes only or whether they contribute the perception of taste that underlies behavioural decisions. How the different chemical cues arising from the receptor level are utilized remains uncertain since tendencies in two different directions of neural processing appear: interneurons responding to just one type of stimulus can transfer this information further to centres of motor or behaviour decisions while other interneurons do the same for combinations of stimuli that might serve the perception of combined repulsive or attractive chemical stimuli for egg-laying behaviour.

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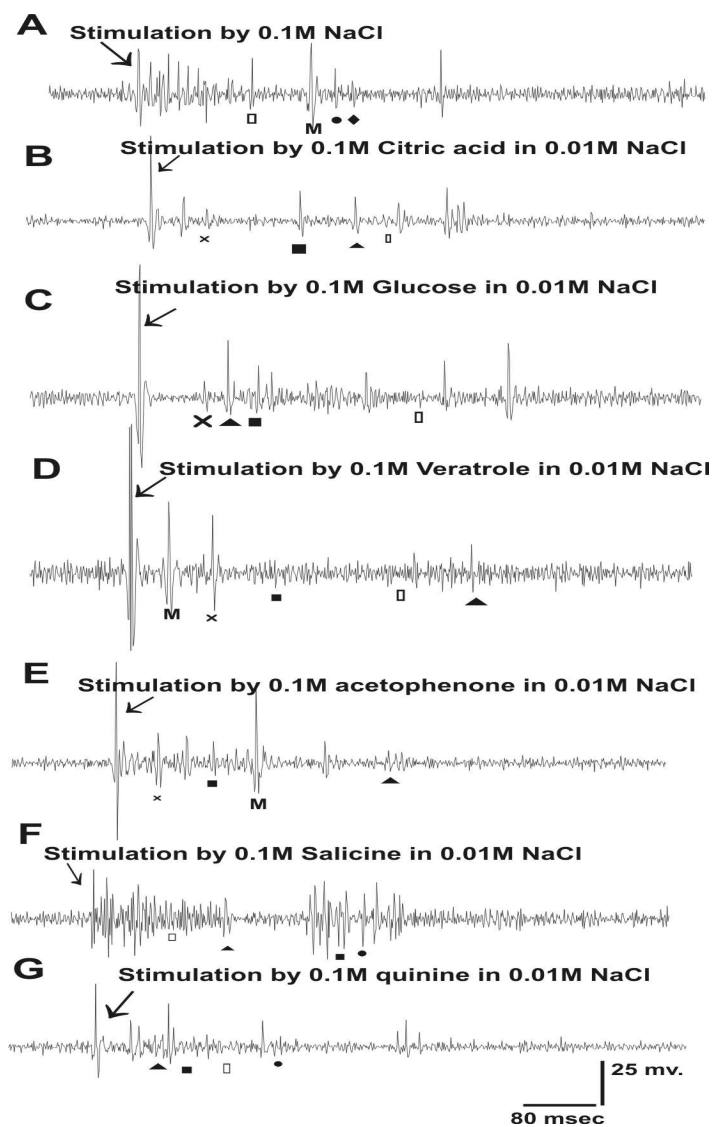
Fig. 1: Extracellular responses (recording from the tip of the ventral valve basiconic sensillum) to ovipositor valve stimulation with different kind of chemical substances. A. Stimulation by 0.1M NaCl; B. Stimulation by 0.1M citric acid in 0.01M NaCl; C. Stimulation by 0.1M glucose in 0.01M NaCl; D. Stimulation by 0.1M veratrole in 0.01M NaCl; E. Stimulation by 0.1M acetophenone in 0.01M NaCl; F. Stimulation by 0.1M salicine in 0.01M NaCl; G. Stimulation by 0.1M quinine in 0.01M NaCl.
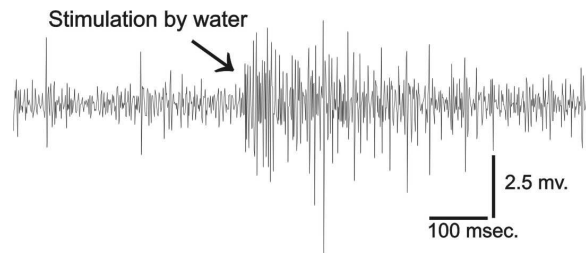
Stimulation by water

2.5 mv.

100 msec.

Fig. 2: Interneuron responses recorded from the ascending connective of the terminal ganglion during stimulation of the ventral ovipositor with water. At least three units respond phasically.

A

ChSIN 1 (- citric acid)

B

ChSIN 2 (- NaCl)

C

ChSIN 3 (+ citric acid)

D

ChSIN 4
(+ NaCl, + citric acid)

E

ChSIN 5
(+ NaCl, + glucose, + acetophenone)

F

100 µm

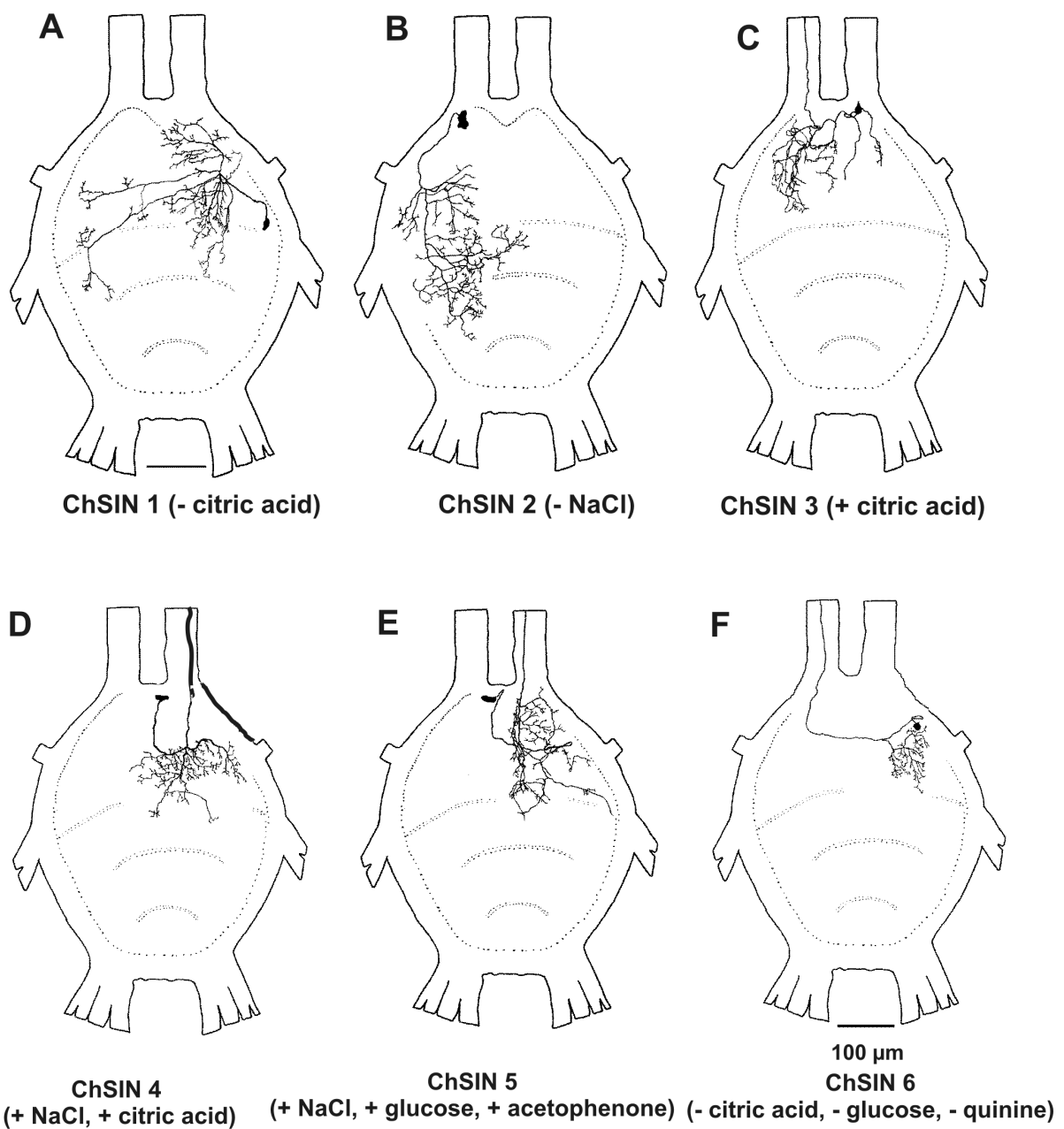ChSIN 6
(- citric acid, - glucose, - quinine)

Fig. 3: Morphological overview of interneurons that integrate chemosensory information (ChSIN) from the locust ovipositor, stained intracellularly with Lucifer yellow in the terminal ganglion (dorsal view). A. ChSIN 1 (inhibited by glucose) is a local interneuron branching ipsilateral to the soma in the posterior 8th, the 9th and anterior 10th neuromere. A single contralateral branch also crosses the midline. B. ChSIN 2 (inhibited by NaCl) is an extensive local interneuron with a very lateral soma (in 9th neuromere) branching ipsi- and contralaterally in the 8th and the 9th neuromere. Two main branches cross the midline and extend there far into the contralateral neuropil. C. ChSIN 3 (excited by citric acid) is a projecting interneuron of with contralateral extensive neuropile branching in the 8th neuromere and a contralateral ascending axon. In the ipsilateral 8th neuromere only few branches extend from the primary neurite of the soma. D. ChSIN 4 (excitedby NaCl and citric acid) is a projecting interneuron with an almost median soma, extensive ipsilateral neuropile branching in the 8th neuromere and some branches in the 9th neuromere and across the ganglion midline. The ascending axon extends ipsilaterally. E. ChSIN 5 (excited by NaCl, glucose and acetophenone), with an almost median soma in the 8th neuromere and all other structures extending contralaterally, branches extensively in the 8th neuromere and sparse branching in the 10th while its axon ascends medially. F. ChSIN 6 (inhibited by citric acid, glucose and quinine) with a lateral soma and restricted neurite branching in the posterior 8th neuropile and with an ascending contralateral axon without branching.