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INTERNEURONES INVOLVED IN STRIDULATORY PATTERN GENERATION IN THE GRASSHOPPER *CHORTHIPPUS MOLLIS* (CHARP.)

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Summary

In tethered grasshoppers, *Chorthippus mollis*, stridulatory leg movements were elicited by d.c. brain stimulation. Stridulatory chirps comprise both slow up-and-down movements and rapid oscillations of the hindlegs. Intracellular recording, stimulation and staining of interneurones within the metathoracic ganglion complex were performed simultaneously with recordings of leg movement.

Five interneurones were identified in the metathoracic ganglion complex. The branching patterns of these interneurones were typical of stridulatory interneurones. Three of these neurones had a structure very similar to stridulatory interneurones already characterized in the species *Omocestus viridulus*.

During stridulation, the spike activity of all interneurones was phasically coupled to the chirp rhythm; two interneurones additionally exhibited coupling to the rapid leg oscillations. Intracellular stimulation of interneurones A1-AC-2 and A1-AI-1 prolonged the duration of the rapid leg oscillations and influenced the generation of the chirp rhythm. Interneurones T3-LI-2 and T3-LC-4 decreased the amplitude of the slow up-and-down movement.

The data indicate that at least part of the metathoracic stridulatory network of *C. mollis* is organized in a structurally and functionally similar way to that of *O. viridulus*.

Key words: grasshopper, *Chorthippus mollis*, stridulation, interneurone, pattern generation.

Introduction

Acoustic communication systems are present in several groups of insects (cicadas, bushcrickets, crickets, grasshoppers) (Elsner and Popov, 1978; Michelsen and Larsen, 1985). The different species use species-specific sound patterns to recognize and localize their mates. In gomphocerine grasshoppers, the stridulatory apparatus consists of a cuticular vein on the forewings and a row of cuticular pegs along the proximal surface of the femora of the hindlegs. Sound is produced by rhythmically rubbing the legs against the vein of the wings (Elsner and Popov, 1978). Although the stridulatory apparatus in different species is very similar, species-specific sound patterns are produced. This is due to species-specific motor patterns which move the hindlegs and activate the sound-producing apparatus (Elsner, 1974*a*, 1975). The underlying stridulatory neuronal network should thus show species-specific modifications for the generation of the particular motor pattern. Acridid grasshoppers, therefore, represent a taxon in which the natural variety of stridulatory networks may offer the chance to analyse species-specific adaptations at the neuronal level.

In gomphocerine grasshoppers, the neuronal network generating the stridulatory motor pattern is located in the metathoracic ganglion complex. The structure, physiology and functional significance of metathoracic stridulatory interneurones have previously been analysed only in *Omocestus viridulus* (Gramoll and Elsner, 1987; Gramoll, 1988; Hedwig, 1992*a*,*b*). These authors identified stridulatory interneurones that drive the thoracic network, change the coordination of the hindlegs and modulate the shape of the stridulatory movements. However, nothing is known about the presence and function of these interneurones in other species. We therefore carried out the present study for comparative purposes.

In the grasshopper *O. viridulus*, the stridulatory movements during the calling and courtship songs consist of rather simple and uniform up-and-down movements. In *Chorthippus mollis*, however, stridulatory movements are more complicated. During a chirp, a slow up-and-down movement of the hindleg is followed by a period of rapid hindleg oscillations (Elsner, 1974*a*). The different stridulatory movement patterns of *O. viridulus* and *C. mollis* raised the questions of whether corresponding stridulatory interneurones to those in *O. viridulus* can be identified in *C. mollis* and/or whether there are specific adaptations of the stridulatory neuronal network. To

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answer this question, intracellular recording, staining and stimulation of metathoracic interneurones were performed in stridulating *C. mollis*.

Materials and methods

Animals

Male grasshoppers *Chorthippus mollis* (Charp.) (Acrididae, Gomphocerinae) were collected from biotopes in low mountain ranges in Franken, Germany, and were kept in the laboratory. A total of 302 animals were used in experiments.

Preparation

A preparation was used which allowed intracellular recording, stimulation and staining of metathoracic interneurones during stridulation by the grasshoppers. Stridulation was elicited by stimulating the brain with direct currents slowly increasing to a maximum of 15–30 μA. The current was applied *via* a suction electrode attached to the protocerebrum. Simultaneously, the activity of metathoracic neurones and the stridulatory leg movements were recorded. For intracellular stimulation of the neurones, depolarising current pulses of 700–1000 ms duration were applied through the microelectrode and any simultaneous changes in the leg movements were analysed. Details of the preparation, experimental procedures and data analysis are given in Hedwig (1992*a*). Neurones were named using the system described by Hedwig (1986*a*); for example, T3-LI-3 refers to the third (3) identified local interneurone with an ipsilateral axon (LI) within the metathoracic ganglion (T3). The terms ipsilateral and contralateral are used with respect to the soma position of the neurones.

Results

Stridulatory leg movements of Chorthippus mollis

During a stridulatory sequence of the grasshopper *C. mollis*, the chirps are generated by two different movement patterns of the hindlegs (pattern I and pattern II), which are performed simultaneously (Fig. 1). Pattern I is characterized by a slow up-and-down movement followed by a phase of rapid leg oscillations. A single slow up-and-down movement lasts for approximately 150–180 ms, whereas the rapid oscillations are of 20 ms duration and are performed for about 300 ms. While one hindleg makes a clear slow up-and-down movement (pattern I) the contralateral hindleg simultaneously produces only a slow upward movement (pattern II), which is then followed by rapid oscillations. There is a fixed assignment of the movement patterns to the hindlegs during a stridulatory sequence but the assignment may change occasionally between sequences. Details of the stridulatory leg movements of *C. mollis* are given in Elsner (1974*a*). During stridulation elicited by brain stimulation, the differences between the two movement patterns may not be clearly expressed, and both hindlegs may perform movements similar to pattern I (see Figs 3–6).

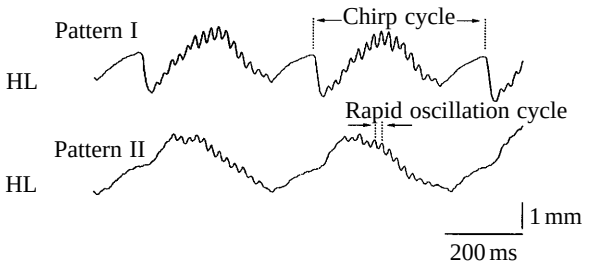

Fig. 1. Stridulatory hindleg movements of *Chorthippus mollis*: section of a courtship sequence. The stridulatory movements during one chirp consist of a slow up-and-down movement followed by a sequence of rapid leg oscillations. Each hindleg (HL) performs a different movement pattern (see text). For calculation of phase diagrams, the upper reversal point of the slow up-and-down movement was used as a reference, since it provided the best triggering possibilities.

Structure and activity of stridulatory interneurone T3-LI-3

Intracellular recordings were obtained from interneurones in the metathoracic ganglion complex from locations corresponding to the arborization pattern of the stridulatory interneurones in *O. viridulus*. Stridulation could be elicited in about 80 % of *C. mollis* prepared for intracellular recordings.

Interneurone T3-LI-3 (LI: local ipsilateral axon) has an anterior ventral soma (Fig. 2A). A thin branch with a varicose axonal appearance projects very laterally in a posterior direction and gives off small and short collaterals. Profuse arborizations with a dendritic appearance occupy an area close to the dorsal surface of the ganglion and run parallel to the midline as far posterior as the second abdominal neuromere. Data on this interneurone are based on seven recordings.

During stridulation, the membrane potential of interneurone T3-LI-3 oscillated in time with the chirp rhythm (Fig. 2B). The interneurone was depolarized above spike threshold at the end of the rapid leg oscillations and was maximally excited during the slow upward movement. The corresponding discharge rates were about 100 action potentials per second ($APs\,s^{-1}$) and 180 $APs\,s^{-1}$, respectively. During the downward movement of the ipsilateral leg, the depolarization rapidly decreased as the interneurone received a barrage of IPSPs (arrows Fig. 2B). Phase diagrams of the chirp cycle and the rapid oscillation cycle showed that the discharge rate of the interneurone was clearly coupled to the end of the chirp cycle and was in phase with the slow upward movement (Fig. 2C, left). There was no spike activity phasically coupled to the rapid oscillation cycle (Fig. 2C, right).

Stimulating the interneurone with injected current had no effect on stridulatory leg movements. In resting *C. mollis*, however, intracellular depolarization with 3 nA for 1000 ms led to a discharge of 50 $APs\,s^{-1}$ in the interneurone and elicited a slow upward movement of the ipsilateral hindleg (Fig. 2D). This demonstrates that the interneurone has access to the motor network involved in the control of ipsilateral hindleg movements.

Structure and activity of stridulatory interneurone T3-LC-4

Interneurone T3-LC-4 (LC: local contralateral axon) was

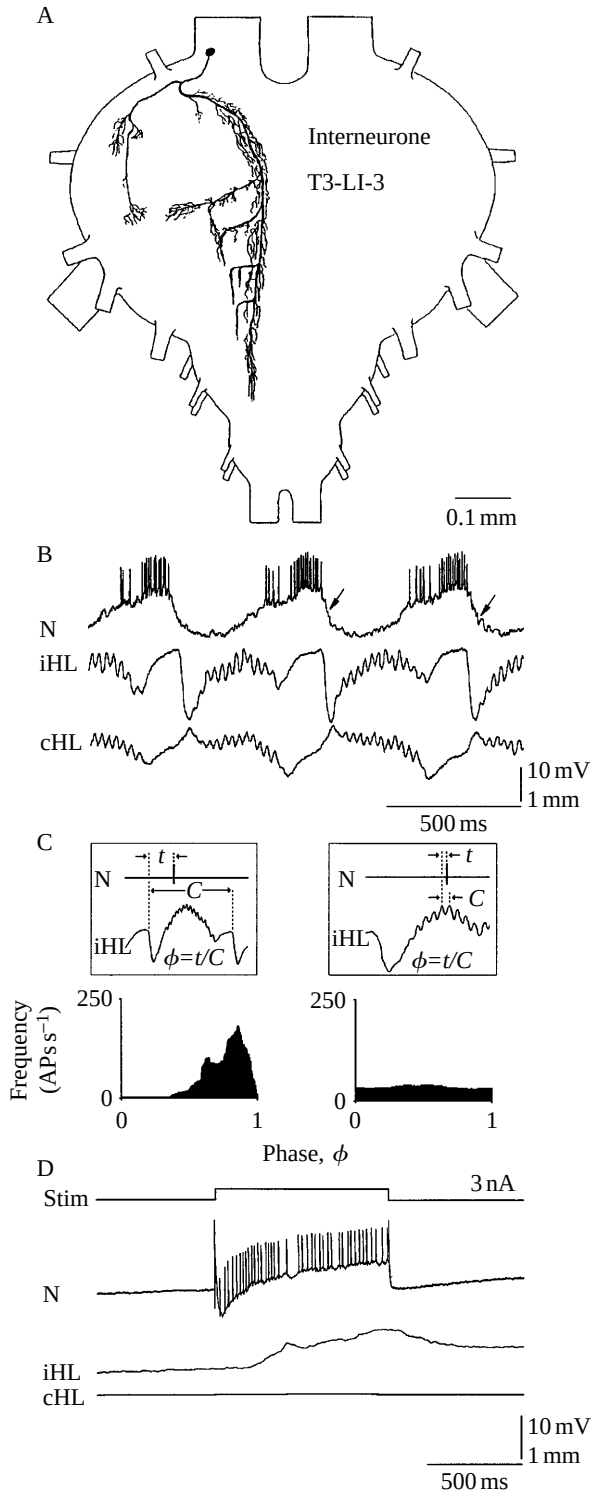

Fig. 2. (A) Structure of interneurone T3-LI-3 of *Chorthippus mollis* in the metathoracic ganglion complex. Note the dense arborization pattern of the neurone orientated parallel to the midline of the ganglion. (B) Activity pattern of the interneurone during stridulation (upper trace). The lower traces show the stridulatory patterns of the hindlegs. The arrows indicate a period of decreased polarization due to IPSPs. (C) Diagrams giving the average instantaneous spike frequency in the chirp cycle (left) and during the rapid leg oscillation cycle (right). (D) Depolarization of interneurone T3-LI-3 in a resting grasshopper caused slow upward movements of the ipsilateral hindleg. N, neuron; iHL, ipsilateral hindleg movement; cHL, contralateral hindleg movement; Stim, stimulus; *C*, chirp cycle; *t*, time between beginning of chirp cycle and the occurrence of an action potential, ϕ, phase; AP, action potential.

recorded and stained 12 times; it has almost symmetrical bilateral arborizations within the metathoracic ganglion complex (Fig. 3A). From the soma, which has a ventral medial position, the primary neurite projects dorsally and then crosses the midline. The ipsilateral arborizations of the neurone have a smooth appearance characteristic of dendrites. Most ipsilateral branches project into the lateral dorsal neuropile, but some fine processes run parallel to the midline as far posterior as the abdominal neuromeres. In the contralateral hemiganglion, the arborizations exhibit a corresponding branching pattern, but have a varicose appearance which may indicate an axonal function (Hedwig, 1992*a*).

The membrane potential of the interneurone oscillated in time with the chirp rhythm of the stridulatory movements (Fig. 3B). During the slow upward movement of the leg, T3-LC-4 was depolarized and thereafter discharged spikes at a rate of about 80–90 APs s^{-1} during both the downward movement and the following rapid leg oscillations. Spike activity stopped before the end of the rapid leg oscillations and the membrane potential of the interneurone was at a minimum during the transition from the rapid leg oscillations to the slow upward movement. The phase diagrams for the chirp cycle and the rapid oscillation cycle show that the spike activity of the interneurone was in phase with the first half of the chirp cycle (Fig. 3C, left) and that there was also a coupling between its spike activity and the rapid leg oscillations (Fig. 3C, right). The discharge rate of T3-LC-4 reached 55 APs s^{-1} in phase with the upper reversal point of the rapid oscillations and only 11 APs s^{-1} at the lower reversal point of the movement. Thus, the spike activity was in phase with both the chirp cycle and the rapid leg oscillations.

In the grasshopper *O. viridulus*, interneurone T3-LC-4 is involved in the coordination of the stridulatory leg movements of both hindlegs (Hedwig, 1992*a*; Ocker, 1994). In *C. mollis*, the effect of depolarizing the interneurone was tested with current pulses of 1000 ms duration and 3 nA amplitude. The stimuli were applied during stridulation while the ipsilateral hindleg was producing pattern II and the contralateral leg was producing pattern I. Stimulation of the interneurone caused an increase in the amplitude of the ipsilateral slow up-and-down movements and simultaneously decreased the amplitude of the contralateral slow up-and-down movements (Fig. 3D). These changes in movement amplitude correspond to a functional change in the stridulatory movement pattern, although it was not complete. Movements of the ipsilateral leg represent a transition from the low-amplitude pattern II to the high-amplitude pattern I, while the contralateral changes represent a transition from pattern I to pattern II. Since this particular animal stridulated with very small amplitude rapid leg oscillations, we could not observe any influence on this section of the chirp.

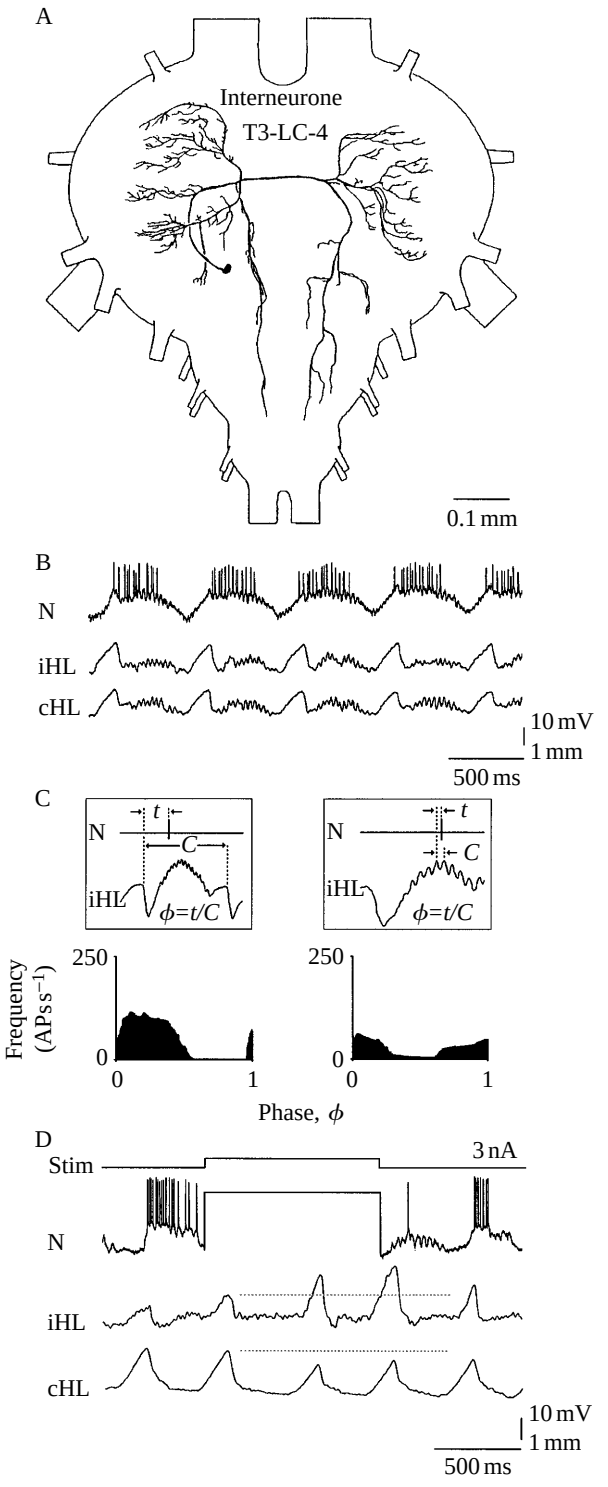

Fig. 3. (A) Structure of interneurone T3-LC-4 in the metathoracic ganglion complex. Branches ipsilateral to the soma had a smooth dendritic appearance, whereas contralateral branches were more varicose. (B) Activity of the interneurone (upper trace) and movements of the hindlegs (lower trace) during stridulation. (C) Phase diagrams giving the average frequency of spikes in the phase of chirps (left) and during the cycles of the rapid leg oscillations (right). (D) Depolarization of interneurone T3-LC-4 during stridulation increased the amplitude of ipsilateral slow up-and-down movements and decreased the amplitude of contralateral movements. Dotted lines indicate the amplitude of the movements before stimulation. The bridge circuit was out of balance during depolarization. Abbreviations as in Fig. 2.

Structure and activity of stridulatory interneurone T3-LI-2

Local interneurone T3-LI-2 is restricted to one side of the metathoracic neuromere (Fig. 4A). The neurone has a very lateral soma position and the neurite projects in a slight curve to the posterior. The branch with a dendritic appearance and the branch with an axonal appearance then separate. The main dendritic-looking branch projects directly posteriorly and gives off numerous side branches. The axonal branch, however, runs towards the midline before turning back to enter the lateral neuropile of the ganglion. Here, the varicose arborizations occupy a more dorsal position than the smooth dendritic branches. A total of 17 recordings were obtained from interneurone T3-LI-2. The structure of this interneurone in *C. mollis* closely resembles a corresponding interneurone in *O. viridulus* (neurone type 2; Gramoll and Elsner, 1987).

During stridulation, spike activity of T3-LI-2 was coupled to the chirp cycle and to the rhythm of the rapid leg oscillations (Fig. 4B). Depolarization of the interneurone started just before the upper reversal point of the slow up-and-down movement and elicited a burst of spikes. Depolarisation and spike activity continued until the end of the rapid leg oscillations. During each rapid oscillation of the hindleg, the interneurone discharged two action potentials. After the rapid leg oscillations, the depolarization decreased. The interneurone did not spike during the slow upward movement. This activity was reflected in the phase diagrams. The peak of activity, with a discharge rate of about 220 APs s^{-1}, occurred at phase 1/0, i.e. at the upper reversal point of the slow movement. During the rapid oscillations, the mean discharge rate fell from about 200 APs s^{-1} to about 100 APs s^{-1} (Fig. 4C, left). There was no activity during the slow upward movement. The spike activity was also modulated in the rhythm of the rapid leg oscillations. The discharge rate reached about 120 APs s^{-1} directly after the upper reversal point of each rapid oscillation but was only about 75 APs s^{-1} at all other phases (Fig. 4C, right).

In *O. viridulus*, stimulation of interneurone T3-LI-2 during stridulation damps the movement amplitude of the ipsilateral hindleg (Hedwig, 1992*b*; Ocker, 1994). To test the functional significance of this interneurone in *C. mollis*, the interneurone was depolarized with current pulses of 900 ms duration and 3 nA amplitude (Fig. 4D). This caused a distinct increase in spike activity. The interneurone now discharged about 6–9 APs (instead of two) during every rapid leg oscillation and some spikes now occurred during the slow upward movement. As a consequence of the increased activity, the slow up-and-down movement of the ipsilateral hindleg was transiently damped to about 35 % of the normal amplitude. After the end of the stimulus, the movement amplitude reached its full magnitude again. Although the interneurone activity was coupled to the rapid leg oscillations, we did not observe any change in the amplitude of these movements. There was no change in the

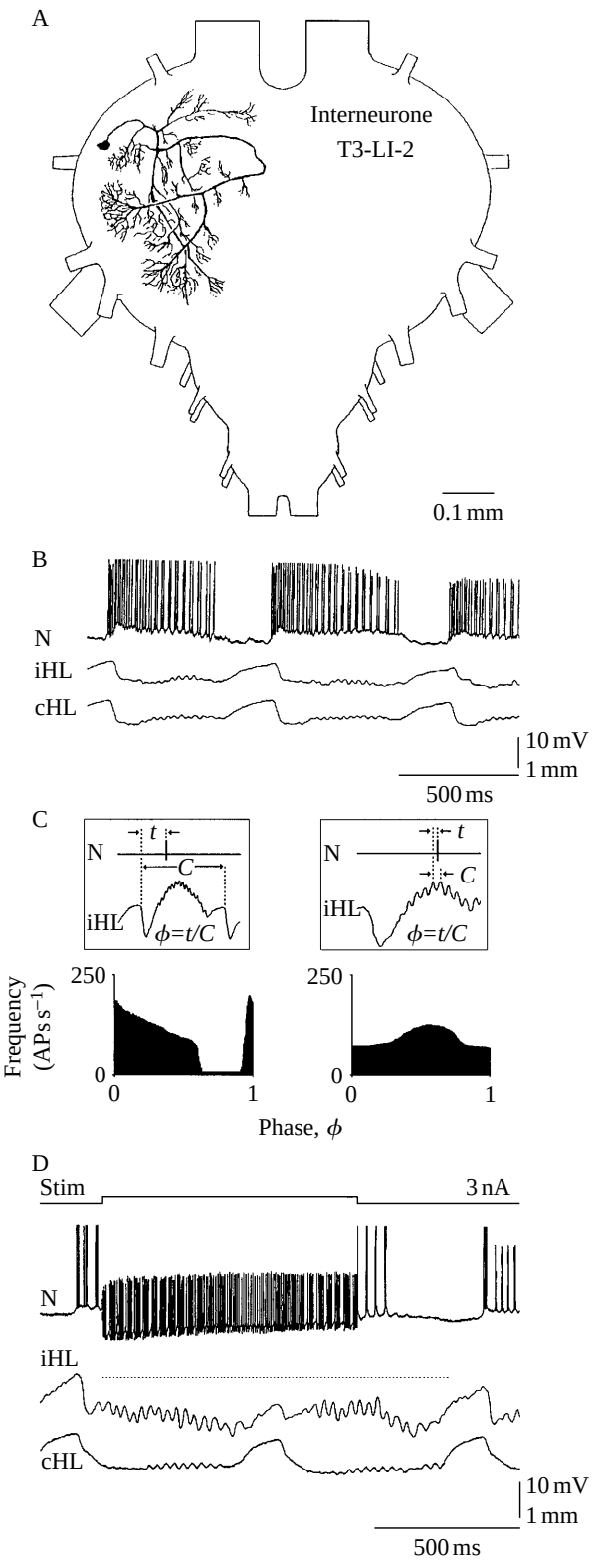

Fig. 4. (A) Structure of interneurone T3-LI-2 in the metathoracic neuromere. Note the hairpin-like course of the axon, which arborizes dorsal to dendritic branches. (B) Activity of the interneurone (upper trace) and movements of the hindlegs (lower trace) during stridulation. Some spikes are clipped as a result of the recording procedure. (C) Phase diagrams giving the average frequency of spikes in the phase of chirps (left) and during the cycles of the rapid leg oscillations (right). (D) Depolarization of interneurone T3-LI-2 during stridulation decreased the ipsilateral slow up-and-down movements but had no effect on the contralateral movements. The amplitude of the movement before stimulation is indicated by the dotted line. Abbreviations as in Fig. 2.

contralateral movements during depolarisation of the interneurone.

Structure and activity of stridulatory interneurone A1-AC-2

Interneurone A1-AC-2 (AC: ascending contralateral axon) has branches on both sides of the metathoracic ganglion complex that have structural features typical of stridulatory interneurones (Fig. 5A). The soma occupies a lateral posterior position in the first abdominal neuromere. Arborizations ipsilateral to the soma exhibit predominantly smooth dendritic structures, whereas contralateral branches are varicose. The dendritic and axonal arborizations mainly occupy a medial region, parallel to the midline, in an area in which other stridulatory interneurones also have their projections. In the abdominal neuromeres, the lateral side branches form a ladder-like arborization pattern. The axon ascends to anterior ganglia contralaterally with collaterals projecting to the contralateral medial neuropile. Data are from one recording.

The membrane potential of A1-AC-2 exhibited oscillations in time with the rhythm of the stridulatory cycle (Fig. 5B). Depolarization was strongest at the upper reversal point of the slow up-and-down movement, when the discharge rate of the neurone reached about 120 APs s^{-1}. The level of depolarisation and spike activity then decreased and the spikes stopped during the middle of the rapid leg oscillations. Depolarization reached a minimum at the transition from the rapid leg oscillations to the slow upward movement. There was no coupling of the spikes to the cycle of the rapid leg oscillations. Correspondingly, the phase diagrams show maximal spike activity at phase 1/0 and during the first part of the chirp cycle (Fig. 5C, left). There was no coupling of the neurone activity in the rhythm of the rapid leg oscillations since the phase diagram shows no maximum (Fig. 5C, right).

The effect of the interneurone on the stridulatory movements was tested during ongoing stridulation (Fig. 5D). The interneurone was constantly hyperpolarized by −1.5 nA and then depolarized for 600 ms with 2 nA. During hyperpolarization, there was little spike activity during stridulation. Depolarization elicited a burst of spikes with a discharge rate of about 130 APs s^{-1}. This significantly prolonged the ongoing rapid leg oscillations of the stridulatory movement pattern. The chirp length was about 424 ms before the stimulus and increased to 1136 ms during depolarization. Such changes never occur during normal stridulation. The ipsilateral hindleg continued performing rapid leg oscillations after the end of the depolarisation, whereas the contralateral leg immediately began a slow up-and-down movement. As a consequence, both hindlegs moved out of phase for one cycle. The subsequent ipsilateral chirp cycle was distinctly shorter

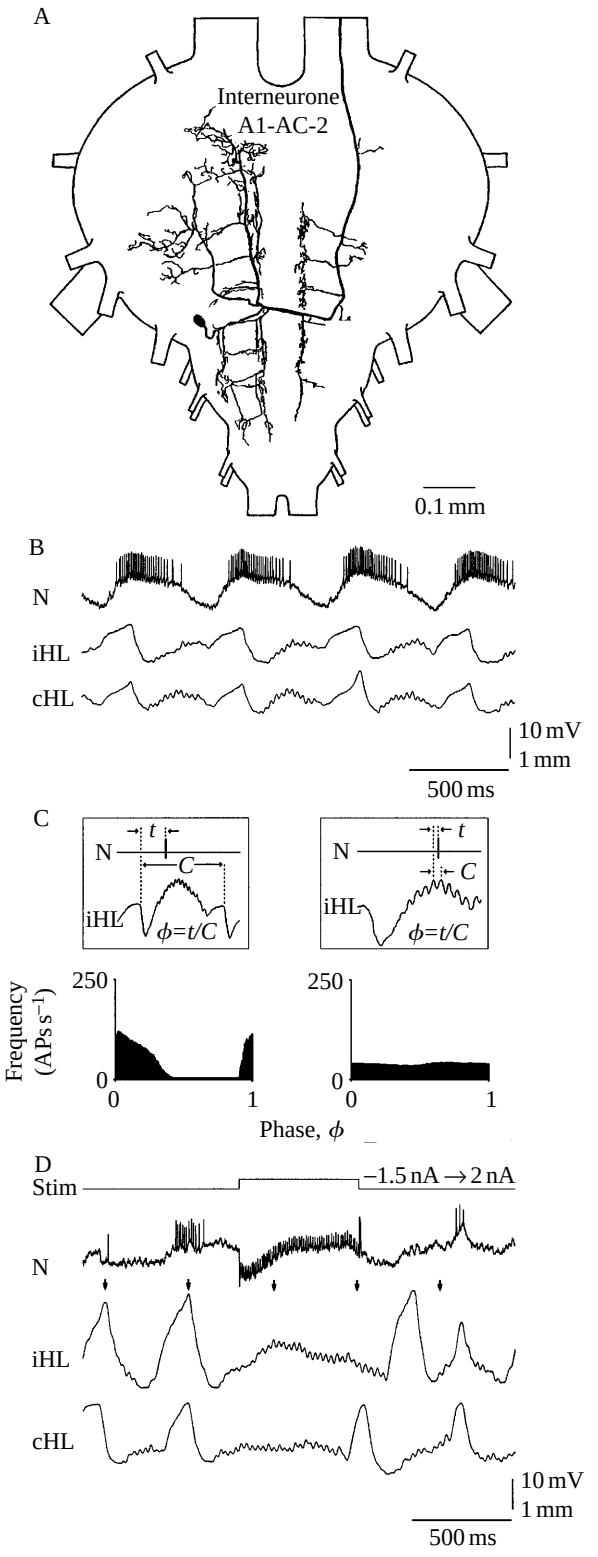

Fig. 5. (A) Structure of interneurone A1-AC-2 in the metathoracic ganglion complex. Note the ladder-like dendritic arborizations in the abdominal neuromeres and the dendritic and axonal arborizations parallel to the midline of the ganglion. (B) Activity of the interneurone (upper trace) and movements of the hindlegs (lower trace) during stridulation. (C) Phase diagrams giving the average frequency of spikes in the phase of chirps (left) and during the cycles of the rapid leg oscillations (right). (D) A pulse of depolarisation (2 nA) applied to interneurone A1-AC-2 during stridulation prolonged the duration of rapid leg oscillations and increased the chirp duration. The neurone was constantly hyperpolarised by −1.5 nA before and after the pulse. Arrows mark the beginning of the chirp cycle predicted from the initial rhythm. Abbreviations as in Fig. 2.

than the contralateral cycle, bringing the legs into phase. Thereafter, both legs moved in phase again and normal chirp cycles were produced. These chirps were not in phase with the chirps before the period of depolarisation (arrows in Fig. 5D indicate the initial chirp rhythm). Depolarisation of this interneurone therefore reset the chirp rhythm.

Structure and activity of the stridulatory interneurone A1-AI-1

Interneurone A1-AI-1 (AI: ascending ipsilateral axon) has a median ventral soma position and a complex structure within the metathoracic ganglion (Fig. 6A). Ipsilaterally, the neurone exhibits a ladder-like arborization pattern in the abdominal neuromeres. In the metathoracic neuromere, the main arborizations project laterally on the ipsilateral side, although some project more medially on the contralateral side. The main neurite runs anteriorly and gives off numerous small smooth branches which form a profuse arborization parallel to the midline. Before the axon enters the ipsilateral connective, an axonal collateral crosses the midline and turns back towards the abdominal neuromeres, giving off lateral varicose branches along its length. The data are based on two recordings.

Recordings of the interneurone were obtained from the anterior axonal branches. At this recording site, membrane potential oscillations caused by synaptic activity are not apparent. However, interneurone A1-AI-1 exhibited bursts of spikes in phase with the slow upward leg movement. The activity of the interneurone reached about 220 APs s^{-1} and ceased before the upper reversal point of the movement. There was little or no spike activity during the rapid leg oscillations. The phase diagrams therefore show a peak in the discharge rate close to the end of the chirp cycle (Fig. 6C, left). There was only weak activity during the rapid oscillation which was not phasically coupled to the rapid oscillation cycle (Fig. 6C, right).

During ongoing stridulation, the interneurone was held hyperpolarized by injection of −0.5 nA and then depolarized for 900 ms by 2 nA (Fig. 6D). This elicited a discharge rate of about 125 APs s^{-1} in the interneurone. The rapid leg oscillations were now performed for much longer and, as a consequence, the ongoing chirp was prolonged from 490 to 780 ms. Spike activity of the interneurone was interrupted before the end of the depolarisation at the same time as a slow upward movement terminated the rapid oscillations. The first chirp cycle following the depolarising pulse was shortened to 340 ms, but subsequent chirps had a normal duration. These chirps were, however, not in phase with the chirp rhythm before stimulation (arrows in Fig. 6D indicate the initial chirp rhythm). Stimulation of interneurone A1-AI-1 therefore reset the timing of the chirp rhythm.

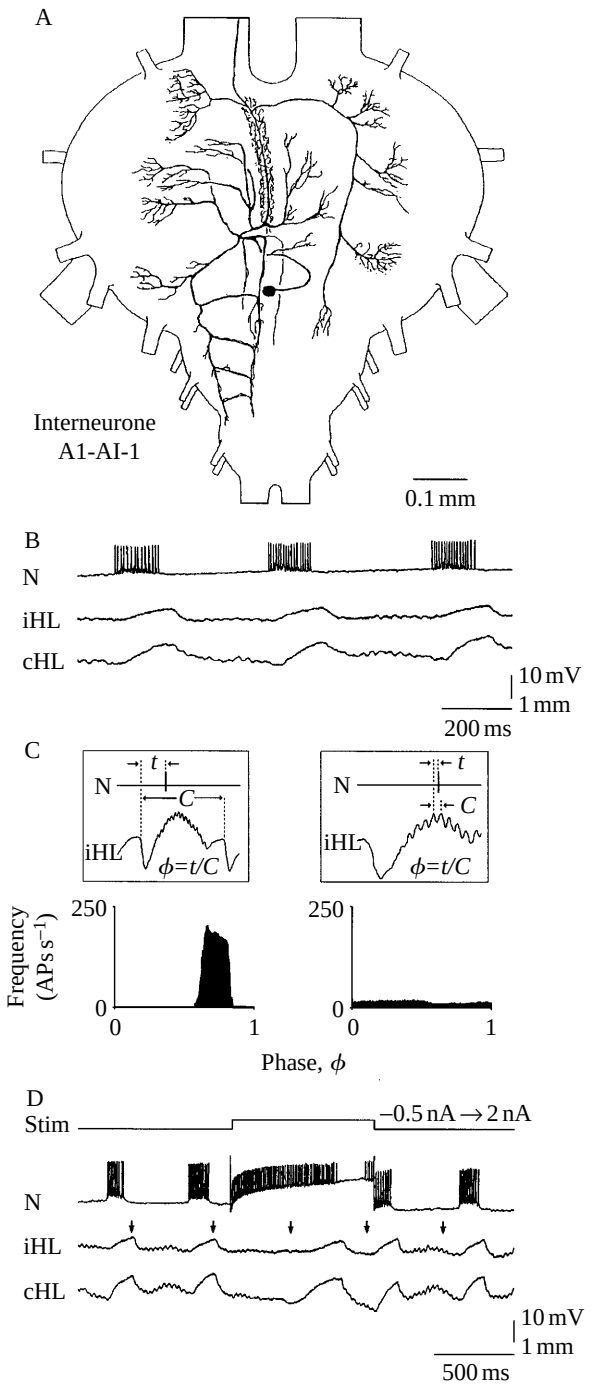

Fig. 6. (A) Structure of interneurone A1-AI-1 in the metathoracic ganglion complex. Note the ladder-like dendritic arborizations in the abdominal neuromeres. (B) Activity of the interneurone (upper trace) and movements of the hindlegs (lower trace) during stridulation. (C) Phase diagrams giving the frequency of spikes in the phase of chirps (left) and during the cycles of the rapid leg oscillations (right). (D) Depolarization of interneurone A1-AI-1 during stridulation prolonged the occurrence of rapid leg oscillations, increased the chirp duration and transiently changed the chirp rhythm. The interneurone was constantly hyperpolarised by −0.5 nA before and after the depolarisation. Arrows mark the beginning of the chirp cycle predicted from the initial rhythm. Abbreviations as in Fig. 2.

Although the activity of A1-AI-1 was coupled to the slow up-and-down movements, depolarisation of the interneurone suppressed this part of the movement and prolonged the sequence of rapid leg oscillations.

Discussion

The aim of the present experiments was to analyse stridulatory interneurones in the grasshopper *C. mollis* and compare them with those identified in the grasshopper *O. viridulus* (Gramoll and Elsner, 1987; Gramoll, 1988; Hedwig, 1992*a*,*b*). Since *C. mollis* performs completely different movement patterns (Elsner, 1974*a*), it was our aim to gain insight into the organization of the stridulatory neuronal network in *C. mollis*. Within this context, stridulatory interneurones are defined as interneurones which are phasically active during the stridulatory motor pattern. However, this does not exclude activation of the neurones during the generation of other motor patterns.

Structure of stridulatory interneurones in Chorthippus mollis

Four of the interneurones identified in *C. mollis* (Figs 2, 3, 5, 6) arborize in at least three neuromeres of the metathoracic ganglion complex and reach from the metathoracic to the abdominal neuromere. All of these interneurones show, at least to some degree, medial arborizations that run parallel to the midline of the ganglion. This is most clearly expressed in interneurone T3-LI-3. Although these branches have either a smooth dendritic or varicose axonal appearance, they may not, however, be interpreted as strict input or output regions of the neurones (Watson and Burrows, 1985). Furthermore, the neurones exhibit ladder-like arborization patterns in the abdominal neuromeres. These general features of the arborization pattern closely correspond to the organization of stridulatory interneurones in *O. viridulus*, where the interneurones also arborize in the metathoracic and abdominal neuromeres; they exhibit ladder-like arborization patterns in the abdominal neuromeres and have a dense arborization pattern parallel to the ganglionic midline (Fig. 7; Gramoll and Elsner, 1987; Hedwig, 1992*a*,*b*).

Apart from the similar spatial arrangement of the stridulatory network within the metathoracic ganglion complex in both species, there are also certain similarities in at least three interneurones. The arborization patterns of interneurones T3-LI-3, T3-LC-4 and T3-LI-2 very closely resemble those of the corresponding interneurones in *O. viridulus* (Fig. 7, see also Figs 5, 4, 7, respectively, in Gramoll and Elsner, 1987; Figs 2, 9 in Hedwig, 1992*a*; Fig. 4 in Hedwig, 1992*b*). Therefore, at least three interneurones in the stridulatory networks seem to be homologous in both species, although direct evidence for homology can only be established by a developmental analysis of cell lines. Corresponding similarities have been reported for descending interneurones of the subeosophageal ganglion and brain that modulate or activate the stridulatory pattern (Lins and Lakes-Harlan, 1994; Hedwig, 1994; B. Hedwig, unpublished data). The general

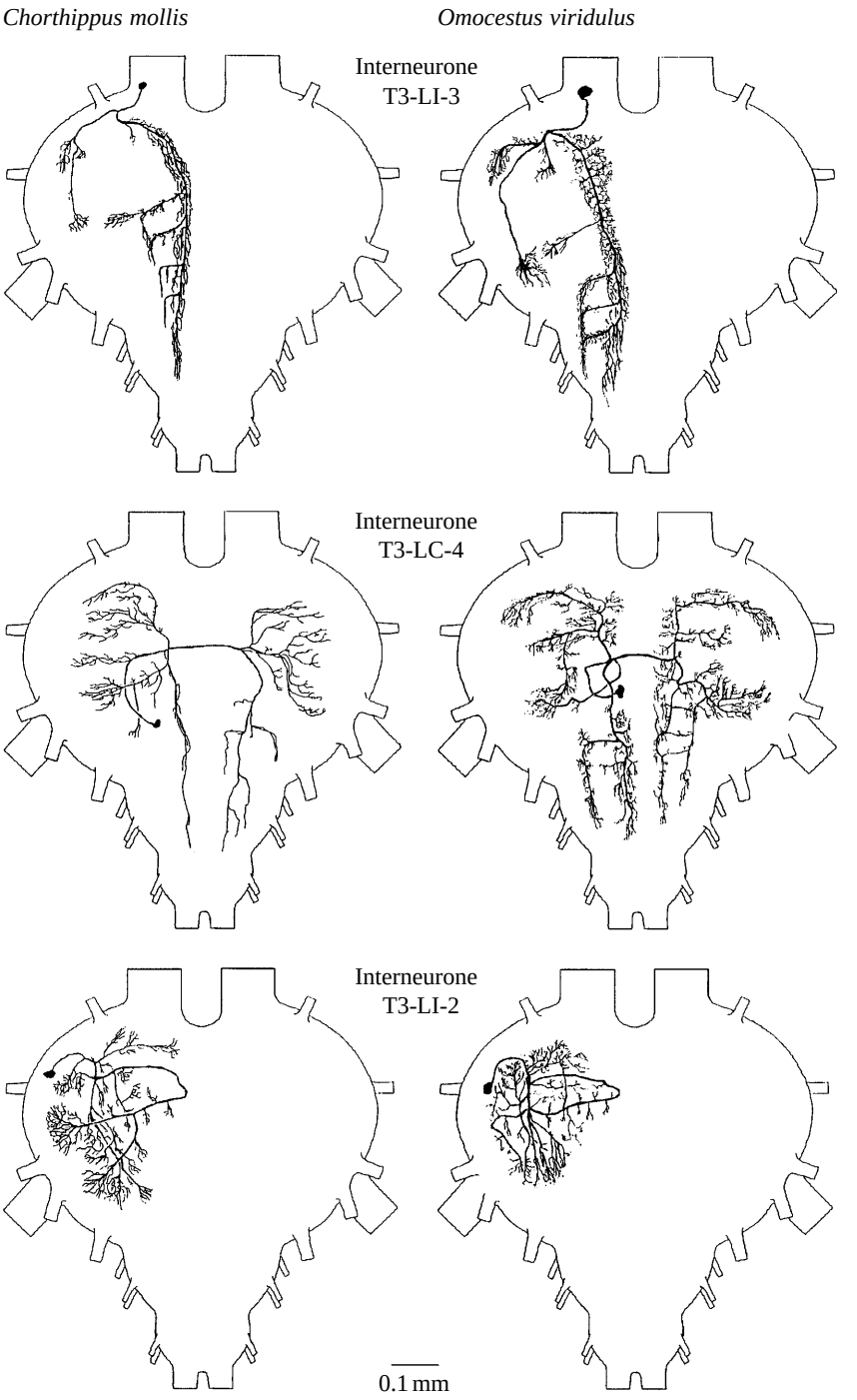

Fig. 7. Structure of the stridulatory interneurones T3-LI-3, T3-LC-4 and T3-LI-2 which exhibit similar branching patterns in the two grasshopper species *C. mollis* (left) and *O. viridulus* (right).

structural features of stridulatory interneurones therefore seem to be preserved in the species-specific stridulatory networks.

Clearly, we have neither identified all the metathoracic stridulatory interneurones nor understand the functional significance of minor changes in the arborization patterns or possible differences in membrane properties. We do not know at what levels of the neuronal network the species-specific characteristics that lead to the observed species-specific motor patterns are expressed. Such adaptations may best be identified by analysing those interneurones that are elements of the central rhythm-generating system. However, in *O. viridulus*, we have not yet identified elements corresponding to interneurones A1-AI-1 and A1-AC-2, which in *C. mollis* can modulate the rapid leg oscillations and the generation of the chirp rhythm.

Phase relationships and functional significance of interneurone activity

The stridulatory movements of *C. mollis* comprise a slow up-and-down movement which is followed by a sequence of rapid leg oscillations. Stridulation always starts with slow up-and-down movements, whereas the rapid oscillations are gradually inserted into the intervals between the slow up-and-

down movements. It therefore seems that two different motor-pattern-generating mechanisms are involved in producing the stridulatory movements. The phase relationships between the spike activity and leg movements should make it possible to determine to which network each neurone probably belongs. As a consequence, this information may suggest which parts of the stridulatory movement pattern in different species are homologous.

The spike patterns of interneurones T3-LI-3, A1-AC-2 and A1-AI-1 (Figs 2, 5, 6) were only coupled to the slow up-and-down movement. These interneurones had different phases of maximum activity within the chirp cycle, but none of them exhibited any coupling of their spike activity with the cycle of the rapid leg oscillations. These neurones, therefore, are not likely to be involved in the generation of the rapid leg oscillations.

The functional significance of the interneurones for the generation of the stridulatory movements was tested by eliciting an enhanced discharge rate during ongoing stridulation. Interestingly, those neurones whose activity was coupled to the slow up-and-down movement (A1-AC-2 and A1-AI-1, Figs 5A, 6A) prolonged the sequence of rapid leg oscillations when their firing rate was artificially increased, but did not influence the stridulatory movement pattern to which they were phasically coupled. This may be explained, at least partly, if neurones A1-AC-2 and A1-AI-1 have an inhibitory effect on the slow up-and-down movement and thereby support the occurrence of the rapid leg oscillation. The effects of interneurone stimulation, however, indicate that the slow up-and-down movement and the rapid leg oscillations can be modulated independently and may thus be controlled by different networks.

Interneurone T3-LI-3 in *C. mollis* is coupled only to the chirp rhythm. In *O. viridulus*, the corresponding interneurone is involved in the initiation of the thoracic stridulatory motor pattern and its activity is strongly coupled to the stridulatory leg movement. In *O. viridulus*, the movements are rather simple, consisting only of up-and-down movements with a cycle length of approximately 80 ms. A comparison of the activity of interneurone T3-LI-3 in the stridulatory networks of both species suggests that the chirps of *C. mollis* and the up-and-down movements of *O. viridulus* are corresponding actions. Within this context, it would be interesting to determine which interneurones are involved in the generation of the rapid leg oscillations in *C. mollis*.

As the phase diagrams indicate, interneurones T3-LI-2 (Fig. 4C) and T3-LC-4 (Fig. 3C) were coupled both to the chirp rhythm and to the rhythm of the rapid leg oscillations. These interneurones may therefore be involved in the generation of both movement patterns. It must be considered, however, that any coupling to the rapid leg oscillations will also be expressed in the phase diagram as a coupling to the chirp rhythm. We may therefore overestimate the functional relationship of these neurones to the generation of the chirp rhythm.

Intracellular depolarization of interneurone T3-LI-2 (Fig. 4) decreased the amplitude of the ipsilateral slow up-and-down movements, whereas depolarization of T3-LC-4 (Fig. 3) altered the coordination of the hindlegs. Corresponding effects can be demonstrated in the grasshopper *O. viridulus* (Hedwig, 1992*a*,*b*; Ocker, 1994). Depolarisation of interneurone T3-LI-2 decreased the amplitude of the ipsilateral leg movement. Depolarisation of interneurone T3-LC-4 changed the coordination of the stridulatory movement pattern of the hindlegs. The structure of this interneurone may be regarded as a prerequisite for this function, since the connection of ipsilateral dendritic arborizations with the contralateral axonal branches suggests a directed flow of information between both sides of the ganglion. As in *C. mollis*, neither of these interneurones influenced the chirp rhythm. Therefore, besides the similar structural features in both species, these two interneurones also have a functional similarity in their effect on the stridulatory pattern. We assume that at least parts of the stridulatory networks in both species are organized similarly in both structural and functional respects.

Future prospects

The evolution of species-specific sound patterns within the gomphocerine grasshoppers could theoretically come about through changes in the sound-producing apparatus and/or the motor pattern used for sound production. Indeed a few species, such as *Stenobothrus rubicundus*, have developed mixed wing stridulation and leg stridulation (Elsner, 1974*b*; Elsner and Wasser, 1995*a*,*b*). All species that use leg stridulation use a similar peripheral sound-producing mechanism that shows only minor species-specific adaptation. Nevertheless, even in closely related species, the motor patterns underlying sound production may be completely different (Elsner, 1975), and these differences in the timing of motor activity give rise to the species-specific sound patterns. Motor patterns underlying sound production are therefore an important element in the evolution of species-specific acoustic signals. Since we now know some of the central stridulatory interneurones, it will be interesting to determine the morphology and role of these neurones in other stridulating and nonstridulating grasshoppers to obtain an insight into the evolution of these neuronal networks (Dumont and Robertson, 1986).

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