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On the control of stridulation in the acridid grasshopper *Omocestus viridulus* L.

I. Interneurons involved in rhythm generation and bilateral coordination

Berthold Hedwig

I. Zoologisches Institut, Universität Göttingen, Berliner Str. 28, W-3400 Göttingen, FRG

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Summary. In tethered, minimally dissected grasshoppers stridulation was elicited by DC brain stimulation. Intracellular recording, stimulation and staining of interneurons in the metathoracic ganglion complex were performed simultaneously with measurements of hindleg movements. The functional significance of interneurons for generation of the stridulation rhythm and bilateral coordination of the hindlegs was tested and quantitatively analysed. Interneurons involved in stridulation were found in the metathoracic and abdominal neuromeres. Typically they included arborizations in a dorsal neuropil parallel to the long axis of the ganglion. Interneuron T3–LI–3 started and drove the ipsilateral rhythm generator. The stridulation rhythm was reset by interneuron A1–AC–1. It was stopped if interneuron T3–AC–1 was stimulated. Bilateral coordination of stridulation movements was based on excitatory and inhibitory pathways between the hemiganglionic networks. Switching in the coordination of hindleg movement patterns was induced by changes in the discharge rate of the bilateral arborizing interneuron T3–LC–4. The hemiganglionic interneurons T3–LI–3 and T3–LI–1 influenced coordination via the activity level within the networks.

Key words: Grasshoppers – Stridulation – Intracellular stimulation – Rhythm generation – Pattern coordination

Introduction

Acridid grasshoppers of the subfamily Gomphocerinae have developed a highly elaborate stridulation behaviour (Faber 1953; Jacobs 1953). Species-specific sound signals are produced by rhythmical hindleg movements causing cuticular pegs on the femur to strike a cuticular rim of the elytra. In *Omocestus viridulus* stridulation is characterized by the repetition rate, the movement form and the coordination of the hindlegs which perform two different movement patterns (Elsner 1974). However, little is known about how these parameters of behaviour are controlled by the nervous system.

The rhythm-producing network for stridulation is organized in two hemiganglionic rhythm generators which are located in the metathoracic ganglion complex (Hedwig 1986a; Ronacher 1989, 1991). A neurophysiological analysis of the neuronal network underlying stridulation was begun by electrical stimulation of certain brain areas (Huber 1959) which elicited stridulation in tethered grasshoppers. Combining brain stimulation and intracellular recordings (Hedwig 1986a, b) characterized plurisegmental interneurons which showed activity clearly correlated with the stridulation motor pattern. Thus, an intracellular approach to the neuronal network underlying stridulation in grasshoppers was possible.

In a dorsally dissected preparation, from which the hindlegs had been removed, the structure and the activity pattern of thoracic stridulation interneurons were described (Gramoll and Elsner 1987; Gramoll 1988). Now, in a new approach, intracellular recording and stimulation of stridulation neurons have been combined with the registration of stridulation leg movements. The objective of the experiments was to examine the effect of modulating the discharge rates of single interneurons for rhythm generation and the coordination of motor patterns. An accompanying paper will describe neurons which are involved in the shaping of the stridulation leg movements (Hedwig 1992).

Materials and methods

Animals. Males of the grasshopper *Omocestus viridulus* L. (Acrididae, Gomphocerinae) were collected during summer in the surroundings of Göttingen (Germany) and Pontresina (Switzerland) and were kept in the laboratory. All experiments were performed at 27–30 °C.

Preparation. The grasshoppers were glued ventral side up into a U-shaped holder. The head, pronotum and prothoracic tergites were rigidly connected with wax. The front and middle legs were restrained but the hindlegs, which perform the stridulation move-

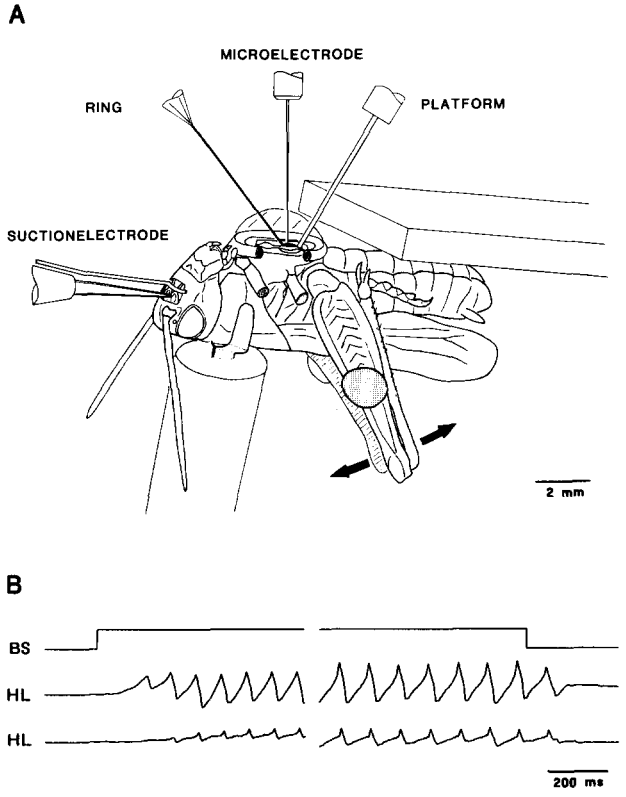

Fig. 1. A Preparation for intracellular recording and stimulation in stridulating grasshoppers. Front and middle legs have been omitted in the drawing. Both hindlegs were free to perform stridulatory movements. **B** Stridulation sequence of 10 s duration elicited by brain stimulation. The *middle* part of the sequence has been omitted. Brain stimulus (*BS*), hindleg (*HL*)

ments, remained completely free to move (Fig. 1A). The inverted body position also occurs under natural conditions.

Leg movements. In *O. viridulus* stridulation movements of the hindlegs consist of relatively simple up- and downstrokes. Depending on temperature one cycle lasts 60–70 ms. Several hundred cycles are continuously linked together and form a stridulation sequence. During stridulation one hindleg produces a large-amplitude movement (pattern 1) and the other a small-amplitude movement (pattern 2), and both legs move with a specific coordination (Elsner 1974). The leg performing pattern 1 reaches the upper reversal point of its movement distinctly before the contralateral leg. The interval between the upper reversal points of patterns 1 and 2 will be referred to as the relative latency. The relative latency is usually stable during sequences but may sometimes change within a sequence. The stridulation movements were recorded with two optoelectronic cameras (von Helversen and Elsner 1977).

Brain stimulation. Details of the brain stimulation technique have been described by Hedwig (1986a). Negative currents of 10–30 μA were delivered by a constant current source (WPI Stimulus Isolation Unit Model 305–2). Usually, DC pulses of 10 s duration were given (WPI Anapulse Stimulator Model 302–T). Stridulation started with the onset of the brain stimulus and ceased at its end (Fig. 1B). Behaviour could be elicited reliably at the ventral side of the protocerebrum in 99% of the animals. Leg movements occurred with the same accuracy as in natural stridulating grasshoppers (Elsner 1974; Hedwig 1986a). However, the repetition rate was reduced to 7–11 $C \cdot s^{-1}$ and in rare cases the generation of the movement patterns could be modified (Fig. 3A). In some experiments the brain stimulus was set to be just suprathreshold to evoke low-amplitude stridulation, since certain effects of single cell stimulation on the motor output were not obvious during full intensity stridulation.

Intracellular recording and stimulation. Intracellular neuropil recordings were obtained from 243 animals from which parts of the

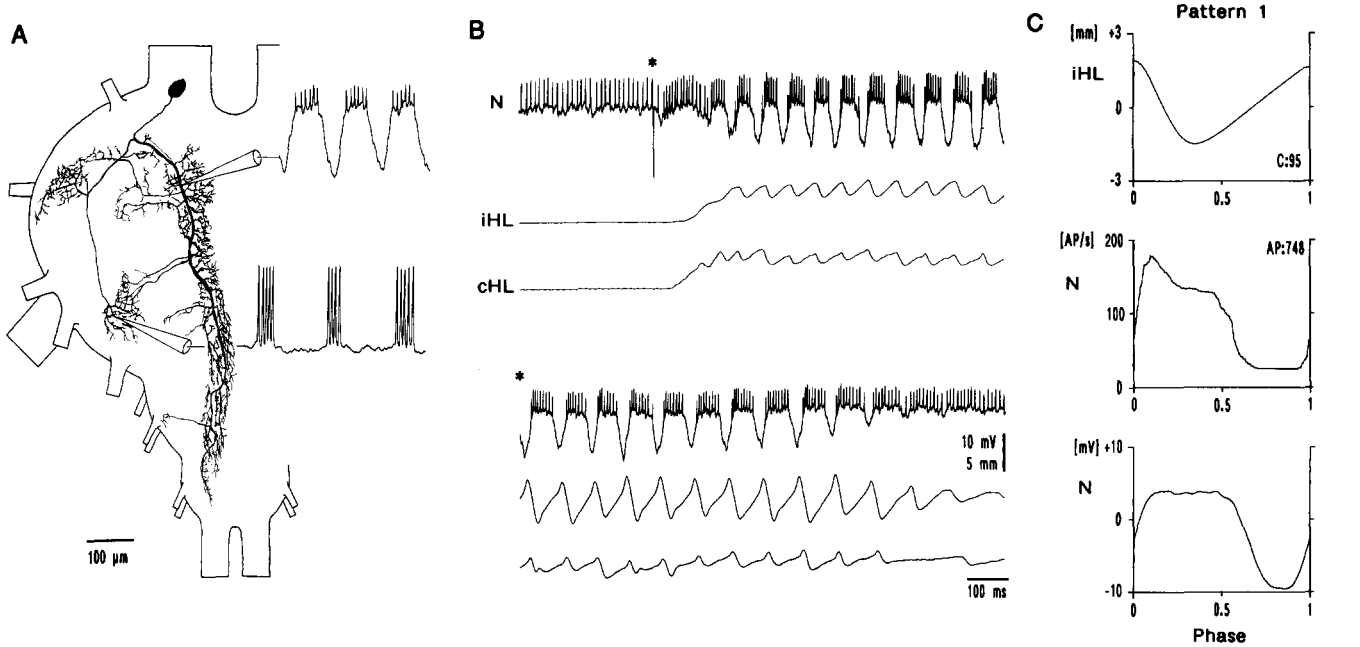

Fig. 2. A Structure of the interneuron T3–LI–3 (dorsal view). EPSPs and spikes were recorded in the main branch of the neuron and only spikes in the lateral axonal branch. **B** Interneuron activity at the beginning and end of a stridulation sequence. Onset and offset of brain stimulation indicated by an *asterisk*. **C** Phase-corrected averages of the ipsilateral hindleg movement ($iHL_{[mm]}$), the instantaneous spike frequency ($N_{[AP/s]}$) and the membrane potential ($N_{[mV]}$) of T3–LI–3. Averages are based on 95 cycles (*C*) and 748 action potentials (*AP*)

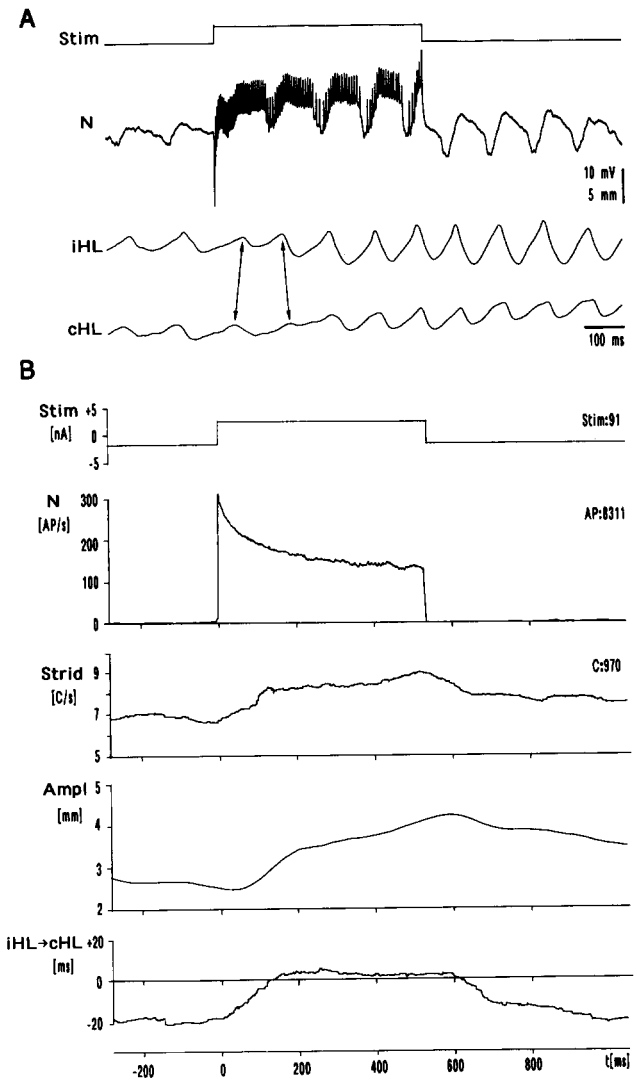

Fig. 3A, B. Intracellular stimulation of T3–LI–3 during spontaneous stridulation increased the repetition rate of stridulation and induced movement pattern 1 in the ipsilateral hindleg: **A** Specimen record, *arrows* indicate the change in relative latency of the hindleg movements. Stimulus (*Stim*), neuron activity (*N*) and movement of the ipsilateral and contralateral hindleg (*iHL*, *cHL*); **B** Averages of the motor effects with the intracellularly applied current ($Stim_{[nA]}$), the instantaneous spike frequency of the neuron ($N_{[AP/s]}$), the repetition rate of stridulation ($Strid_{[C/s]}$), the amplitude of the ipsilateral hindleg movement ($Ampl_{[mm]}$) and the relative latency of the hindlegs ($iHL \rightarrow cHL_{[ms]}$). A total of 91 stimuli (*Stim*), 8311 action potentials (*AP*) and 970 cycles (*C*) were evaluated. **A** and **B** are aligned to the same time scale

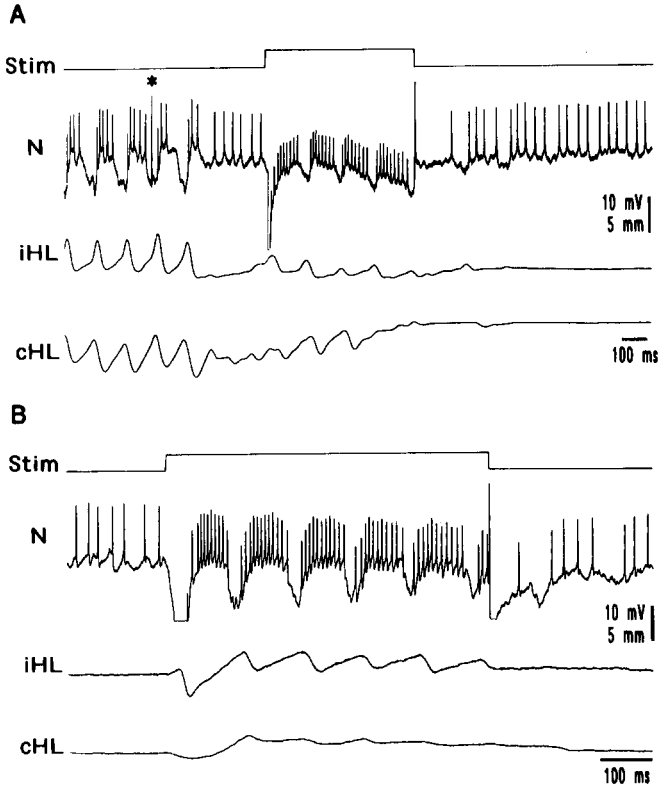

Fig. 4. **A** Intracellular depolarization of T3–LI–3 after a stridulation sequence elicited rhythmic membrane potential oscillations and stridulation movements. The end of the brain stimulus is indicated by an *asterisk*. **B** Simultaneous brain stimulation and intracellular depolarization of T3–LI–3. The brain stimulus was set to be just subthreshold for stridulation. Depolarization of T3–LI–3 elicited rhythmic membrane potential oscillations and stridulation movements. To obtain a distinct change in the discharge rate T3–LI–3 was continuously hyperpolarized with −1.5 nA and repeatedly depolarized with +3 nA pulses of 600 ms duration

meso- and metathoracic sternites had been removed. A silver ring supported the metathoracic ganglion and also served as a reference electrode. The central aperture of the ring allowed recordings to be obtained in the most dorsal region of the ganglion without breaking the electrode. To establish stable recording conditions an additional small ring was gently pressed on top of the ganglion (Wolf and Pearson 1987). Drops of saline (Robertson and Pearson 1982) prevented the recording site from drying. Thin-walled microelectrodes filled with a solution of 5% lucifer yellow CH in distilled water (resistances between 40 and 60 MΩ) were used for intracellular recordings.

After penetration of candidate interneurons stridulation was repeatedly elicited. The neuron's activity was first recorded during stridulation, then the cell was depolarized during rest and afterwards during stridulation. To obtain a maximum change in discharge rate some neurons were constantly hyperpolarized via the amplifier and then depolarized for 400–600 ms with an additional current source (WPI Stimulus Isolation Unit Model 305–2) connected to the amplifier. The diameters of primary neurites of the interneurons were typically about 4 µm. Penetrations of such neurites had to be stable for at least 5–10 min to perform all the required tests.

Staining and reconstruction. Neurons were stained with lucifer yellow CH by application of hyperpolarizing currents of −1 to −3 nA for 2–5 min. After staining, the meso- and metathoracic ganglia were dissected out and processed by standard histological techniques. The whole mounts were viewed, drawn and photographed with a Leitz epifluorescence microscope. In the description of structures and physiological effect the terms ipsilateral and contralateral refer to the soma position of neurons.

Data analysis. All data were recorded on magnetic tape and subsequently digitized (sampling rate 10 kHz per channel, amplitude resolution 12 bit). A specially developed software (Hedwig and Knepper 1991) was used to display, plot and evaluate the recordings.

Diagrams refer to the movement of the ipsilateral hindleg. For phase-corrected averaging all cycles were normalized to the mean cycle duration and then averaged. The evaluation of membrane

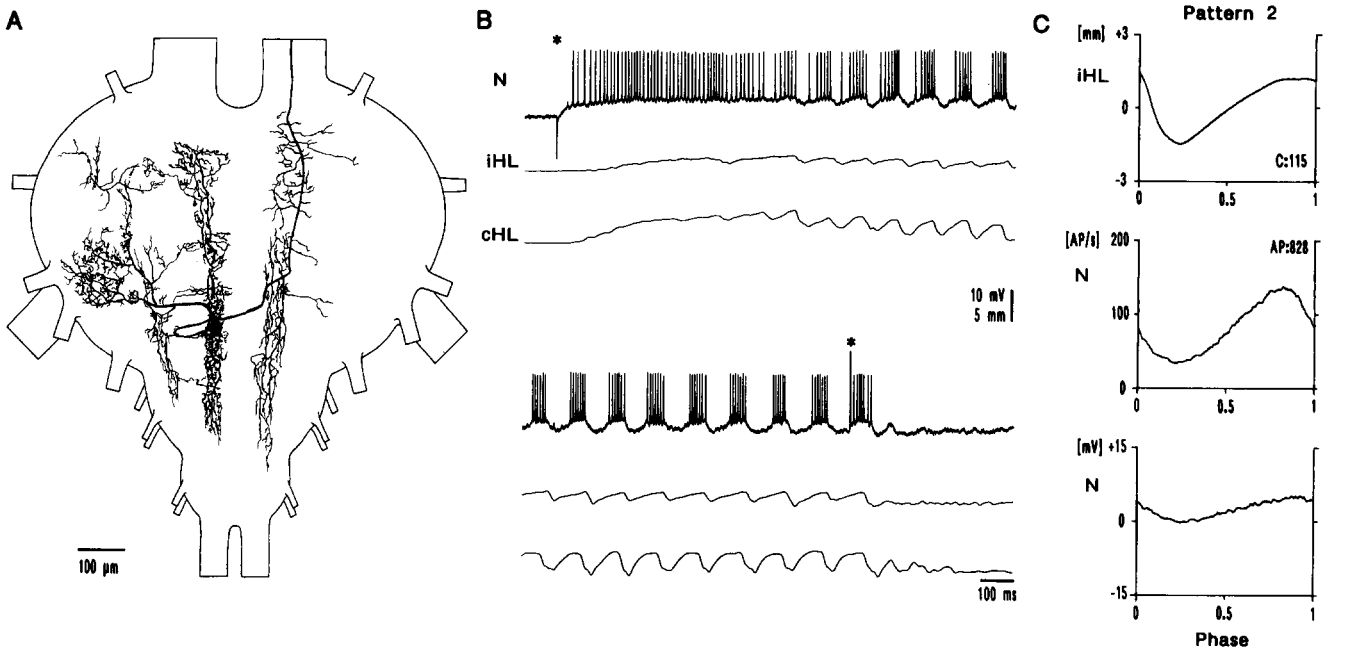

Fig. 5. **A** Structure of the interneuron A1–AC–1. Soma position is ventral. **B** Interneuron activity at the beginning and end of a stridulation sequence. Onset and offset of brain stimulation are marked by an *asterisk*. **C** Phase-corrected averages of the ipsilateral hindleg movement ($iHL_{[mm]}$), the instantaneous spike frequency ($N_{[AP/s]}$) and membrane potential (N_{mV}) of A1–AC–1. Averages are based on 115 cycles (*C*) and 828 action potentials (*AP*)

potentials was done after cutting off spikes at their base with a software filter function. The instantaneous repetition rate of events, i.e. spikes and leg oscillations, could be calculated. The coordination of the hindlegs was described by the relative latency between the upper reversal points of their movements. Positive intervals indicate a lead of the leg ipsilateral to the soma of the recorded neuron. For determination of the movement amplitude the connecting lines were calculated between all upper reversal points and between all lower reversal points of the leg movement, respectively. The amplitude of stridulation was obtained by subtraction of the lower polygon from the upper polygon. All parameters were expressed as monotonic functions and could be averaged simultaneously with the time functions of the recordings. Data for phase response diagrams were obtained directly from the computer screen. Calculation of the diagrams was performed with a commercial program (Quattro Pro, Borland GmbH).

For nomenclature of neurons the system described by Hedwig (1986a) was used. It was supplemented with "L" as an abbreviation for local intraganglionic interneurons.

Results

When there were long pauses between the stridulation sequences, the spike activity of the described interneurons was very low. However, their activity could be increased if subsequent sequences were elicited at short intervals. The efficacy of intracellular stimulation depended on the intensity of stridulation. Generally, effects

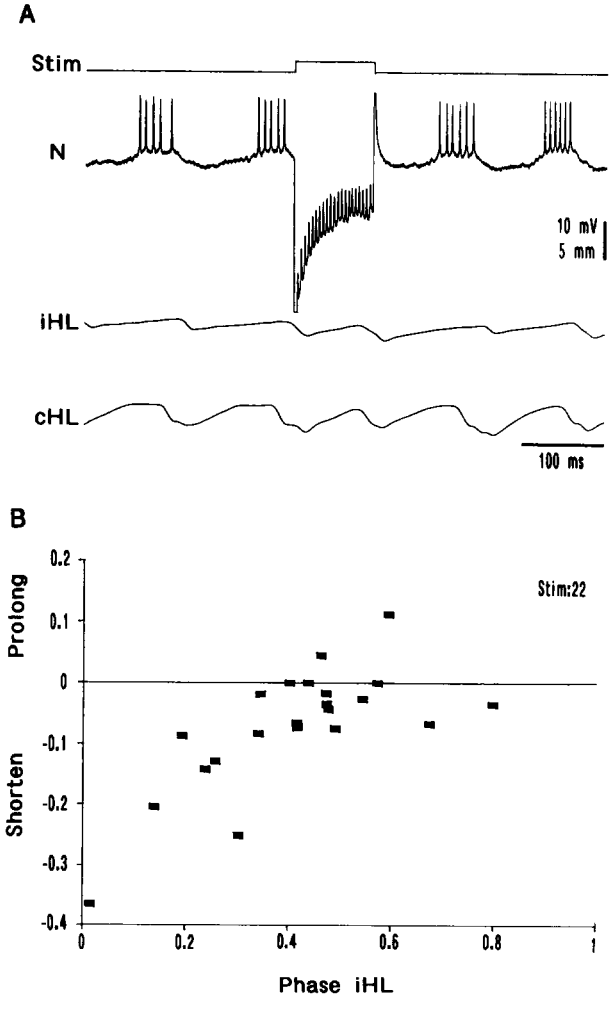

Fig. 6. **A** Intracellular stimulation of A1–AC–1 with a current pulse (3 nA, 105 ms) elicited a high-frequency burst in the interneuron and reset the stridulation rhythm. **B** Phase response diagram calculated for 22 current pulses delivered at different phases of the stridulation cycle

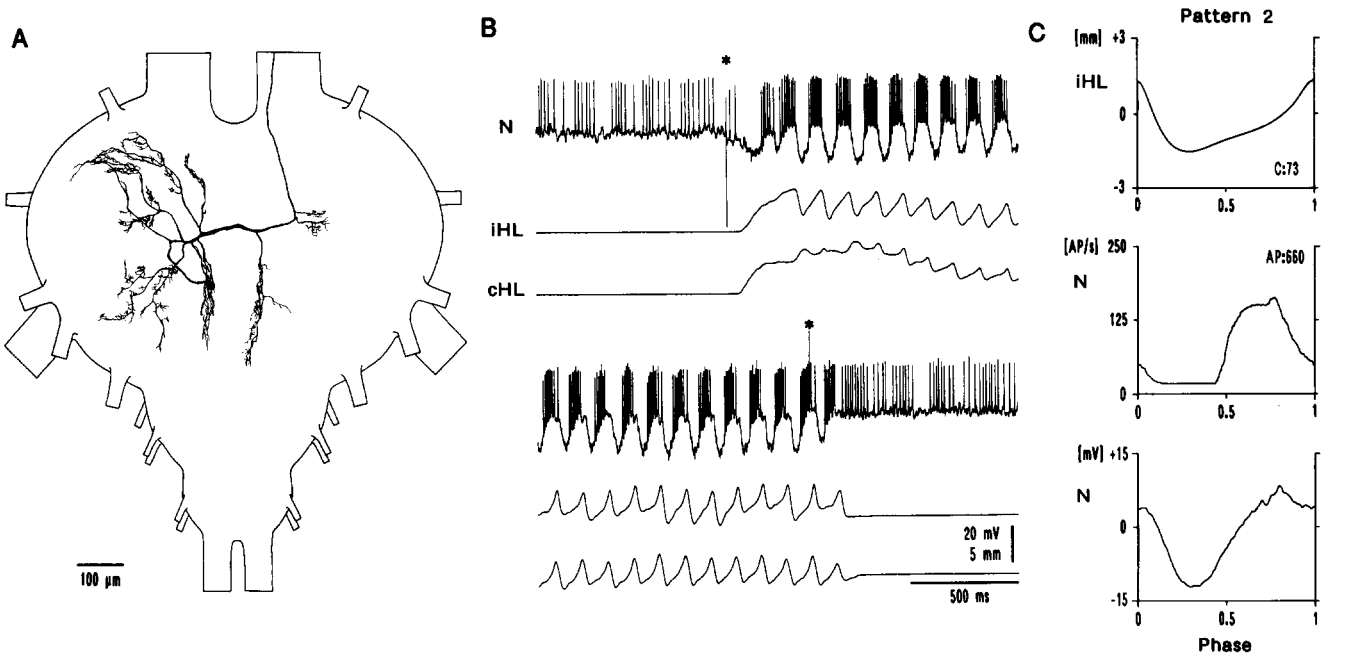

Fig. 7. **A** Structure of the interneuron T3–AC–1. Soma position is ventral. Only small branches occur in the dorsal neuropil, parallel to the long axis of the ganglion. **B** Interneuron activity at the beginning and end of a stridulation sequence. Onset and offset of brain stimulation are marked by an *asterisk*. **C** Phase-corrected averages of the ipsilateral hindleg movement ($iHL_{[mm]}$), the instantaneous spike frequency ($N_{[AP/s]}$) and the membrane potential ($N_{[mV]}$). Averages are based on 73 cycles (*C*) and 660 action potentials (*AP*)

were small during intense stridulation and significant during stridulation with low amplitude and reduced repetition rate. The effects observed were always qualitatively consistent in different preparations.

The impact of neurons on the generation of the stridulation rhythm and coordination of movement patterns is described below.

Influence of interneuron T3–LI–3 on rhythm generation and coordination

This interneuron has been described as type 3 by Gramoll and Elsner (1987). Data are based on 32 recordings. Multiple fills show that in each hemiganglion there are at least three identical siblings of T3–LI–3. The main process runs close below the dorsal surface in line with the medial edge of the connectives and had numerous smooth, fine arborizations (Fig. 2A). Anteriorly one pro-

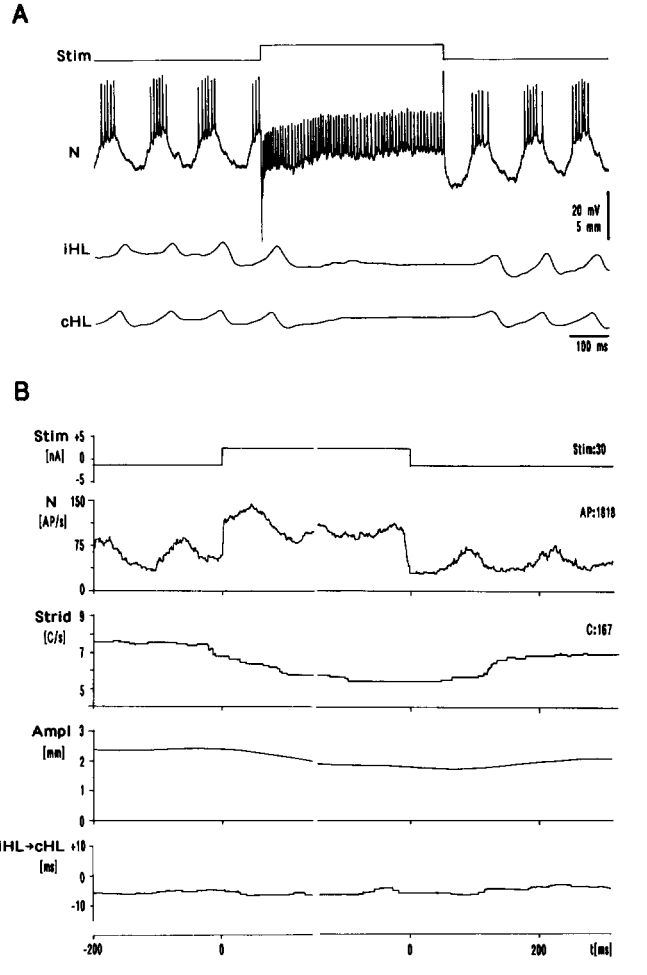

Fig. 8A, B. Intracellular stimulation of T3–AC–1 stopped stridulation for the duration of the stimulus: **A** Specimen record. The interneuron was hyperpolarized by −1.5 nA and repeatedly depolarized with pulses of +2.5 nA of either 470 ms or 290 ms duration; **B** To combine both stimuli durations in one diagram, averaging was performed independently for the beginning and the end of intracellular stimulation. Averages of the motor effects with the intracellularly applied current ($Stim_{[nA]}$), the instantaneous spike frequency of the interneuron ($N_{[AP/s]}$), the repetition rate of stridulation ($Strid_{[C/s]}$), the amplitude of the ipsilateral movement ($Ampl_{[mm]}$) and the relative latency of the hindlegs ($iHL \rightarrow cHL_{[ms]}$) are given

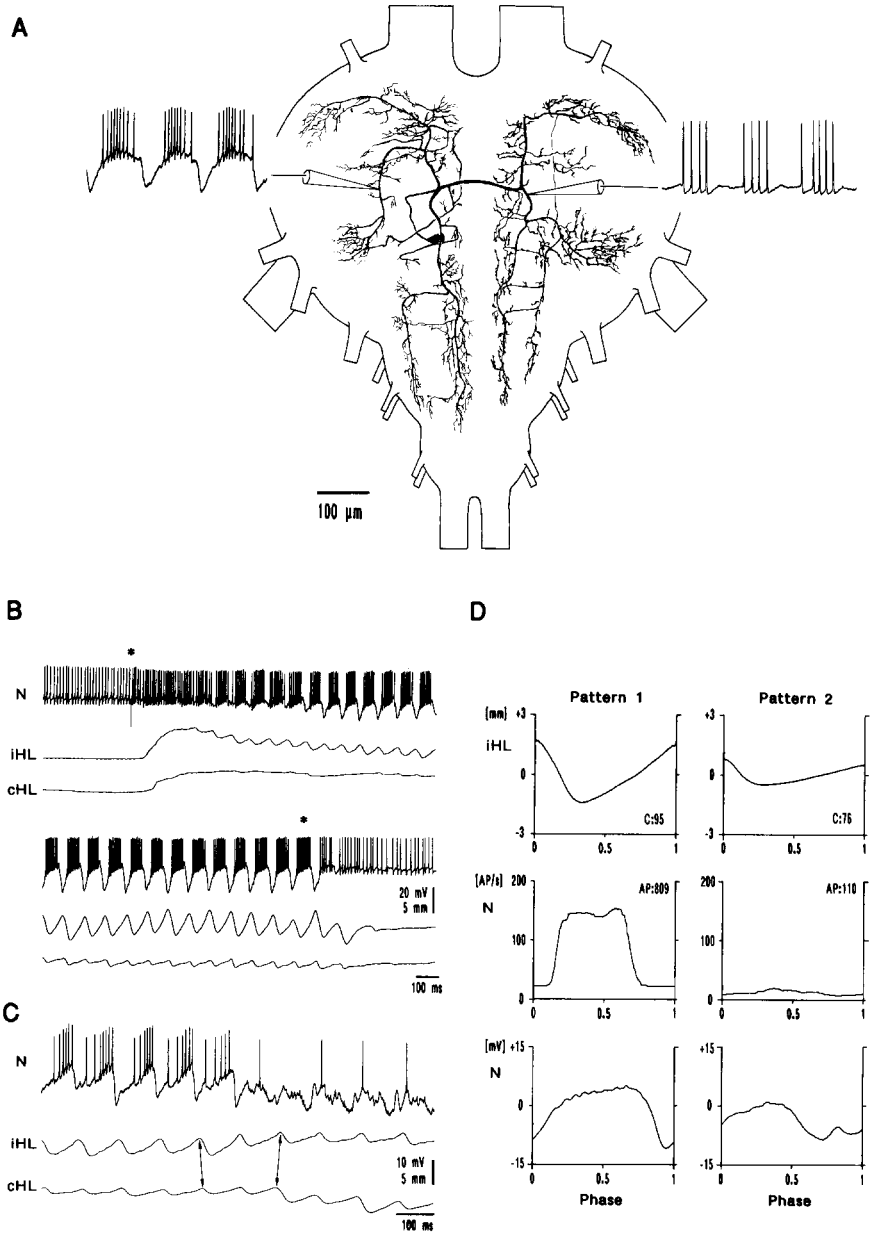

Fig. 9. A Structure of the interneuron T3–LC–4. Soma position is ventral. All ipsilateral arborizations have a smooth appearance and contralateral branches have a varicose structure. EPSPs and spikes were recorded from ipsilateral branches, whereas only spikes were obtained from contralateral arborizations. **B** Interneuron activity at the beginning and end of a stridulation sequence. Onset and offset of brain stimulation are marked by an *asterisk*. **C** Activity of T3–LC–4 during a spontaneous change in coordination. *Arrows* indicate changes in the relative latency of the hindlegs. **D** Phase-corrected averages of the ipsilateral hindleg movement ($iHL_{[mm]}$), the instantaneous spike frequency ($N_{[AP/s]}$) and membrane potential (N_{mV}) during pattern 1 and pattern 2

minent branch with varicose arborizations loops laterally and more ventrally. In the main branch of the neuron synaptic potentials and spikes were obtained, whereas only spikes were recorded in the lateral branch (Fig. 2A), indicating the dendritic and axonal regions of T3–LI–3.

During stridulation rhythmic membrane potential oscillations were seen in T3–LI–3 in phase with the stridulation cycle (Fig. 2B, C). The interneuron was depolarized in downstroke phase and hyperpolarized in the upstroke phase. A mean of 7.9 ± 0.6 AP was elicited during each cycle. On average, the instantaneous frequency was highest (178 AP $\cdot$ s^{-1}) just after the upper reversal point of the leg movement.

In a spontaneously stridulating grasshopper a distinct increase in spike activity of T3–LI–3 was obtained by depolarization with $+2.5$ nA after tonic hyperpolarization with -2 nA (Fig. 3A). The instantaneous spike rate of the neuron was almost zero during hyperpolarization and reached 305 AP $\cdot$ s^{-1} at the beginning of depolarization; thereafter, it decreased to about 150 AP $\cdot$ s^{-1}. Before stimulation the contralateral leg produced pattern 1. It led the ipsilateral leg by 19 ms although it performed a smaller movement amplitude (Fig. 3B). Stimulation of T3–LI–3 had considerable effects on stridulation. The repetition rate increased from 6.8 to 9 C $\cdot$ s^{-1} at the end of the stimulus. Within the first 150 ms the cycle duration decreased from 147 to 120 ms. Thus, the interneuron accelerated the stridulation rhythm. The movement amplitude of both hindlegs initially was slightly decreased but then enhanced. Ipsilaterally it increased by 60%, from 2.7 to 4.3 mm. The relative latency of the leg movements changed by about 23 ms, with the ipsilateral leg now leading the contralateral leg by 4 ms. These effects on relative latency and amplitude indicate that the enhanced activity in T3–LI–3 induced movement pattern 1 in the ipsilateral leg. The effects on the repetition rate

and amplitude gradually increased during the stimulus. The change in coordination was completed within one stridulation cycle and remained almost constant for the whole stimulus (Fig. 3B). After stimulation the effects gradually decreased.

T3–LI–3 sometimes started stridulation if high frequency discharges were elicited at microelectrode penetration. In resting grasshoppers intracellular stimulation of single T3–LI–3 neurons generally had no effect. However, under certain conditions intracellular stimulation initiated stridulation. The behaviour started again with a smaller movement amplitude if the neuron was depolarized just after a stridulation sequence (Fig. 4A). Also, the brain stimulus could be set to a subthreshold level so that stridulation was not started. If the spike rate of the interneuron was then increased to 150 AP · s^{-1} by intracellular current injection stridulation was repeatedly initiated for the duration of the current pulse. During stimulation the ipsilateral leg always performed pattern 1; it moved with a greater amplitude and led the contralateral leg. However, stimulation of T3–LI–3 also elicited small contralateral leg movements which always resembled pattern 2. In both situations (Fig. 4A, B) the activity of T3–LI–3 changed from a tonic discharge pattern to rhythmic membrane potential oscillations in phase with stridulation.

The data demonstrate that T3–LI–3 interneurons can initiate and drive stridulation activity. Therefore, they are clearly associated with the rhythm-generating network. It was not possible to reset stridulation with short depolarizations of a single T3–LI–3 interneuron. Subsequent recordings of different T3–LI–3 neurons in one hemiganglion always revealed similar discharge activity of the interneurons and did not indicate a functional difference between the siblings.

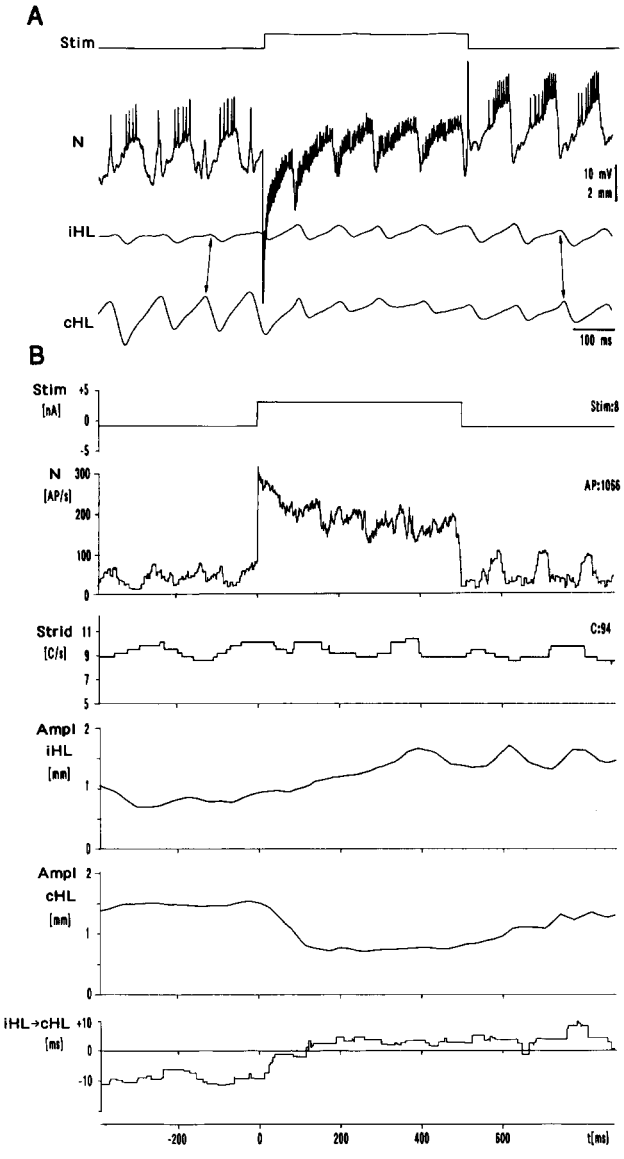

Fig. 10A, B. Intracellular stimulation of T3–LC–4 during ipsilateral stridulation of pattern 2 caused a switching in the coordination of the movement patterns: **A** Specimen record, the change in relative latency is indicated by *arrows*. Stimulus (*Stim*), neuron activity (*N*) and movement of the ipsilateral and the contralateral hindleg (*iHL, cHL*); **B** Averages of the motor effects with the intracellularly applied current ($Stim_{[nA]}$), the instantaneous spike frequency of the interneuron ($N_{[nAP/s]}$), the repetition rate of stridulation ($Strid_{[C/s]}$), the amplitude of the ipsilateral hindleg movement ($Ampl\ iHL_{[mm]}$), the amplitude of the contralateral hindleg movement ($Ampl\ cHL_{[mm]}$) and the relative latency of the hindlegs ($iHL \rightarrow cHL_{[ms]}$). **A** and **B** are aligned to the same time scale

Reset of the stridulation rhythm by interneuron A1–AC–1

The soma of this interneuron has a ventral position in the first abdominal neuromere (Fig. 5A). The primary neurite projects to the dorsal neuropil where the neuron forms three areas of arborization. At the ipsilateral side of the soma smooth as well as varicose branches occur along the long axis of the ganglion. Predominantly varicose arborizations project laterally. At the contralateral side the ascending axon has varicose arborizations parallel to the long axis of the ganglion.

Only one recording was made: the ipsilateral hindleg exhibited pattern 2. During stridulation A1–AC–1 was excited in upstroke phase (Fig. 5B, C). In each cycle 7.2±0.9 APs were elicited with an average highest instantaneous frequency of 136 AP · s^{-1} during the upstroke.

Intracellular stimulation of A1–AC–1 indicated a reset of the stridulation rhythm (Fig. 6A, B). The phase-response diagram shows that stimuli which were delivered during the downstroke (phase values 0–0.3) considerably shortened the cycle, whereas stimuli at other phases had no significant effect on the rhythm. Corresponding with the bilateral structure of the interneuron the motor effects were expressed in both hindlegs simultaneously.

Termination of stridulation by interneuron T3–AC–1

The structure of this interneuron is characterized by a ventral posterior soma location in the metathoracic neuromere and a contralateral ascending axon (Fig. 7A). It

has ipsilateral and contralateral small branches in the dorsal neuropil along the long axis of the ganglion. Most other arborizations project ipsilaterally to the lateral dorsal neuropil.

The neuron was recorded once with movement pattern 2 stridulated by the ipsilateral hindleg. The membrane potential of T3–AC–1 oscillated in phase with stridulation (Fig. 7B, C). The neuron was inhibited at the lower reversal point of the movement. It received an excitatory input which evoked a mean of 9.1 ± 0.8 APs during the upstroke. The mean maximum spike rate reached 160 $AP \cdot s^{-1}$ during the upward movement. There were almost no spikes during the downstroke. The activity peak of T3–AC–1 coincided with the inhibition of T3–LI–3.

Depolarization enhanced spike activity in T3–AC–1 from 68 to 143 $AP \cdot s^{-1}$ (Fig. 8A, B). The obvious effect of T3–AC–1 on stridulation was a reduction in repetition rate and amplitude of stridulation. During intense stridulation only the amplitude was decreased and the repetition rate was not influenced (data not shown). At weak stridulation the neuron completely stopped the leg movements during stimulation. The relative latency of the hindlegs was not influenced. Rhythm generation recovered almost instantaneously at the end of the stimulus. An immediate onset of stridulation of this sort never occurs at the beginning of any sequence and may in this case have been due to the brain stimulus which was continuously driving the cephalic descending activity. Since, in these experiments, stridulation was always elicited by brain stimulation, no data are available on the discharge activity of T3–AC–1 at the end of normal sequences.

Switching of motor patterns by interneuron T3–LC–4

The soma position of this neuron is at the ventral surface in the posterior region of the metathoracic neuromere (Fig. 9A). The arborizations extend almost over the entire ganglion complex but could be absent in A3. The ipsilateral arborizations have a predominantly smooth appearance, whereas the contralateral ones have a varicose structure. Correspondingly, in the ipsilateral branches EPSPs and spikes were recorded and in the contralateral branches only spikes. Thus, in T3–LC–4 input and output regions may be assigned to one hemiganglion, respectively; this is necessary in order to convey information for bilateral coordination. Double stainings demonstrated that at least two T3–LC–4 interneurons originated in each hemiganglion. The interneuron was described as type 4 by Gramoll and Elsner (1987). Data are derived from 21 recordings.

The activity pattern of T3–LC–4 was clearly correlated with the movement pattern of the ipsilateral hindleg (Fig. 9B–D). During pattern 1 the neuron showed membrane potential oscillations with a broad depolarization during the upstroke and a prominent inhibition before the upper reversal point. T3–LC–4 generated 8.5 ± 0.7 APs in each cycle and reached a mean maximum spike rate of 153 $AP \cdot s^{-1}$ during the upward movement (Fig. 9B, D). A spontaneous change in coordination of the movement patterns is shown in Fig. 9C. Simultaneously with the change in relative latency, the activity of the interneuron decreased. During pattern 2 the average maximum excitation occurred at the beginning of the upward movement, and an inhibition for half of the cycle duration followed. The neuron generated only

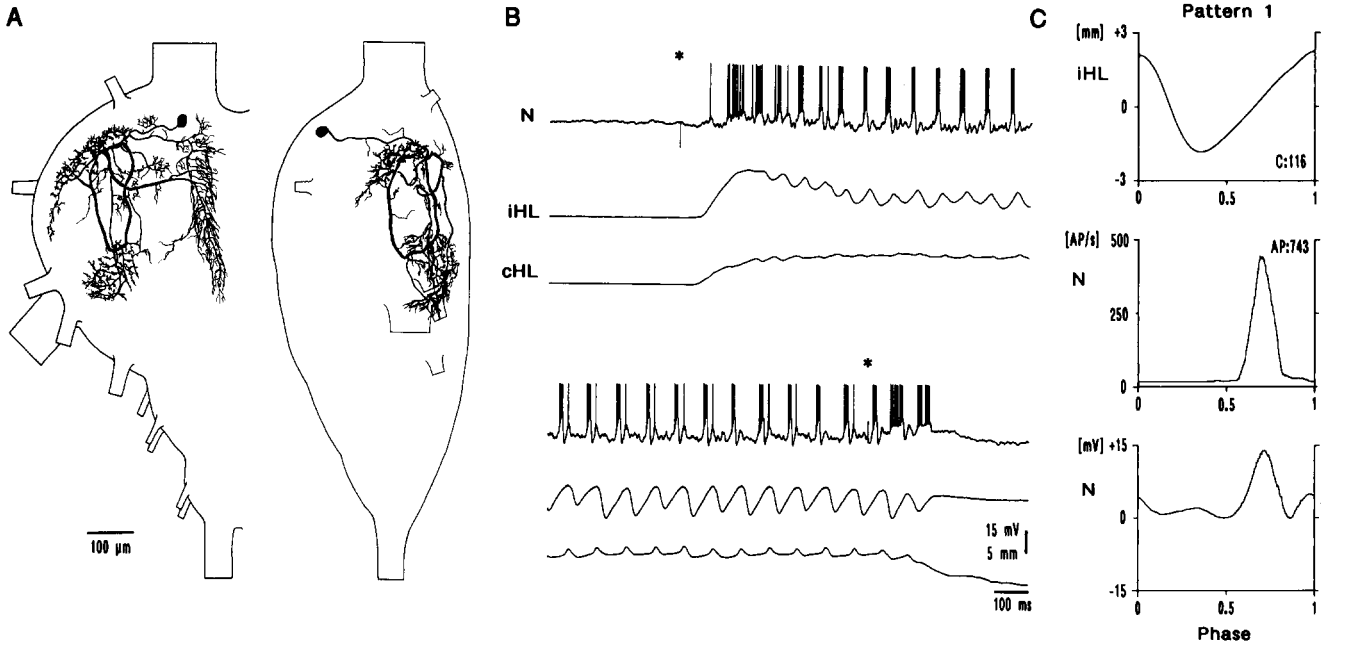

Fig. 11. **A** Dorsal (*left*) and lateral (*right*) view of interneuron T3–LI–1 with an almost circular arrangement of the axon. **B** Interneuron activity at the beginning and end of a stridulation sequence. Onset and offset of brain stimulation are marked by an *asterisk*. **C** Phase-corrected averages of the ipsilateral hindleg movement ($iHL_{[mm]}$), the instantaneous spike frequency ($N_{[AP/s]}$), and the membrane potential ($N_{[mV]}$) of T3–LI–1

1.4 ± 1.6 APs per cycle and reached a mean maximum spike rate of 20 AP · s^{-1} (Fig. 9D). This is an extreme example; the movement patterns were not always so drastically reflected in the activity of T3–LC–4. However, during pattern 1 at least twice as many APs were generated as during pattern 2.

If the activity pattern of T3–LC–4 plays an important causal role in coordination, intracellular depolarization should change the movement patterns of the legs. Since depolarization enhances spike activity a change in coordination could only be expected if the ipsilateral hindleg was performing pattern 2. T3–LC–4 was stimulated with pulses of +3.5 nA, while the ipsilateral hindleg performed pattern 2 during weak stridulation (Fig. 10A, B). During stimulation the interneuron remained rhythmically active with a spike rate of about 200 AP · s^{-1}. The enhanced activity had no effect on the repetition rate of stridulation. However, the amplitude of the ipsilateral and contralateral movements and their relative coordination were distinctly influenced. During stimulation after about 50 ms the relative latency of the movements changed from about −10 ms to 3 ms and the contralateral amplitude was reduced. Furthermore, the ipsilateral amplitude gradually increased. These specific changes indicate a switch in the coordination of the movement patterns. The only difference from a spontaneous change of the movement patterns was the relatively slow increase of the ipsilateral movement amplitude. Stimulation of T3–LC–4 during pattern 1 decreased the contralateral movement amplitude but did not modulate the movement patterns (Hedwig et al. 1990).

Effects of interneuron T3–LI–1 on coordination of movement patterns

Interneurons like T3–LI–1 (Fig. 11A) have been called Type 1 interneurons (Gramoll and Elsner 1987). However, variability in axon structure indicates that they may not be a uniform group. Recordings shown (Figs. 11–13) are from T3–LI–1 neurons with a circular axon structure within the lateral neuropil. The axon first projects posteriorly, branching close to the offspring of Nv 5. In a more ventral lateral position it then runs anteriorly again and ends below the neuropil region where it started. Data are derived from eight recordings.

Interneuron T3–LI–1 had a peculiar discharge pattern. During the middle of the upstroke it exhibited a short burst of activity with an average of 6.4 ± 0.7 APs during the upstroke and a mean maximum instantaneous spike frequency of 439 AP · s^{-1} (Fig. 11B, C). This was the highest spike rate so far encountered among stridulation neurons. The underlying synaptic potential was a brief rapid depolarization which was shut off by a hyperpolarization. The spike pattern of T3–LI–1 changed with the movement pattern. During pattern 1, but not during pattern 2, the neuron was depolarized again after the inhibition and single spikes were elicited (Fig. 11B, upper and lower records).

Intracellular stimulation of T3–LI–1 with +4 nA during pattern 1 elicited discharge rates of 400–500

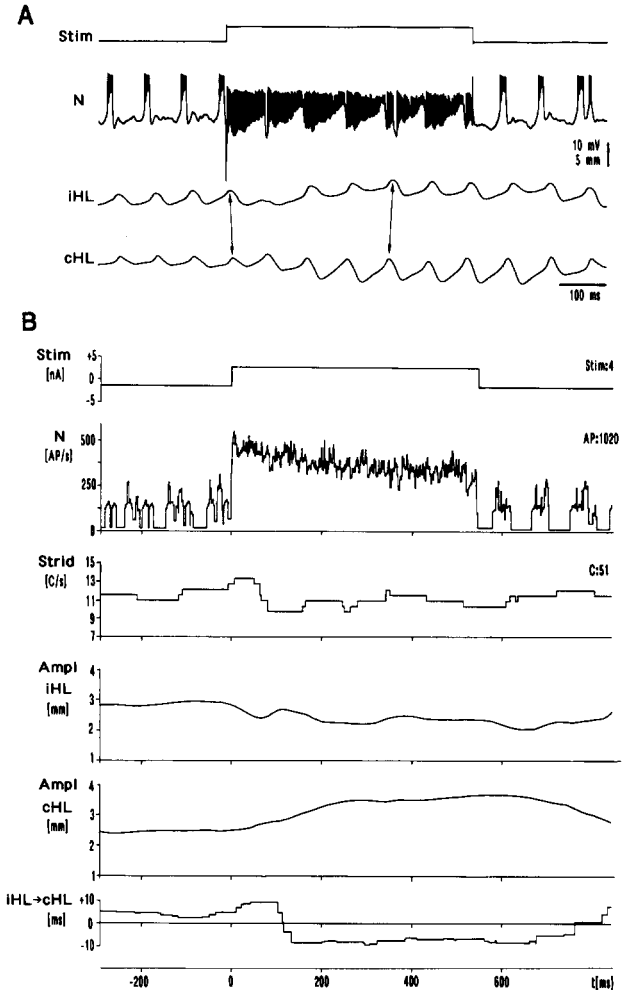

Fig. 12A, B. Intracellular stimulation of T3–LI–1 during ipsilateral stridulation of pattern 1 modulated the amplitude and coordination of stridulation: **A** Specimen record, *arrows* indicate the change in relative latency of the leg movements; **B** Averages of the motor effects with the intracellularly applied current ($Stim_{[nA]}$), the instantaneous spike frequency of the interneuron ($N_{[AP/s]}$), the repetition rate of stridulation ($Strid_{[C/s]}$), the amplitude of the ipsilateral hindleg ($Ampl\ iHL_{[mm]}$), the amplitude of the contralateral hindleg ($Ampl\ cHL_{[mm]}$) and the relative latency of the hindlegs ($iHL \rightarrow cHL_{[ms]}$). **A** and **B** are aligned to the same time scale

AP · s^{-1} (Fig. 12A, B). The repetition rate of stridulation did not change and was only transiently influenced at the beginning of stimulation. However, this may be an effect of the decreased movement amplitude in the ipsilateral hindleg, which made the determination of the upper reversal point less exact. During stimulation the ipsilateral movement amplitude gradually decreased by 18% and the contralateral amplitude increased by 50%. About 150 ms after onset of current injection the relative latency of the legs changed by about 14 ms, from the ipsilateral leg leading to the contralateral leg leading. After stimulation the effects on amplitude and relative latency gradually decreased again. Obviously stimulation of T3–LI–1 elicited a change from pattern 1 to pattern 2. However, this change had a delay which was almost 100 ms greater than the changes in coordination caused by T3–LI–3 and T3–LC–4. So far there are no corre-

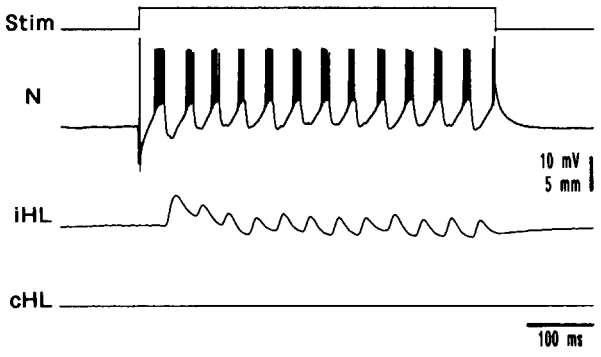

Fig. 13. Tonic intracellular stimulation of T3–LI–1 with +2.5 nA in resting grasshoppers elicited rhythmic high-frequency bursts in T3–LI–1 which were accompanied by leg oscillations

sponding data on the effects of T3–LI–1 on the performance of pattern 2.

Interestingly, stimulation in resting grasshoppers also caused rhythmic activity in T3–LI–1. It elicited bursts of 400–450 AP · s^{-1} which were very similar to those during stridulation (Fig. 13). The interneuron activity was accompanied by rhythmic movements of the ipsilateral leg which corresponded 1:1 to the bursts of the interneuron. However, their form differed from that of normal stridulation movements. There were no movements of the contralateral leg.

Discussion

Intracellular stimulation of interneurons elicited changes in rhythm generation and bilateral coordination in stridulating *O. viridulus*. The repetition rate and the relative latency are two important parameters which characterize stridulation behaviour.

On the significance of single cell stimulation

The efficacy of intracellular stimulation was high if stridulation was performed with low amplitude and reduced repetition rate (Figs. 3, 8, 10). A similar observation was described by Pearson et al. (1985) for the stimulation of flight-initiating interneurons and may not be surprising. During intense behaviour the level of excitation within the network is high and therefore probably less disturbed by changes in single cell activity. The existence of stridulation interneurons with two or three siblings further indicates parallel processing of information. Under these circumstances the significance of single neurons can probably only be demonstrated if the activity in the parallel neurons is not maximal.

On the control of the stridulation rhythm

Intracellular stimulation of single neurons can initiate flight in the locust (Pearson et al. 1985), swimming in the leech (Weeks and Kristan 1978; Weeks 1982; Nusbaum and Kristan 1986) and in *Tritonia* (Getting 1977) and feeding behaviour in *Heliosoma* (Granzow and Kater 1977), *Lymnaea* (Rose and Benjamin 1981; Elliot and Benjamin 1985) and *Pleurobranchaea* (Gillette et al. 1978). Unlike the flight-initiating interneuron 404 (Pearson et al. 1985) which is tonically active, all other interneurons initiating motor activity exhibit a rhythmic modulation of their activity pattern due to feedback from the rhythm-generating network. Some of these interneurons, such as the SO neuron in *Lymnaea* (Rose and Benjamin 1981) and the neurons 204 and 205 in the leech (Weeks 1982), can additionally alter the cycle duration of the motor rhythm. Activity in T3–LI–3 elicited stridulation and increased its repetition rate. Therefore, it functionally corresponds to the interneurons mentioned above. However, there was no direct relation between the spike rate of the interneuron and the cycle time of the leg movement as described in the leech and *Lymnaea*. In *O. viridulus* the stridulation rate continued to increase slowly, even though the spike rate of the stimulated interneuron T3–LI–3 was decreasing due to adaptation to the injected current (Fig. 3). Thus, the decrement in cycle length is not a linear function of the instantaneous frequency of the interneuron.

Stimulation of single T3–LI–3 neurons (Fig. 4) can initiate stridulation, but inhibition of the interneuron does not stop the behaviour (Fig. 3). Thus, the activity of single T3–LI–3 neurons is not necessary for maintenance of stridulation. Since T3–LI–3 neurons initiate stridulation and drive the rhythm generator they have to be considered as a part of the rhythm-generating network.

So far the only interneuron found to reset the stridulation rhythm is A1–AC–1. This interneuron receives its maximum depolarization during the upstroke when T3–LI–3 is inhibited (Fig. 5). Therefore, the two interneurons may influence the rhythm-generating network in different ways. The structure and soma position of interneuron A1–AC–1 emphasizes the importance of the abdominal neuromeres for stridulation.

Interneuron T3–LI–1, which elicited rhythmic leg movements in resting grasshoppers (Fig. 13), may also be of importance for rhythm generation. The bursting activity of the interneuron occurred even without muscle activity (Gramoll 1988) and resembled that during stridulation. Thus, T3–LI–1 is obviously involved in a local circuit which has the ability to generate rhythmic activity. This circuit may be an elementary part of the stridulation rhythm-generating network.

During stridulation T3–LI–3 received inhibition after every burst of activity (Fig. 2). This inhibition may be an elementary process in the stridulation network and determine the cycle duration. Interestingly, interneuron T3–AC–1, which stopped stridulation, exhibited maximum activity in phase with the inhibition of T3–LI–3 (cf. Figs. 2C, 7C). It may therefore be a source for inhibition of T3–LI–3 and in this way also terminate the behaviour. An inhibitory function of the interneuron is supported by its ventral medial soma position. In the locust flight system such a soma position correlates highly with an inhibitory output (Pearson and Robertson 1987).

Bilateral coordination of stridulation movements

During stridulation the hindlegs of *O. viridulus* produce different movement patterns. Bilateral coordination of the movements may change between sequences and occasionally within a sequence (Elsner 1974). After hemisections of the metathoracic ganglion complex coordination of the movements is lost in *Chorthippus biguttulus* and *C. dorsatus* (Ronacher 1989, 1991). Therefore, coordination must be based on connections between the hemiganglionic networks.

The results indicate excitatory and inhibitory interactions between the hemiganglionic networks. Intracellular stimulation of T3–LI–3 in resting grasshoppers not only elicited ipsilateral leg movements but also started less intense stridulation on the contralateral side (Fig. 4). This activation of the contralateral network must be due to an excitatory coupling between the hemiganglionic rhythm generators. Interneurons like A1–AC–1 with bilateral arborizations and bilateral effects on the stridulation rhythm may mediate such excitation.

If T3–LI–3 was stimulated during ipsilateral production of pattern 2, this led to an increased activation of the ipsilateral network. As a consequence pattern 1 was produced ipsilaterally and the contralateral side changed from pattern 1 to pattern 2 (Fig. 3). The hemiganglionic network, which was more active due to intracellular stimulation of T3–LI–3, obviously dominated the contralateral network. Therefore, inhibitory connections between the hemiganglionic rhythm generators are required. Since T3–LI–3 interneurons exhibit only ipsilateral arborizations other interneurons must provide this connection.

T3–LC–4 interneurons have separate input and output regions in both hemiganglia and a discharge activity which correlated highly with the movement patterns (Fig. 9). Therefore, they are well suited for coordinating functions. If the neuron was stimulated during pattern 2 it caused a complete change in coordination of the movement patterns (Fig. 10). Depolarization of the neuron during pattern 1 reduced the contralateral movement amplitude (Hedwig et al. 1990). Thus, T3–LC–4 interneurons probably form inhibitory connections between the hemiganglionic networks. Such mutual inhibition, if not tempered by adaptation, leads to unilateral dominance. Increased activity in the pattern 2-producing network, such as obtained during stimulation of T3–LI–3 (Fig. 3), would increase the inhibition of the contralateral network via T3–LC–4 interneurons. This in turn would also reduce the contralateral inhibition and as a consequence pattern coordination would change. Correspondingly, a reduction of activity in the pattern 1 producing network also would result in a change of coordination. This may have happened during stimulation of T3–LI–1 which changed pattern 2 to pattern 1 (Fig. 12). T3–LI–1 obviously first reduced the activity within the hemiganglion since the ipsilateral movement amplitude decreased. As a consequence a new state of coordination then resulted.

The significance of bilateral neurons such as T3–LC–4 for coordination of the movement patterns is emphazised by mediosagittal incisions which transsect all anterior commissures of the metathoracic neuromere, including the axon of T3–LC–4. They lead to synchronous leg movements in *O. viridulus* (N. Elsner and B. Ronacher personal communication). Since corresponding incisions in *Chorthippus dorsatus* (Ronacher 1991) have no effect, species-specific differences in the organization of the neuronal pathways for coordination are indicated.

Interneurons which function in bilateral synchronization of hemiganglionic pattern generators in the crustacean swimmeret system have been described by Paul and Mulloney (1985). These interneurons influence the swimmeret rhythm on both sides and therefore may have similar properties to the interneuron A1–AC–1. A switching of motor programs can be induced by single-cell stimulation in the swimmeret system of the crayfish (Heitler 1985) and in the ventilatory system of the crab (DiCaprio 1990). In *O. viridulus* changes in bilateral coordination can be induced by at least three different interneurons. The movement patterns of the legs seem to be determined by the different activity levels in the hemiganglionic rhythm generators which are connected via a mutual inhibitory pathway.

The interneurons described here modulated rhythm generation and coordination of stridulation. The following paper will describe interneurons which form the stridulation movement without modulating these parameters (Hedwig 1992).

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References

Dicaprio RA (1990) An interneurone mediating motor programme switching in the ventilatory system of the crab. J Exp Biol 154:517–535

Elliot CJH, Benjamin PR (1985) Interactions of pattern-generating interneurons controlling feeding in *Lymnaea stagnalis*. J Neurophysiol 54:1396–1411

Elsner N (1974) Neuroethology of sound production in gomphocerine grasshoppers (Orthoptera: Acrididae). I. Song patterns and stridulatory movements. J Comp Physiol 88:67–102

Faber A (1953) Laut- und Gebärdensprache bei Insekten: Orthoptera (Geradflügler) Teil 1. Mitt Mus Naturk 287, Stuttgart

Getting PA (1977) Neuronal organisation of escape swimming in *Tritonia*. J Comp Physiol 121:325–342

Gillette R, Kovac MP, Davis WJ (1978) Command neurons in *Pleurobranchaea* receive synaptic feedback from the motor network they excite. Science 199:798–801

Gramoll S (1988) Activity of metathoracic interneurons during stridulation in the acridid grasshopper *Omocestus viridulus* L. J Comp Physiol A 163:813–825

Gramoll S, Elsner N (1987) Morphology of local "stridulation" interneurons in the metathoracic ganglion of the acridid grasshopper *Omocestus viridulus* L. J Comp Neurol 263:593–606

Granzow B, Kater SB (1977) Identified higher-order neurons controlling the feeding motor program of *Helisoma*. Neuroscience 2:1049–1063

Hedwig B (1986a) On the role in stridulation of plurisegmental interneurons of the acridid grasshopper *Omocestus viridulus* L. I. Anatomy and physiology of descending cephalothoracic interneurons. J Comp Physiol A 158:413–427

Hedwig B (1986b) On the role in stridulation of plurisegmental interneurons of the acridid grasshopper *Omocestus viridulus* L.

II. Anatomy and physiology of ascending and T-shaped interneurons. J Comp Physiol A 158:429–444

Hedwig B (1992) On the control of stridulation in the acridid grasshopper *Omocestus viridulus* L. II. Shaping of hindleg movements by spiking and non-spiking interneurons. J Comp Physiol A 171:129–140

Hedwig B, Knepper M (1991) Neurolab, ein Programm zur Analyse neurobiologischer Daten. In: Elsner N, Penzlin H (eds): Synapse Transmission Modulation. Proc 19th Göttingen Neurobiology Conference, p 597

Hedwig B, Gramoll S, Elsner N (1990) Stridulatory interneurons in the metathoracic ganglion of the grasshopper *Omocestus viridulus* L. In: Gribakin FG, Wiese K, Popov AV (eds) Sensory systems and communication in arthropods. Birkhäuser, Basel, pp 183–187

Heitler WJ (1985) Motor programme switching in the crayfish swimmeret system. J Exp Biol 114:521–549

Helversen O von, Elsner N (1977) The stridulatory movements of grasshoppers recorded with an opto-electronic device. J Comp Physiol 122:53–64

Huber F (1959) Auslösung von Bewegungsmustern durch elektrische Reizung des Oberschlundganglions bei Orthopteren (Saltatoria: Gryllidae: Acrididae). Verh Dtsch Zool Ges 52:248–269

Jacobs W (1953) Verhaltensbiologische Studien an Feldheuschrekken. Z Tierpsychol 10 [Suppl 1]

Nusbaum MP, Kristan WB (1986) Swim initiation in the leech by serotonin-containing interneurons, cells 21 and 61. J Exp Biol 122:277–302

Paul DH, Mulloney B (1985) Local interneurons in the swimmeret system of the crayfish. J Comp Physiol A 156:489–502

Pearson KG, Robertson RM (1987) Structure predicts synaptic function of two classes of interneurons in the thoracic ganglia of *Locusta migratoria*. Cell Tissue Res 250:105–114

Pearson KG, Reye DN, Parsons DW, Bicker G (1985) Flight-initiating interneurons in the locust. J Neurophysiol 53:910–925

Robertson RM, Pearson KG (1982) A preparation for the intracellular analysis of neuronal activity during flight in the locust. J Comp Physiol 146:311–320

Ronacher B (1989) Stridulation of acridid grasshoppers after hemisection of thoracic ganglia: evidence for hemiganglionic oscillators. J Comp Physiol A 164:723–736

Ronacher B (1991) Contribution of abdominal commissures in the bilateral coordination of the hindlegs during stridulation in the grasshopper *Chorthippus dorsatus*. J Comp Physiol A 169:191–200

Rose RM, Benjamin PR (1981) Interneuronal control of feeding in the pond snail *Lymnaea stagnalis*. I. Initiation of feeding by a single buccal interneurone. J Exp Biol 92:187–201

Weeks JC (1982) Segmental specialization of a leech swim-initiating interneuron, cell 205. J Neurosci 2:972–985

Weeks JC, Kristan WB (1978) Initiation, maintenance and modulation of swimming in the medicinal leech by the activity of a single neurone. J Exp Biol 77:71–88

Wolf H, Pearson KG (1987) Intracellular recordings from interneurons and motoneurons in intact flying locusts. J Neurosci Meth 21:345–354