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Neuromuscular activity during stridulation in the cricket *Teleogryllus commodus*

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Summary. The central nervous control of singing behavior in male crickets (*Teleogryllus commodus*) has been investigated using electromyograms (EMG) and intracellular recording techniques. A preparation is described in which the species-specific stridulatory behavior can be elicited with the normal chirp and trill pattern. Stridulation was induced routinely in intact crickets as well as in crickets prepared for intracellular analysis (Fig. 1).

1. EMG recordings were taken from opener muscle 99 (subalar) and closer muscle 90 (remotor coxae) of intact singing animals. The intervals between bursts in both opener and closer muscles vary with cycle duration (opener to opener) and maintain their phase constancy. During the chirp the closer muscle activity (burst duration, number of spikes per burst) increases considerably as compared with the trill, but opener muscle activity remains unchanged (Fig. 2).

2. The preparation necessary for the intracellular analysis of motor patterns involved deafferentation of the thoracic ganglia, in which the stridulatory pattern generator resides. This resulted in an increase of the period duration in chirp and trill by about 20% (Fig. 3). Otherwise, only minor changes in the motor pattern were noted after deafferentation except for a marked increase in opener burst duration (Fig. 3), which did not affect the phase relationship described for the intact preparation.

3. The investigation of synaptic input to identified motoneurons 99 (opener) and 90 (closer) revealed differences in the burst patterns in these neurons during the chirp as compared with the trill, but also some common synaptic components (Figs. 6 and 7). This suggests that some interneurons are activated during the chirp and trill, whereas others are only activated during the chirp.

Abbreviations: EMG electromyogram; *MN* motoneuron

Introduction

The stridulatory behavior of male crickets is well suited to investigation of neuronal organization, because it is expressed in a stereotyped manner, the nervous system is readily accessible, and the behavior itself is well understood. The mechanical properties of the wings and their role in sound generation have been described (Koch et al. 1988), and the activity of specific muscles has been correlated with wing movement during sound production (Bentley and Kutsch 1966; Kutsch 1969; Bentley 1971). Sensory feedback from wing hinge and wing surface receptors is important for efficient sound production (Elliott 1983; Elliott and Koch 1983; Schäffner and Koch 1987), although pattern generation itself appears to be independent of this feedback (Kutsch and Huber 1970; Möss 1971; Schäffner and Koch 1987). Electrical stimulation of selected parts of the central nervous system such as the brain (Huber 1960), the cervical connectives (Otto 1971) and single fibres within these connectives (Bentley 1977) showed that the neural circuitry generating the song pattern is located in the thoracic nervous system (Kutsch and Otto 1972), whereas the brain orchestrates the different song patterns (Huber 1960; Otto 1971; Bentley 1977). Bentley (1969) has described the activity of motoneurons and interneurons with the meso- and metathoracic ganglia during stridulation in *Gryllus campestris*.

Muscular and motoneuronal activity during cricket stridulation poses several questions relevant to the understanding of motor pattern generation. Different species of crickets have evolved different calling songs, in order to attract only conspecific females. What are the properties of the song pattern generator in each case and what are the physiological factors responsible for the species-specific differences in the song? Some species of the same

genus (*Teleogryllus*) produce calling songs which contain two different rhythms (Bentley 1971). Are two pattern generators used during the expression of one behavior?

The first step in an attempt to answer these questions involves a description of the muscular and motoneuronal activity during stridulation. The cricket *Teleogryllus commodus* was chosen for two reasons: firstly, the *T. commodus* calling song contains two rhythms: the chirp and the trill. During the latter the syllables are produced at double the repetition rate of those in the chirp (Bentley 1971). Secondly, most studies on muscular and motoneuronal activity during cricket stridulation have focussed on *G. campestris*, which has a distinctly different stridulatory pattern from *T. commodus*. Thus a comparison between the neuronal control of the calling songs of the two species might reveal common principles in the neuromuscular control of muscular and motoneuronal activity during cricket stridulation.

In order to induce stridulation routinely in intact as well as in dissected animals, a method recently developed to elicit stridulation in grasshoppers (Hedwig 1986) was modified for the cricket. This technique involved applying DC-current to the brain and proved to be superior in reliability to the focal lesions and pulsed trains of electrical stimuli used previously in eliciting stridulation in male crickets (brain stimulation: Huber 1960; connective stimulation: Otto 1971; Bentley 1977). In this paper the pattern of discharge of opener and closer muscles during stridulation in *T. commodus* is first described quantitatively, and this is followed by a description of the intracellular activity of some of the motoneurons active during stridulation. Motoneurons innervating opener muscle 99 (subalar) and closer muscle 90 (remotor coxae) were chosen for investigation because their activity has been used previously to describe the stridulatory motor pattern in *G. campestris*. The investigation of synaptic input to closer and opener motoneurons in this present study indicated that some premotor interneurons were activated during the chirp and the trill, whereas others were active only during the chirp.

Materials and methods

Animals. Experiments were performed on adult male crickets, *Teleogryllus commodus* (Walker), from 1 to 5 weeks post-ecdysis. Crickets were either caught in the field near Canberra, Australia, or taken from a breeding colony not more than two generations old, originating from the same area.

Eliciting stridulation. All legs were removed and the crickets were restrained, dorsal side up, by pinning the head capsule onto a plasticine cushion supported by foam. The anterior head capsule, including the antennae, the clypeus, and the labrum, was removed to expose the brain (Fig. 1B, C). These were the only dissections on the otherwise intact animal. A suction electrode, consisting of a plastic tube mounted onto the front of a shortened syringe needle and filled with saline, was applied to the ventral part of the brain, just anterior to the circumoesophageal connectives (Fig. 1B). The inner diameter of the suction electrode was approximately 0.5 mm. Electrodes of smaller diameter elicited stridulation less readily. The indifferent electrode was placed approximately 0.5 mm posterior to the suction electrode.

To elicit stridulation a negative DC current of 20–40 μA (generated from an 18 V battery) was applied to the brain. As soon as current was applied the animal showed an increase in ventilatory and other motor activity and often struggled. Within 1–5 min the animals usually began to stridulate, sometimes continually for up to 3 h. At least a short period of singing was induced in every cricket stimulated in this way. The current passed evoked a range of motor patterns (respiratory activity, flight, vigorous leg movements) and it was then necessary to adjust the current appropriately to maintain stridulation. Occasionally this involved reversing the polarity of the current. Depending upon electrode position and the current applied, the 3 song patterns which *T. commodus* males normally produce (i.e. calling song, aggressive song, and courtship song) could all be induced. This study concentrates exclusively on the calling song pattern. All experiments were performed at room temperature ($23 \pm 1°$ C).

Electromyograms. Once the cricket stridulated continually, electrodes (50 μm silver wire, insulated except at the tip) were inserted into the posterior mesothoracic subalar muscle 99 (opener) and the remotor coxae muscle 90 (closer). Recordings from these electrodes were then amplified, displayed on an oscilloscope, and stored on magnetic tape for later analysis. Recordings of stridulatory sounds were made with a microphone placed just above the animal and then preamplified with a Sony recorder (WM-D6). The position of the EMG wires was confirmed by dissection following each experiment. The EMG recordings were analyzed using a PDP-11 computer after first passing them through a trigger circuit.

Preparation for intracellular recordings. Stridulation was induced as described above and maintained throughout the dissection procedure. The wings and the posterior part of the pronotum were removed. The abdomen and thorax were opened via a dorsal midline incision. The meso- and metathoracic ganglia were exposed by removing the gut and overlying tissue (Fig. 1C; see also Robertson 1987), and then stabilized on a Nichrome platform. Particular care was taken not to damage or stretch the abdominal connectives, since eliciting stridulation then proved much more difficult and often impossible. All sensory and motor nerves to the thoracic ganglia were cut so as to reduce body movements and to obtain a preparation in which the thoracic nervous system generating the motor pattern was as completely deafferented as possible. This operation had some effect on the EMG and central neuronal recordings, most likely due to removal of sensory feedback from proprioceptors in the wingstumps and on the wing surface. Sensory input from the cerci (Dambach et al. 1983) was probably also lacking under these circumstances, because no wing movement or muscular contractions were present.

To monitor ongoing stridulation, usually nerve 3A3 in the mesothoracic ganglion (which innervates the anterior closer muscle 89 (Fig. 1C)) was lifted onto a 75 μm silver wire hook electrode and insulated from body fluids with a mixture of vaseline and mineral oil. Sometimes nerve 3A was used as a

monitor and those recordings then contained background activity of motoneurons innervating muscles other than muscle 89 as seen in some of the recordings (see Kutsch and Huber 1970 for details of innervation and the nomenclature used in this study). The preparation was continuously flooded with TES-buffered saline (in m*M*: NaCl 128, KCl 1, $CaCl_2$ 2, $NaHCO_3$ 2, TES 4).

Glass capillary micropipettes pulled to a resistance of 40–60 MΩ (when filled with 1 *M* potassium acetate) were backfilled with a 3% solution of Lucifer Yellow in 0.5 *M* lithium chloride for intracellular staining. Motoneurons 90 and 99 were penetrated in their neuropilar segments and intracellular activity was recorded using standard techniques and stored on magnetic tape. Recorded motoneurons were then stained by applying constant hyperpolarizing current (5–10 nA) for 5–15 min. The ganglion was dissected from the preparation, fixed in 3% phosphate buffered (pH = 7.2) formaldehyde, dehydrated, cleared, and viewed and drawn as a wholemount under a fluorescence microscope. The intracellular recordings presented in this study are representative of those obtained for each motoneuron type in more than 20 preparations.

Results

Eliciting stridulation with electrical DC-stimulation of the brain

Stridulation in *T. commodus* consists of a repetition of phrases (Fig. 1 A), each phrase usually commencing with a short chirp (4–6 syllables at about 20 Hz) and ending with one to three trills (10 to 30 syllables at about 35 Hz). Stridulation was most successfully induced by locating the suction electrode on the ventral side of the brain just anterior to the circumoesophageal connectives (Fig. 1 B), and then applying constant, negative DC-current (Fig. 1 C). The stridulatory behavior evoked appeared normal in that the forewings were raised to the normal angle, and each syllable in the typical calling song was produced by closing movements of the forewings. The stridulatory pattern evoked by electrical stimulation clearly shows the same phrase structure as above, but the chirp was often extended (>10 syllables) and the trill drastically shortened (Fig. 2 A). Male crickets produce such a song pattern normally during a warm-up period before a continuous song can be observed. Sometimes the stridulation elicited was indistinguishable from normal stridulation in having short chirps and long trills. There appeared to be no significant difference between the period of sound pulses in normal and electrically induced stridulation (chirp: 51.1 ± 3.3 ms and 51.8 ± 4.3 ms respectively; trill: 31.4 ± 1.1 ms and 29.9 ± 1.2 ms respectively; from 5 animals in each group).

Neuromuscular activity during stridulation

EMG recordings in intact singing crickets were taken from muscle 99 (opener) and muscle 90 (closer) together with sound recordings. The sound

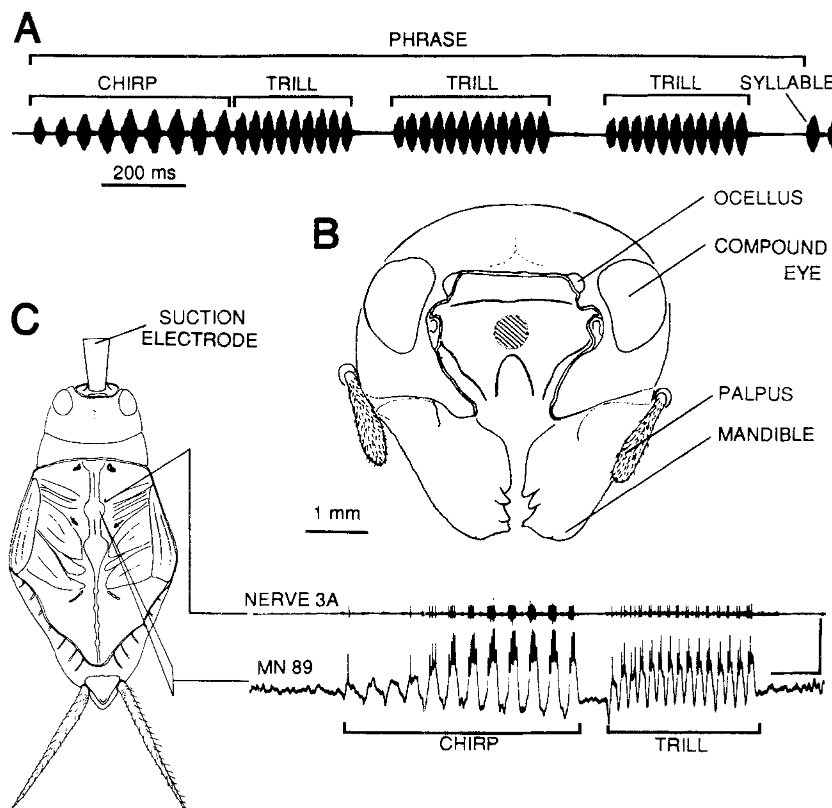

Fig. 1 A–C. Cricket song structure and the experimental arrangement for electrophysiological stimulation and recording. **A** Calling song of an unrestrained voluntarily singing cricket. The two different rhythms (chirp, trill) are indicated and together they form a phrase which is continuously repeated.
B The ventral surface of the brain as seen from the animal's anterior. The placement and the size of the inner diameter of the current delivering suction electrode is indicated by the shaded area.
C Stridulation in intact (not shown) and dissected cricket males was induced by passing a negative DC current (~20–40 μA) and intracellular recordings from motoneurons during stridulation were made from the mesothoracic ganglion. A hook electrode placed under nerve 3A, which innervates closer muscle 89 was used as a monitor for ongoing stridulation. The motoneuronal recording, from a motoneuron 89, shows an electrically induced chirp/trill pattern. Calibration: Vertical 20 mV; horizontal 100 ms

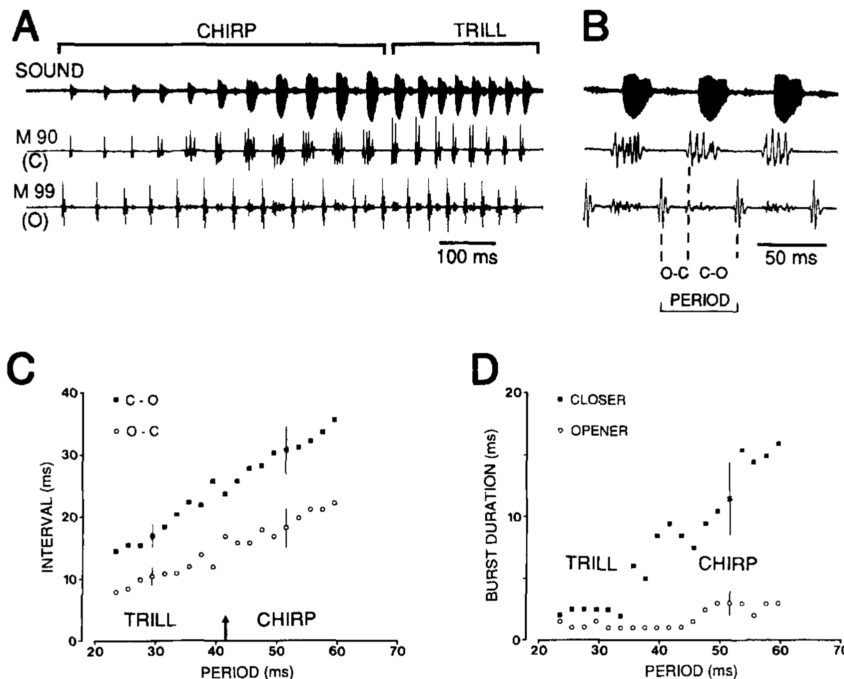

Fig. 2A–D. Electromyogram analysis of the stridulatory pattern produced by an intact singing cricket male.
A Sound pattern of the *T. commodus* calling song and the underlying activity of closer muscle 90 (upper trace) and opener muscle 99 (lower trace) induced by electrical stimulation. Only the closing wing stroke produces a sound pulse. Stridulation begins with an opener burst and ends with a closer burst.
B Indication of the parameters analysed from EMG recordings. Period is measured from opener to opener burst. The intervals were measured from the beginning of a burst to the beginning of the antagonistic burst. *O*, Opener; *C*, Closer.
C Both intervals (O–C, C–O) vary with changing period durations. Arrow indicates period durations occurring at the transition from chirp to trill.
D Opener burst duration changes only little with varying periods, whereas closer burst duration increases during the chirp

pulses reflect the initially increasing excitation seen in closer bursts during the chirp (Fig. 2A). In order to characterize the pattern of activity in these muscles during stridulation the intervals between the opener burst and the closer burst and vice versa were measured as indicated in Fig. 2B. Both intervals vary with period duration (Fig. 2C). The opener to closer interval is always the shorter interval and thus the period is unevenly divided. Both intervals occur at approximately constant phases within the stridulatory cycle (opener to closer: 0.37 ± 0.06; closer to opener: 0.63 ± 0.08). A phrase always starts with an opener burst and ends with a closer burst (Fig. 2A). Measuring the burst durations reveals that the opener burst is very short (often consisting of only a single spike) in trill and chirp (Fig. 2A, D). By contrast the closer burst duration increases strongly in the chirp at longer periods (Fig. 2B, D). This increase in burst duration during the chirp is similar to the one observed for the closer to opener interval (Fig. 3C), which results in an approximately constant interval from the end of the closer burst to the beginning of the opener burst (18.7 ± 3.9 ms).

Dissection of the cricket for intracellular analysis resulted in removal of peripheral feedback from thoracic proprioceptors and the cerci to the pattern generator. In order to assess the impact of this deafferentation on the motor output, the period, interval, and burst durations of activity in opener and closer muscles were determined. Intracellular recordings of opener motoneuron 99 were compared with extracellular recordings from the motoneurons innervating closer muscle 89 (Fig. 3A), because the timing of excitatory input to closer muscles 89 (recorded extracellularly) and 90 (recorded intracellularly; Fig. 3B) is identical. The data from these recordings can then be directly compared with the recordings from muscles 90 and 99 in intact animals (Fig. 2). After deafferentation, the period shifts towards longer durations during chirp and trill (by ca. 20%), as compared with EMG recordings from intact animals (Fig. 3C). The intervals still vary with the changes in period duration, the opener to closer interval remaining the shorter interval (Fig. 3D). Again both intervals occur at approximately constant phases within the cycle (opener to closer: 0.37 ± 0.09; closer to opener: 0.63 ± 0.11). The closer burst duration also shows little change upon deafferentation, but opener bursts increase dramatically during a chirp (cf. Figs. 2B, 3A), something not observed in intact animals (Fig. 2A, D). Measuring the interval from the end of the opener burst to the beginning of the closer burst revealed constant values (O_L–C: 8.2 ± 2.2 ms) independent of variations in period (Fig. 3E), and irrespective of whether O_L–C occurs during the chirp or the trill. The transition phase from chirp to trill was also compared in intact and deafferented animals. Measuring the periods just prior to and after the chirp/trill transition shows that there is a fast, but not instantaneous, shift

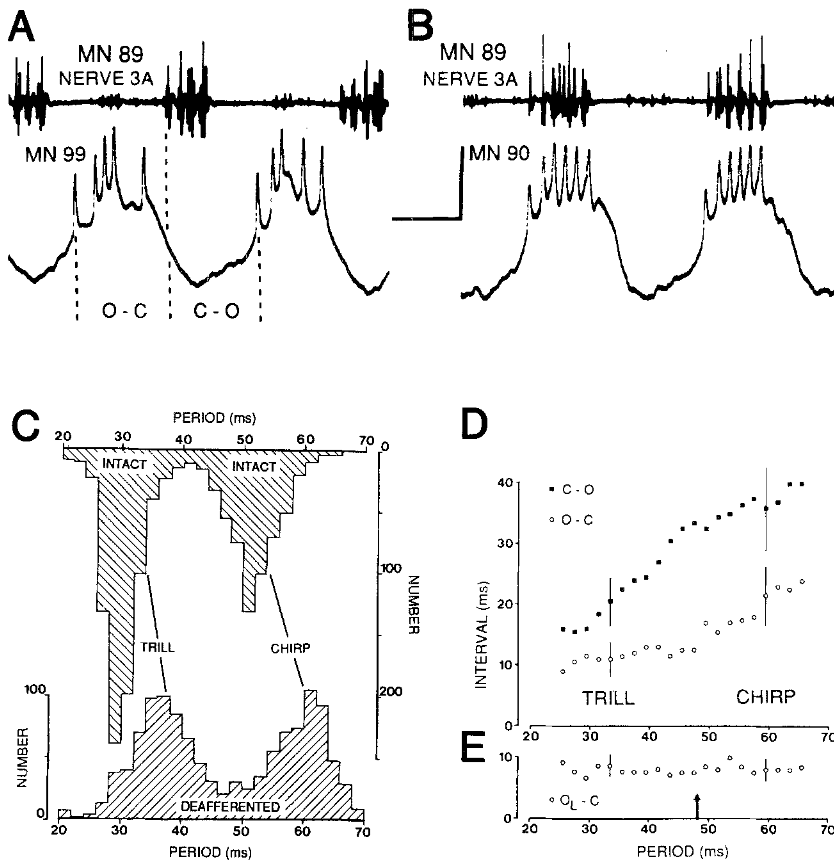

Fig. 3A–E. Stridulation after deafferentation of the thoracic nerve cord. **A** Measurements were taken from recordings from nerve 3A which innervates closer muscle 89 and intracellular recordings from opener MN 99.
B Closer MN 90 and closer muscle 89 are activated simultaneously thus allowing a comparison with data from intact animals (see Fig. 2). Calibration for A, B: Vertical 15 mV, horizontal 25 ms.
C Deafferentation causes an increase in period duration by ca. 20% (upper histogram: intact; lower histogram: deafferented).
D Both intervals vary with period.
E Measuring from the last spike of the opener burst to the beginning of the closer burst (O_L–C) reveals intervals of constant value irrespective of period. Arrow indicates period durations occurring at the transition from chirp to trill

in repetition rate (Fig. 4). Although the durations of cycle periods in intact and deafferented animals differ (Fig. 3C) the pattern of change occurring at the chirp/trill transition is very similar in both conditions (Fig. 4). Extrapolating the chirp period revealed no apparent phase preference for the commencement of trill bursts in neither intact nor deafferented animals (not shown).

Structure of motoneurons

Only the pattern of synaptic input to motoneurons innervating the opener muscle 99 and its direct antagonist, closer muscle 90, has been investigated in this study. The structure of these motoneurons has been described previously for *G. campestris* (Elliott 1983) and *Teleogryllus oceanicus* (Wang and Robertson 1989) and as their structures are very similar, will only briefly be outlined for *T. commodus* (Fig. 5).

The cell bodies of the two motoneurons innervating muscle 99 (MN 99) lie ventrally at the posterior edge of the ganglion (Fig. 5A). The two neurons are very similar in structure, and as there are no consistent distinguishing features they are here

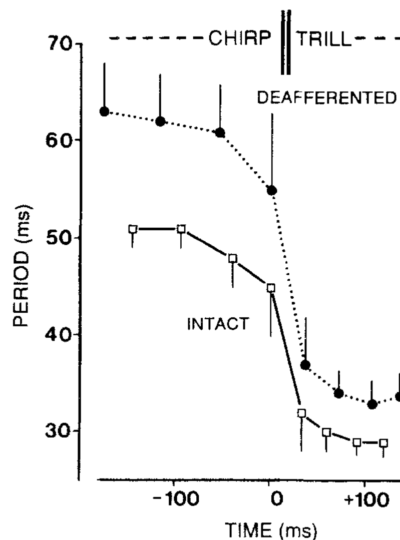

Fig. 4. Changes in period observed during transitions between chirp and trill in intact and deafferented animals. In order to align the transition from chirp to trill in intact and deafferented preparations the period measured from the last chirp burst was given the value zero in time. The transition from chirp to trill is fast but not instantaneous. In intact singing males as well as in dissected animals the last chirp interval is shorter than the previous ones and the first trill interval is longer than the following ones. Data from 5 animals in each group

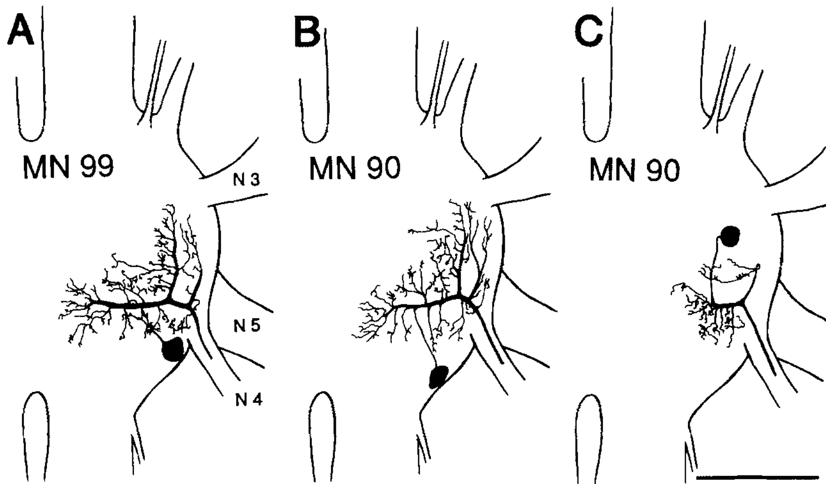

Fig. 5A–C. Drawings showing the morphology of mesothoracic MNs 90 and 99 as seen in wholemount following intracellular staining with Lucifer Yellow.
A Subalar muscle 99 is innervated by 2 MNs, which appeared morphologically indistinguishable. Nerve roots N3, N4, and N5 are indicated.
B, C Remotor coxae muscle 90 is innervated by 5 MNs, 4 with a posterior (**B**), one with a more anterior cell body (**C**). Scale: 200 μm

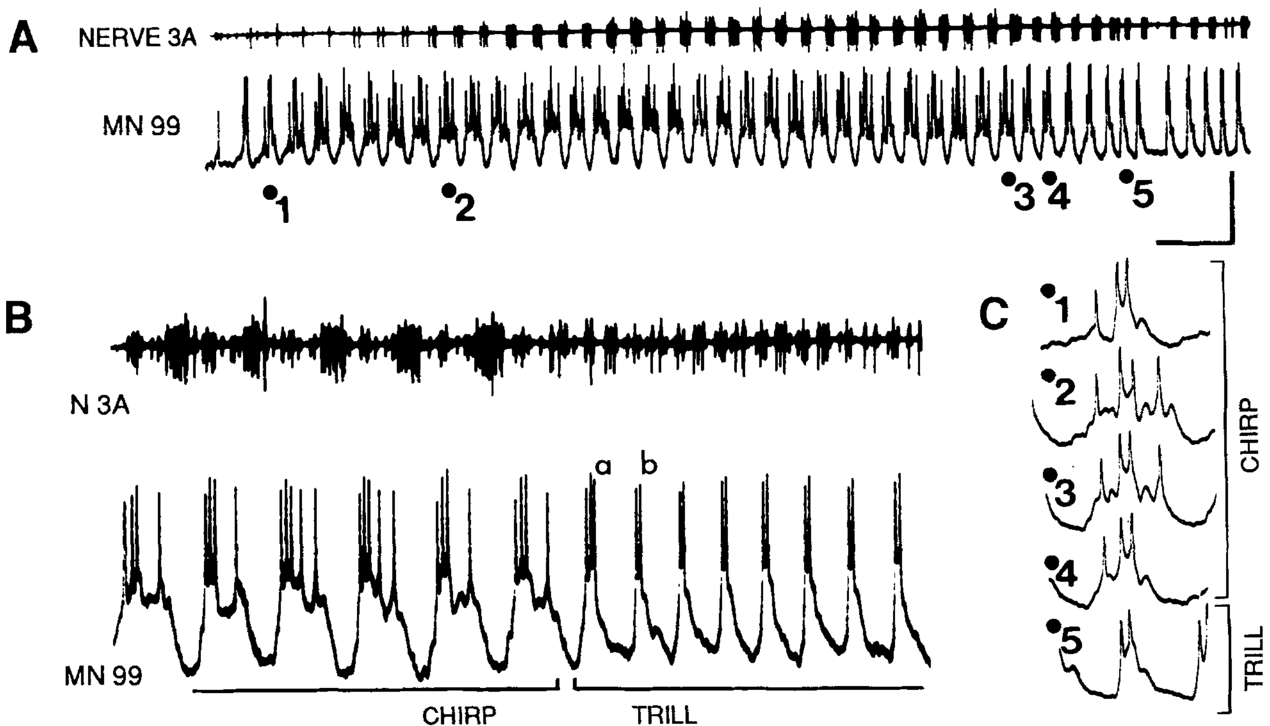

Fig. 6A–C. Intracellular recordings from opener motoneuron 99 during electrically induced stridulatory activity.
A Complete stridulatory phrase. The extracellular recording of nerve 3A monitors activity of closer muscle 89 (see Fig. 1C). The chirp shows a typical fast depolarization which supports a variable number of spikes.
B Expanded section of a chirp/trill transition in a different preparation. During the transition the late depolarization disappears first (see first trill burst (a)), before the excitation generating the first spike in this example disappears (see second trill burst (b)).
C Aligning several chirp bursts above a trill burst taken from the recording in **A** (the numbers indicate the respective bursts) shows the additional synaptic input which distinguishes chirp from trill bursts. Calibration: Vertical **A, C** 20 mV; **B** 10 mV; horizontal **A** 200 ms; **B** 65 ms; **C** 40 ms

discussed as a single motoneuron. MN 99 has extensive arborizations into the posterior half of the ganglion and there are also strong branches in the anterior area of the ganglion. The axon leaves the mesothoracic ganglion via nerve 4D to innervate muscle 99.

Muscle 90 (closer) is innervated by 5 motoneurons which can be grouped into two types (Fig. 5B, C; Elliott 1983; Wang and Robertson 1989). One type appears to be indistinguishable on purely morphological grounds from MN 99 described above (Fig. 5B). The other type (Fig. 5C) has an anterolaterally located cell body and dorsal arborizations which overlap with the branches of the

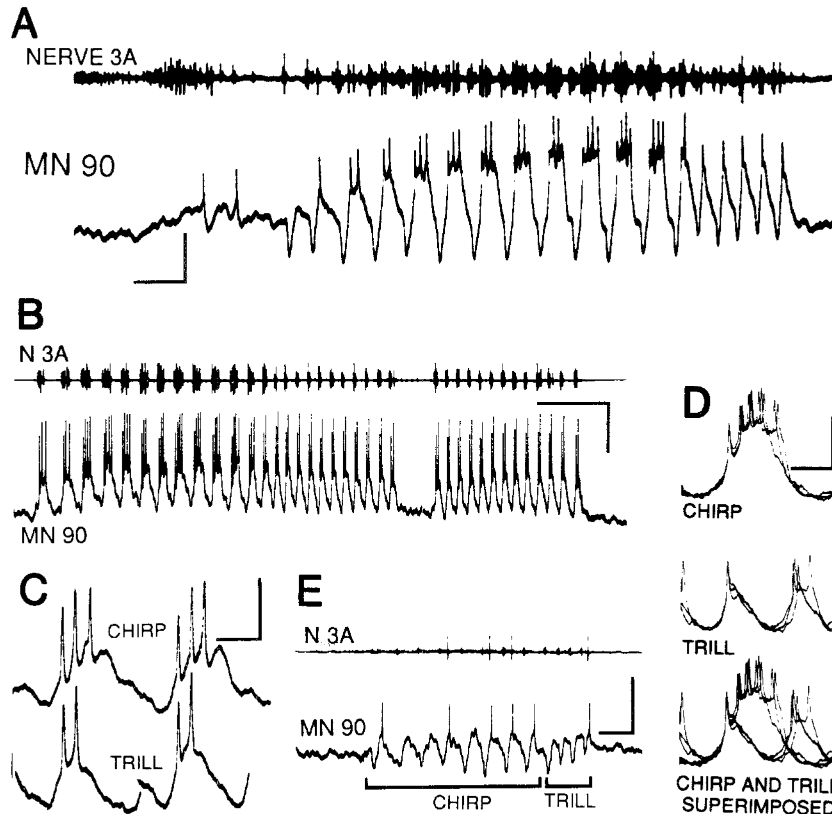

Fig. 7A–E. Activity of closer motoneuron 90 recorded intracellularly during electrically induced stridulatory activity. **A, B** Sequence of a complete stridulatory phrase. The extracellular recording of nerve 3A monitors closer activity of muscle 89 (see Fig. 1C). Note the variation in stridulatory pattern and amplitude of synaptic events between these two examples. **C** Enlarged chirp and trill bursts illustrate the synaptic input (from **B**). **D** Superimposing chirp and trill sections illustrates the common pattern of synaptic input to this MN during chirp and trill. **E** Excitation in closer MNs is not necessary for switching from chirp to trill. Calibration: Vertical **A, B, C** 10 mV; **D, E** 15 mV; horizontal **A, E** 100 ms; **B** 200 ms; **C** 20 ms; **D** 25 ms

other type of MN 90. There are also ventral ramifications. The axons of all motoneurons 90 leave the ganglion via nerve 4D. During stridulation no consistent difference in synaptic input to these two types of MN 90 was noted.

Patterns of synaptic input to motoneurons during stridulation

To record the activity of identified motoneurons 99 (opener) and 90 (closer), stridulation was first induced electrically in an *intact* animal. Once stridulation continued regularly the animal was dissected as described above (Fig. 1C). The intracellularly recorded activity in motoneurons reflects the chirp/trill sequences typical of electrically induced singing (Figs. 1C, 2A). A continuous stridulatory motor pattern with brief chirps and long trills occurred only rarely.

The pattern of synaptic input to opener and closer motoneurons was examined to determine whether motoneurons receive different input during the chirp and trill, or whether the pattern of synaptic input during a chirp merely represents a slowed version of that occurring during the trill.

Opener motoneurons. The opening movement of the wings is soundless (Fig. 2A, B). The recordings of intracellular activity of opener motoneurons (Fig. 6A, B) show a broad depolarization during the chirp (10–20 ms) and a rather short (2–8 ms) depolarization during the trill.

A typical chirp burst in opener motoneurons (Fig. 6B, C) consists of a strong, rapid depolarization generating 2, or sometimes 3, spikes (intraburst frequency: ca. 200 Hz) superimposed on a broader excitation which both precedes and follows this fast depolarization, and generates a variable number of spikes (Fig. 6A, B). At the onset of closer activity the repolarization almost returns to the resting potential. During a trill, a rapid, strong depolarization occurs, followed by a fast repolarization (Fig. 6C) which results in short opener bursts.

Although chirp and trill bursts of opener motoneuron 99 reveal quite different time courses of excitation, the fast depolarization may be due to a synaptic input present during both chirp and trill. This is indicated in Fig. 6C where several chirp bursts have been aligned above a trill burst. The chirp bursts just prior to the chirp/trill transition also show a decrease in the broad excitation leaving only the central depolarization to form the trill burst (Fig. 6B).

Closer motoneurons. Only the closing wing stroke generates the sound pulses in an intact animal (Fig. 2A, B). During the chirp the syllables initially

show a progressive increase in amplitude and duration (Fig. 2A). Intracellular recordings made during a chirp (Fig. 7A, B) reveal inhibitory and excitatory input to closer motoneurons. The synaptic input to MN 90 during a chirp (Fig. 7A, C) consists of a repetition of an IPSP followed by a strong excitation. The excitatory input shows a progressive increase and results in an increasing number of up to 6 spikes (intra-burst discharge frequency: ca. 200 Hz). By contrast, during the trill part of the song (Fig. 7A, B, C), burst durations are shorter and show a weaker excitation following the initial fast depolarization (amplitude: chirp: 22 mV; trill: 16 mV; duration: chirp: 30 ms; trill: 16 ms in the example shown (Fig. 6D)). This corresponds to the softer syllables generated during a trill. The IPSPs are also apparent in the trill and are of similar amplitude to that recorded during the chirp (chirp: 6.9 mV; trill: 6.1 mV from Fig. 7A). The stridulatory pattern as well as the amplitude of the synaptic components may vary somewhat between recordings in different preparations (cf. Figs. 7A and 7B).

Superficially the synaptic input to the closer motoneurons appears to be entirely different during the chirp and trill (Fig. 7A, B). However, a comparison of the excitation generating the first spike in each type of burst shows the rising phases to have very similar slopes in the chirp and trill bursts (Fig. 7C). Superimposing several chirp bursts shows the constancy of the synaptic input generating the rising slope of the burst (Fig. 7D). The same can be seen for trill bursts, although only a single spike may result. Superimposing the chirp and trill bursts reveals little variability in the rising slope of all bursts. This indicates that it is mostly the additional excitatory component which distinguishes the chirp and the trill (apart from their different periods) in closer motoneuron activity.

The additional excitation accounts for the increasing number of spikes observed in the response of MN 90 as the chirp progresses, because the duration of the excitation increases. Note that the excitation of closer motoneurons is not a necessary condition for the switching between chirp and trill, since switching occasionally occurred without any strong excitation in the motoneurons (Fig. 7E) and the burst periods under these circumstances were normal.

In conclusion, one aspect of the synaptic input to MN 90 appears to be common to the chirp and the trill. An IPSP is followed by a fast depolarization which generates one spike during both the chirp and trill. This initial depolarization is quickly followed by an even stronger excitation during the chirp but not during the trill (Fig. 7D).

Discussion

Electromyograms and the effects of deafferentation

The calling song of *T. commodus* contains two rhythms with different periods: the chirp, during which pulse duration and amplitude initially increase, and the trill, which is softer and shows a more constant pulse duration and amplitude (Figs. 1A, 2A; Bentley 1971). The EMG patterns recorded in intact singing *T. commodus* (Fig. 2) show that the opener to closer and the closer to opener intervals both vary with changes in period and each interval maintains a characteristic phase during the chirp and the trill. The opener to closer interval is always shorter in duration than the closer to opener interval (Fig. 2C). These results are consistent with the studies on *G. campestris* stridulation (Bentley and Kutsch 1966; Kutsch 1969), although the calling song pattern of this species is entirely different.

Stridulation in *G. campestris* has also been investigated with regard to the role of peripheral feedback in modifying the central motor output (Kutsch and Huber 1970; Möss 1971; Elliott and Koch 1983; Schäffner and Koch 1987). All studies reported only minor changes in the motor pattern after deafferentation. This is also the case for *T. commodus* stridulation (Fig. 3). Although deafferentation slowed the motor pattern expression by about 20% (Fig. 3C) and increased opener burst duration (cf. Figs. 2B, 3A), the general characteristics of the pattern, such as variation of intervals (Figs. 2C and 3D) and the chirp/trill transition, hardly changed (Fig. 4). Deafferentation also revealed a strong coupling of the closer burst to the opener burst (Fig. 3E). These results support Bentley's (1969) report of a slower rhythm in deafferented preparations and the appearance of time locking which he termed 'opener-closer burst couplets'. The observation of minor effects of deafferentation on the stridulatory behavior contrasts with the findings of investigations on other insect motor patterns (locust flight: Pearson and Wolf 1987; stick insect walking: Bässler 1983; cockroach walking: Delcomyn 1984; cockroach ventilation: Farley and Case 1968) where peripheral feedback was shown to significantly modify the centrally generated motor pattern.

Synaptic input to motoneurons

Bentley (1969) presented intracellular recordings from opener and closer motoneurons during stri-

dulation in *G. campestris*. In general his recordings (obtained from second basalar (opener) and promotor coxae (closer) motoneurons) reveal similar synaptic events occurring during stridulation as those reported here for subalar (opener) and remotor coxae (closer) motoneurons in *T. commodus*, although the stridulatory pattern of *G. campestris* does not contain the two different rhythms seen in *T. commodus* (Fig. 1A). The investigation of patterns of synaptic input to opener and closer motoneurons revealed that (1) the same motoneurons show different burst patterns during the chirp and the trill; and (2) there nevertheless appear to be synaptic components which occur during both the chirp and the trill. For opener motoneurons this synaptic component is a short but fast depolarization (Fig. 6), for closer motoneurons it is an IPSP followed by an excitation (Fig. 7). These components can also be observed when the stridulatory pattern becomes irregular and they appear as 'single events' during the recordings. Thus it seems they most likely originate from interneurons which are activated during the chirp and the trill.

However, the chirp rhythm is not simply a slowed version of the trill. The major changes occurring during the expression of the chirp rhythm consist of an accurately timed additional excitation following the initial action potential in the closer motoneuron burst (Fig. 7D). This additional excitation is the basis for the chirp syllables which have a different amplitude and duration from those in the trill (Figs. 1A, 2A). This excitation may reflect the activity of an interneuron which accumulates excitation, possibly only at low repetition rates. This proposed interneuron is most likely part of the closer premotor pathway, rather than the oscillator mostly because these effects are only observed in closer motoneurons. The source of excitation to this interneuron could originate from the oscillator, or even from the closer motoneurons themselves. The neuronal mechanism responsible for switching the motor output from chirp to trill is independent from accumulating excitation fed back from motoneurons (Fig. 7E) or from peripheral receptors (Fig. 4). The occurrence of two such different repetition rates in the *T. commodus* calling song pattern raises the question of how many oscillators generate the stridulatory motor pattern. Such oscillators are likely to contain driver interneurons (which determine the frequency of the oscillator), resetting interneurons, and premotor interneurons (transforming the frequency of the oscillator into the synaptic pattern shaping the motoneuronal discharge; see Grillner 1977 for this concept). Conclusive evidence, however, about the nature of the central oscillations can only come with recordings from identified driver, resetting, and premotor interneurons, provided that they exist as such entities (Selverston 1980).

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