The Journal of Experimental Biology 202, 2203–2216 (1999)
Printed in Great Britain © The Company of Biologists Limited 1999
JEB2061

NEUROCHEMICAL CONTROL OF CRICKET STRIDULATION REVEALED BY PHARMACOLOGICAL MICROINJECTIONS INTO THE BRAIN

B. WENZEL[1] AND B. HEDWIG[2,*]

[1]*Institut für Zoologie und Anthropologie, Berliner Straße 28, D-37073 Göttingen, Germany* and [2]*Department of Zoology, Downing Street, Cambridge CB2 3EJ, UK*

*Author for correspondence (e-mail: bh202@cam.ac.uk)

Accepted 18 May; published on WWW 19 July 1999

Summary

Neuroactive substances were administered into the frontal protocerebrum of tethered male *Gryllus bimaculatus* by pressure injections from microcapillaries. All three types of species-specific song pattern (calling song, rivalry song and courtship song) could be elicited by injection of acetylcholine and cholinergic agonists. Injection of nicotine led to short bouts of calling song that occurred after a short latency. In contrast, muscarine elicited long-lasting stridulation that took longer to develop. The pharmacologically induced song patterns showed transitions from rivalry song to calling song and from calling song to courtship song, which also occur during natural behaviour. Stridulation induced by a cholinergic agonist could be immediately blocked by microinjection of γ-aminobutyric acid (GABA) into the same neuropile sites. Administration of picrotoxin in resting crickets led to enhanced motor activity that incorporated the three different song patterns. We propose that, in the brain of the cricket, acetylcholine and GABA are putative transmitters involved in the control of stridulation. Histological analysis located the stimulation sites to an area between the pedunculus and the α-lobe of the mushroom body in which the command neurons for calling song have dendritic arborizations.

Key words: cricket, *Gryllus bimaculatus*, brain, stridulation, neuropharmacology.

Introduction

Microinjection of neurotransmitters and neuroactive substances into the central nervous system is a powerful neurobiological method for analysing the neurochemical properties of the targeted networks. In comparison with electrical stimulation, this method can be used to activate specific populations of neurons that carry certain ligand-activated ion channels or G-protein-linked receptors in their surface membrane. By choosing different pharmacological agents, the neurochemical bases of excitation and inhibition within a neuronal network can be investigated.

Microinjections of neuroactive substances have been used successfully in different vertebrate and invertebrate species to analyse the neuronal pathways controlling vocalization and sound production. In the squirrel monkey *Saimiri sciureus*, sequences and elements of the vocal repertoire can be elicited by administering neurotransmitters and neuroactive substances into the midbrain vocal pathway (Jürgens and Richter, 1986; Jürgens, 1994). Similarly, midbrain stimulation with neuroactive substances in cats elicits vocalization (Hernandes-Peon et al., 1963; Bandler, 1982; Bandler and Carrive, 1988). In addition to these excitatory effects in squirrel monkeys, injections of inhibitory transmitters block electrically evoked vocalization (Jürgens and Lu, 1993a,b).

Among the insects, sound production is highly developed in crickets, grasshoppers and cicadas. The neurobiological basis of sound production in this group has been best analysed in crickets and grasshoppers (for reviews, see Kutsch and Huber, 1989; Elsner, 1994). Microinjections of putative neurotransmitters into specific neuropile areas of the brain were based on the arborization pattern of the brain neurons controlling grasshopper stridulation (Hedwig, 1994). After administering acetylcholine (ACh), stridulation could be elicited in different species that perform very different species-specific movement patterns (Ocker et al., 1995). Moreover, in the grasshopper *Omocestus viridulus*, the three different stridulatory motor patterns of courtship behaviour could be evoked by injection of ACh and its agonists (Hedwig and Heinrich, 1997). There is strong evidence that acetylcholine and its agonists are the substances most effective in releasing stridulation in grasshoppers (Heinrich et al., 1997). However, stridulation can also be inhibited. Microinjection of the inhibitory transmitters γ-aminobutyric acid (GABA) and glycine during singing immediately blocks stridulatory activity (Heinrich et al., 1998).

In crickets, the central role of the brain in the initiation and inhibition of singing behaviour was demonstrated by Huber (1964). A preliminary study of the pharmacology of cricket stridulation was performed by Otto (1978) in *Gryllus campestris*. Otto (1978) demonstrated the release of singing by

injection of ACh into the brain. The stimulation sites, however, were not characterized in detail in these experiments. Meanwhile, the command neuron controlling calling song stridulation has been identified in the brain of *Gryllus bimaculatus* (Hedwig, 1996). On the basis of the arborization pattern of the command neuron within the protocerebrum, we performed localized microinjections of neuroactive substances into the brain of *G. bimaculatus*. It was our aim to analyse the pharmacological specificity of the control system for singing in the cricket brain in more detail and thus to further our understanding of the neurochemical basis of a complex type of insect behaviour. A short note on these experiments has been published previously (Wenzel et al., 1998).

Materials and methods

Animals were taken from a colony of *Gryllus bimaculatus* (de Geer) maintained at the Department of Zoology at the University of Göttingen. The crickets were kept at 25 °C with a 12 h:12 h L:D photoperiod. Males that had shown singing behaviour in the cages were selected preferentially for experiments. The animals were first immobilized by cooling to 4 °C and then fixed to a block of Plasticine in a normal upright position by fastening all the legs with small metal clamps. The head was glued into a U-shaped holder and immobilized. Experiments were performed at an ambient temperature of 20 °C.

The head capsule was opened frontally by removing a small piece of cuticle, leaving the antennae and ocellar nerves intact. The opening was constantly superfused with saline (Fielden, 1960). Standard single-barrelled borosilicate microcapillaries were produced using a microelectrode puller (David Kopf 700C). Double-barrelled capillaries were made from tethaglass (Clark Electromedical Instruments TGC 200-10) using a Narishige PE-2 puller. The tips of the capillaries were removed under a microscope to obtain outer diameters of approximately 10 μm. Capillaries filled with neuroactive substances (acetylcholine, muscarine, pilocarpine, nicotine, γ-aminobutyric acid or picrotoxin, all from Sigma Aldrich) were positioned in the brain neuropile using a Leica micromanipulator. A picopump (WPI type PV820) was used for pressure injections. The amplitude and duration of pressure pulses were set to administer volumes of 0.01–3.5 nl.

During stridulation, the forewings perform a rotational opening/closing movement. Sound is produced during the closing movement (Koch et al., 1988). As a consequence of the rotational movement, the lateral part of the right wing is lowered during opening and raised during the closing movement. A piece of retroreflecting foil 2 mm in diameter (3M, Scotchlite type 7610) was glued to the lateral wing flap of the right forewing, allowing the up/down movement of the wing to be detected by an optoelectronic camera (von Helversen and Elsner, 1977). The movement recording also monitored the overall elevation of the wing during any other type of behaviour. The sound pattern produced by the wing movements was monitored simultaneously using a piezo-microphone and a custom-designed amplifier.

The wing movements, the sound pattern and the stimulus monitor of the pressure injection system were A/D-sampled on-line (Real Time Devices board, ADA3300) at a sampling rate of 3 kHz per channel using the software Turbolab 4.3 (Bressner Technology) and an IBM-compatible PC. All data were stored using a magneto-optical disk drive (Sony SMO E502) and evaluated using the software NEUROLAB 8.1 (Hedwig and Knepper, 1992; Knepper and Hedwig, 1997). The software supported *inter alia* the evaluation of chirp durations, syllable durations and corresponding repetition rates. All graphical data were finally processed using Corel Draw 4.0 and printed on an HP Laserjet 4.

Localization of injection sites

Stimulation sites within the brain were marked using double-barrelled microcapillaries, which contained ACh or a cholinergic agonist in one barrel and a dye in the other. Pressure pulses could be delivered independently to each barrel. As a marking substance, we used Lucifer-Yellow-coupled dextrans with a molecular mass of 70 kDa (Molecular Probes D-1827). Owing to the large size of the molecules, the dextrans are not taken up by neurons and stay at their release site (Heinrich et al., 1998). Once an effective stimulus site had been found by injection of ACh, the position of the electrode tip was marked by a pressure pulse delivered to the dye-containing barrel of the microcapillary.

Immediately after the experiments, the brains were removed, fixed in paraformaldehyde, dehydrated in ethanol and embedded in polyester wax. Sagittal sections (14 μm) of the tissue were cut using a microtome (Reichert Jung, model 1130/Biocut) and mounted on microscope slides. The sections were viewed, photographed and drawn using a microscope with an epifluorescent illumination (Leica Dialux 20).

Values are presented as means ± S.D.

Results

Pharmacological release of song patterns

Microinjections of ACh or its agonists muscarine, pilocarpine or nicotine into anterior regions of the protocerebrum were tested for their effectiveness in eliciting stridulatory behaviour in *G. bimaculatus.* The injections were successful in 60 % of the experiments (N=100). At appropriate stimulation sites (67 %), stridulation could be elicited several times with successive application of the neuroactive substance. In one-third of the experiments, only a single bout of singing was elicited. All three song types of *G. bimaculatus* could be elicited, and songs were identified by comparing the pharmacologically evoked songs and wing movements with the natural songs (Huber, 1960; Otto, 1971). In the following section, a brief description of the natural song patterns will be given, with which the pharmacologically released song patterns will be compared.

Eliciting calling song stridulation

At the beginning of a calling song, males gradually raise their elytra into the singing position and start stridulation. Initially,

chirps are of low sound amplitude and contain only 1–3 syllables. After a few seconds, the full sound amplitude is reached. Each chirp now consists of 4–5 syllables and each syllable is 15–20 ms long (Fig. 1A). During a chirp, the mean syllable interval increases gradually from 35 to 42 ms. At a temperature of 20 °C, the chirp interval is approximately 350–400 ms. Under natural conditions, calling song activity may last for many minutes and is under circadian control (Loher, 1989).

After injection of ACh (10^{-3} mol l^{-1}) into the anterior region of the brain, the crickets did not immediately respond with stridulation. Some animals first displayed a short-lasting struggling movements of the legs and body, which calmed down within approximately 5 s. Generally, stridulation built up gradually. The mean latency to the start of stridulation was 14.6±24.0 s (*N*=101). The wings were slowly raised, and small movements started with a soft sound. The number of syllables per chirp gradually increased to four. In the example presented in Fig. 1B, singing activity reached its full intensity after 11 s and lasted for another 50 s, with the chirp rate declining towards the end. Calling songs elicited by ACh injection lasted on average 40.2±81.5 s (*N*=101).

A comparison of the chirp intervals, syllable durations and syllable intervals of natural and pharmacologically elicited calling songs was performed for five individuals (Fig. 1C,D). Caged, freely moving males repeated their chirps at intervals of 398.8±117.3 ms (*N*=159). The same males were then used

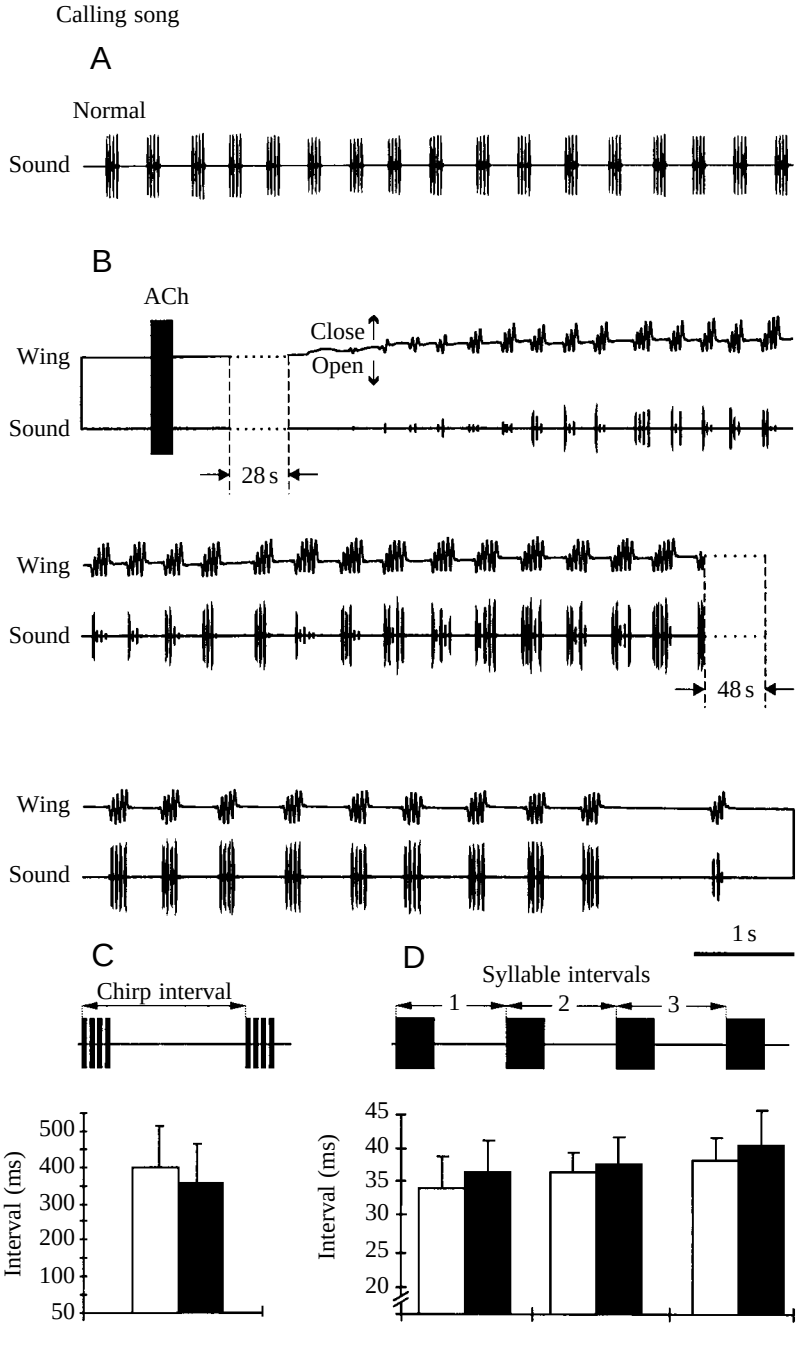

Fig. 1. (A) Section of a continuous normal calling song of *Gryllus bimaculatus* with chirps consisting of four syllables. (B) Continuous sequence of calling song elicited by microinjection of acetylcholine (ACh) into the frontal protocerebrum. The moment of injection is indicated by a filled vertical bar, and interruptions in the presentation are indicated by arrows. Closing movements of the wing correspond to upward deflections of the wing position trace and opening movements to downward deflections. (C) Comparison of the chirp intervals in normal (open columns) and pharmacologically released (filled columns) calling song sequences. (D) Analysis of the syllable intervals of normal and pharmacologically released chirps. Values are means ± S.D. Wing, movement of the right forewing; Sound, sound pattern of stridulation.

for pharmacological experiments. After injection of ACh, the chirp interval was only 355.7±107.0 ms, *N*=117). These values for the mean chirp interval are significantly different (*t*-test, *P*=0.01). A comparison of the syllable intervals revealed that the intervals were slightly longer in pharmacologically released stridulation. Under normal conditions, the first, second and third syllable intervals were 34.4±4.6 ms, 36.9±2.7 ms and 38.7±3.3 ms (*N*=170 for each), whereas after injection of ACh the corresponding values were 36.8±4.3 ms, 38.0±3.7 ms and 41.0±4.9 ms (*N*=122 for each). A comparison of the corresponding mean values for all five animals again showed a significant difference in the syllable interval duration between normal and elicited chirps (*t*-test, *P*=0.01). Despite these differences in the timing of the song parameters, the data and the behavioural performance indicate that the pharmacological stimulation elicited the species-specific motor activity underlying calling song stridulation in *G. bimaculatus*.

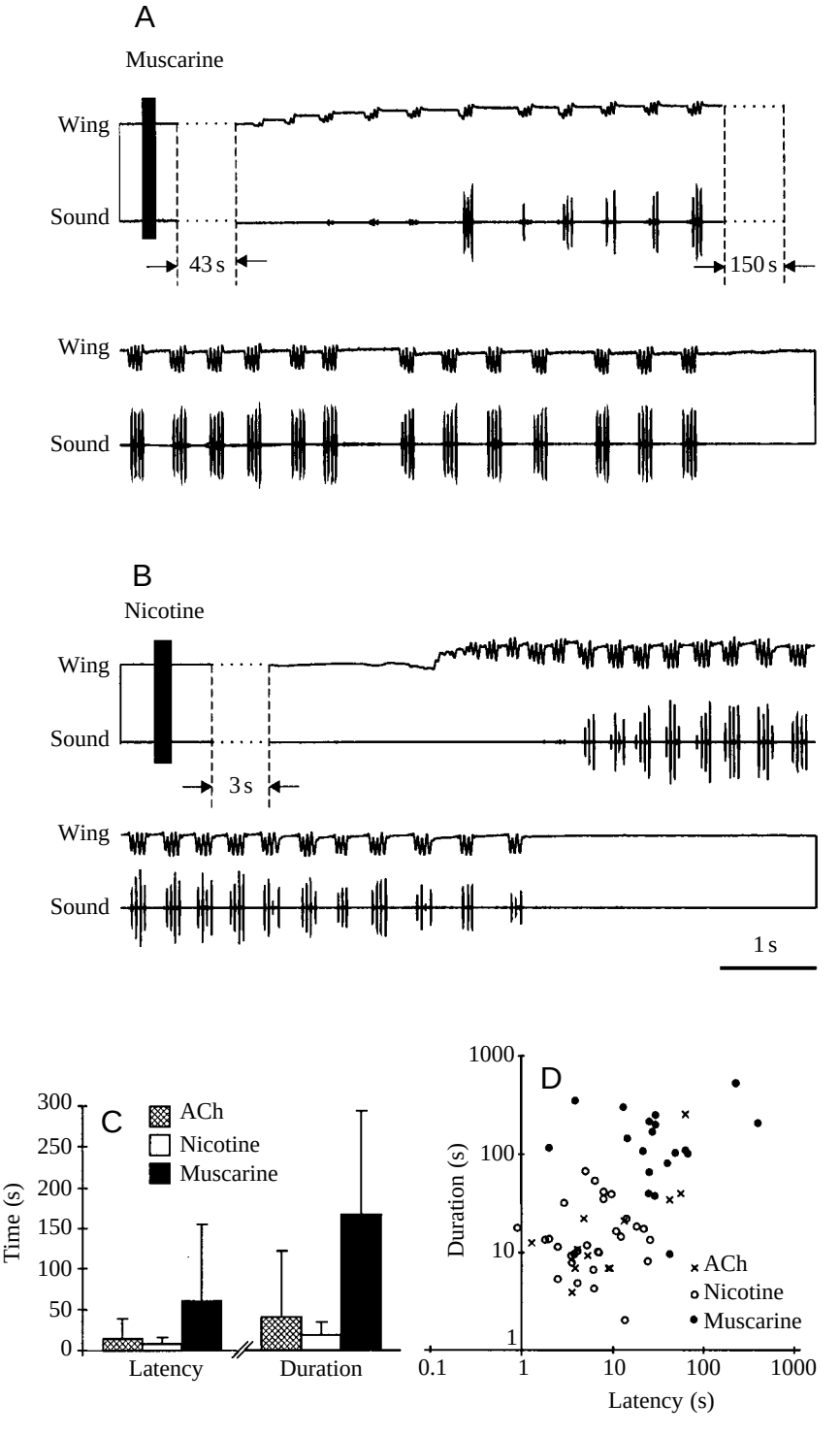

Fig. 2. Comparison of muscarinic- and nicotinic-initiated calling song. (A) Microinjection of muscarine elicited a long countinuous sequence of calling song stridulation after a latency of 43 s. (B) Microinjection of nicotine released a short bout of calling song after a latency of approximately 6 s. (C) Comparison of the latency and duration of calling song sequences elicited by acetylcholine (ACh), nicotine and muscarine. Values are means ± S.D. (D) A scattergram indicating the relationship between latency and duration for calling song sequences released by ACh, nicotine and muscarine. Wing, movement of the right forewing; Sound, sound pattern of stridulation.

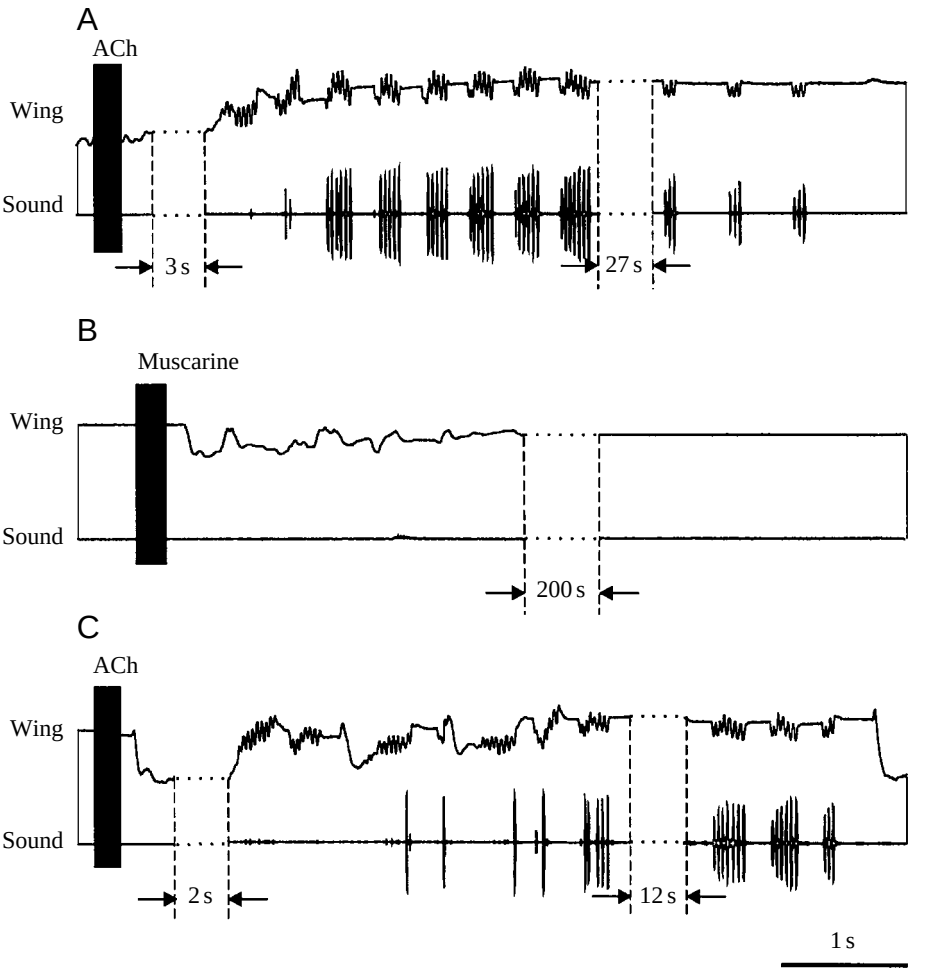

Fig. 3. Effect of acetylcholine (ACh) on the ability of muscarine to elicit calling song. (A) Injection of ACh elicits a typical calling song sequence. (B) Injection of muscarine at the same site increased motor activity slightly but did not initiate calling song. (C) A subsequent injection of ACh at the same stimulus site initiated a sequence of singing activity containing calling song chirps and rivalry song chirps. Wing, movement of the right forewing; Sound, sound pattern of stridulation.

Muscarinic and nicotinic effects on calling song production

Calling song could also be elicited by both muscarinic and nicotinic agonists of ACh. There was, however, a difference in the time course over which muscarine and nicotine initiated and maintained song production. After injection of muscarine (10^{-3} mol l^{-1}), sound was produced after a long latency of 59.8±95.6 s (*N*=19). Stridulation was built up very gradually, with low-amplitude stridulation lasting for several seconds (Fig. 2A). Once full-intensity stridulation had been achieved, it lasted on average for 166.4±127.5 s (*N*=19), and in chirps with four syllables the chirp intervals were 417.5±81.6 ms (*N*=484). Similar results were obtained using pilocarpine (10^{-3} mol l^{-1}) (not shown).

In comparison with the effect of muscarine, the effects of nicotine had a clearly faster time course (Fig. 2B). After injection of nicotine (10^{-3} mol l^{-1}), bouts of singing behaviour lasting 18.3±15.9 s (*N*=29) were released after a short latency of only 8.3±6.8 s (*N*=29). Stridulation reached its full intensity after 1–2 s, and chirp intervals were 356.7±90.1 ms (*N*=369). This is significantly shorter than the chirp interval after muscarine injection (*t*-test, *P*=0.001).

Thses results show that ACh and nicotine had the most immediate effects on sound production. Both elicited short bouts of calling song after a short latency, and the calling songs elicited did not differ significantly for the two subtances. The results for muscarine, however, were significantly different (Fig. 2C). Calling song activity followed injections of muscarine with a long latency and was performed in long sequences. This is also demonstrated in a two-dimensional scatterplot of all latency/duration values, in which the data points for nicotinic and muscarinic song release form two different scatter clouds (Fig. 2D). The values for ACh, however, largely overlap with the values for nicotine, and only one data point for ACh corresponds clearly to a muscarine-like reaction. This indicates that ACh preferentially elicited nicotine-like effects in the neuropile controlling singing behaviour. Since, in these experiments, the stimulation sites for ACh, nicotine and muscarine were not identical, these differences may have been caused by local differences in receptor types. It is possible that ACh stimulation may have occurred at neuropile locations with a very low muscarinic ACh receptor density compared with the surrounding tissue, but this seems to be unlikely.

In two double-barrelled stimulation experiments using ACh and muscarine, we found some evidence that ACh decreased the effect of the muscarinic pathway (Fig. 3). In these experiments, injections of ACh elicited singing behaviour with a latency and duration corresponding to those of ACh-evoked sequences (Fig. 3A). After the ACh-induced behaviour had ceased, we waited 5 min and then injected muscarine at the

same position in the neuropile. No singing behaviour was evoked and only some nonspecific motor activity occurred (Fig. 3B). A subsequent injection of ACh 5 min later was again effective and led to a bout of sonorous stridulation (Fig. 3C). Thus, muscarine was not effective at a site where ACh had already elicited stridulation.

Control experiments

To ensure that the injection of ACh and its agonists did not simply act mechanically on the neurons in the vicinity of the injection site, the following control experiments were carried out (data not shown). ACh, saline and ACh were injected sequentially at locations where calling song could be reliably elicited. The microinjection of saline always failed to elicit stridulation, whereas the injections of ACh before and after the saline injection always elicited stridulatory behaviour, indicating that the mechanical impact of the injection on the neuronal tissue was not responsible for the release of stridulation.

We also tested the specifity of nicotine for the release of singing. In these cases, a location was first established at which nicotine elicited singing behaviour (Fig. 4A). A mixture of nicotine and *d*-tubocurarine (10^{-3} mol l^{-1}), which is known selectively to block the nicotinic ACh receptors, was then injected (Fig. 4B). In this case, the initial short struggling response that followed the injection of nicotine was seen, but singing was not initiated. A subsequent injection of nicotine again initiated singing behaviour (Fig. 4C). The releasing effect of injected nicotine on stridulatory behaviour is therefore specific and is coupled to the activation of nicotinic ACh receptors.

Release of rivalry song

Rivalry song is produced by male crickets encountering other males. The rivalry song has a rather variable chirp pattern (Fig. 5A). Chirps may contain 6–15 syllables but can contain up to 40 syllables. Within a rivalry song, the number of syllables per chirp can change very abruptly. The syllable intervals are similar to those during calling song and are 35–40 ms long.

Sequences of rivalry song were elicited pharmacologically in 11 males and lasted on average 39.5±54.0 s. In the example given in Fig. 5B, rivalry stridulation was elicited by muscarine. Stridulation started after a latency of 27 s and lasted for 160 s. During the sequence, the number of syllables per chirp varied between six and 30. For a detailed analysis, we compared the rivalry song elicited by injection of muscarine with the normal rivalry song obtained from a different male. The mean chirp interval of the pharmacologically elicited song was 618.8±337.4 ms (N=77), which was significantly shorter than the mean normal chirp interval of 749.5±393.1 ms (N=96) (t-test, P=0.05). As during a calling song, the syllable interval gradually increased during the chirps. In the normal sequence, the syllable interval increased from 33.2±6.2 ms (N=112) to

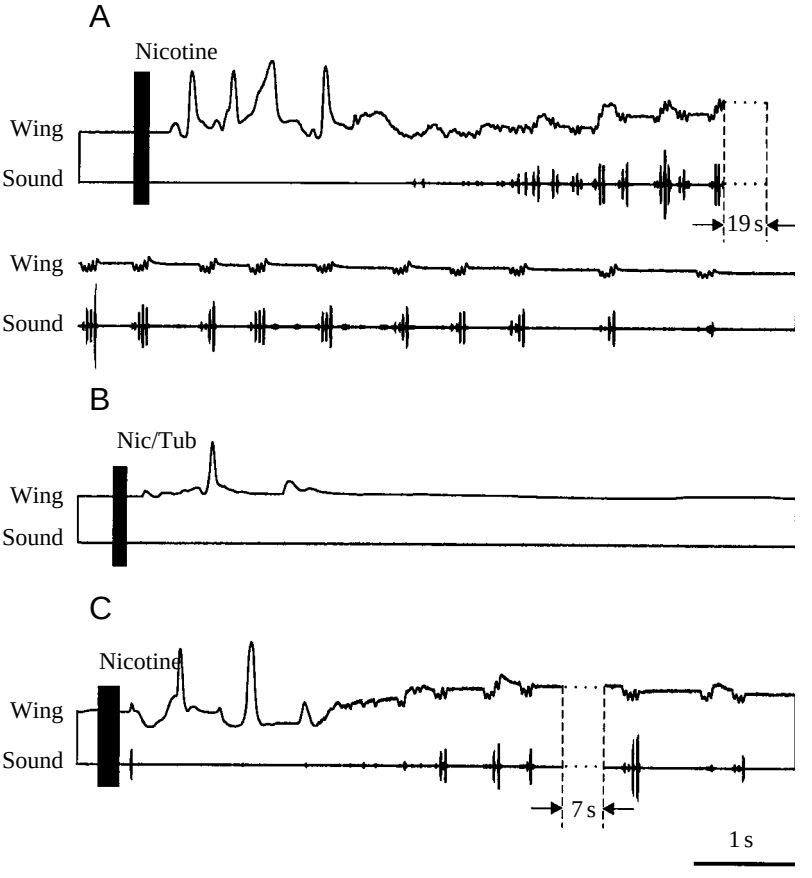

Fig. 4. Control experiment testing the release of calling song by nicotine. (A) Microinjection of nicotine elicited a bout of motor activity followed by a sequence of calling song. (B) A combined injection of nicotine and *d*-tubocurarine (Nic/Tub) at the same injection site after 10 min led to a short-lasting increase in motor activity but did not initiate calling song. (C) A subsequent injection of nicotine after 20 min again evoked calling song stridulation. Wing, movement of the right forewing; Sound, sound pattern of stridulation.

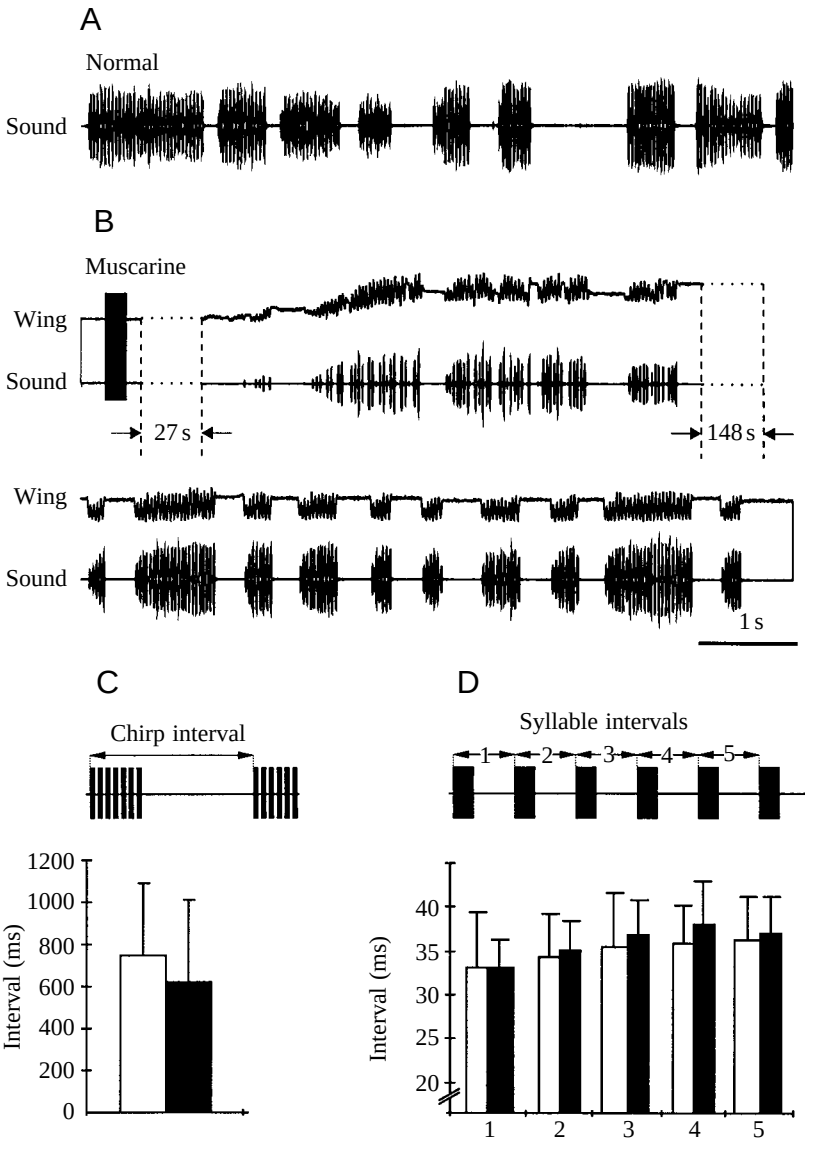

Fig. 5. Pharmacological release of rivalry song. (A) Sound pattern of normal rivalry song recorded in an unrestrained animal. (B) A continuous sequence of rivalry stridulation elicited by microinjection of muscarine into the frontal protocerebrum. (C) Comparison of the chirp interval of normal (open columns) and elicited (filled columns) rivalry songs. (D) Comparison of the first five syllable intervals of chirps from normal and elicited rivalry songs. Values are means ± S.D. Wing, movement of the right forewing; Sound, sound pattern of stridulation.

36.3±5.1 ms (*N*=104). During pharmacologically elicited rivalry song, the syllable interval increased from 33.1±3.1 ms (*N*=83) to 37.1±4.2 ms (*N*=73). The syllable intervals of muscarine-elicited song were significantly longer (*t*-test, *P*=0.05) than the corresponding intervals of the natural rivalry song (Fig. 5D).

Release of courtship song

In the vicinity of a female, male crickets may produce a courtship song. During courtship stridulation, the wings are held in a less elevated position than during calling songs. Each chirp is accompanied by rocking movements of the whole body. Courtship songs consist of chirps with a single syllable (Fig. 6A,C). The frequency of the courtship song is approximately 12 kHz and it is clearly distinguishable from the other two song types. Under normal conditions, the syllable interval of continuous courtship song was 245.0±71.3 ms (*N*=515) (Fig. 6A).

Courtship songs were elicited by pharmacological injection of ACh in six animals. The wings were held in the typical position, but only rather short bouts of courtship song occurred (Fig. 6B), lasting for an average of 23.0±20.7 s. In all elicited courtship songs, the mean courtship syllable interval was 366.4±75.3 ms, which is significantly longer than the value for normal courtship song (*t*-test, *P*=0.001) (Fig. 6C). This difference in the performance of courtship song may occur because the crickets were fixed to a holder and rocking movements of the body were prevented.

Transition between song patterns

During natural singing behaviour, male crickets sometimes produce transitions and alternations between different song types. During courtship behaviour, a calling song can change abruptly or gradually into courtship song (Kutsch and Huber, 1989). On encountering other males, a change from calling song to rivalry song may occur, but transitions from rivalry song to calling song are also possible. These types of change are quite frequent, whereas

transitions between courtship and rivalry song have never been observed.

During pharmacologically released singing behaviour, transitions between the song types were also frequent. A transition between calling song and courtship song is shown in Fig. 7A. After the injection of nicotine, short bouts of calling song alternated with short sequences of courtship song. These changes between the different song patterns were abrupt and occurred within a chirp cycle. Transitions between rivalry song and calling song were also observed (Fig. 7B). These transitions were more gradual and occurred as a steady reduction in the number of syllables per chirp. We never encountered transitions between rivalry song and courtship song in pharmacologically released song patterns.

Effects of GABA on singing behaviour

Earlier experiments indicated that inhibitory mechanisms in the brain are involved in the control of stridulation (Huber, 1955, 1964; Otto, 1971), and so we tested the effect of GABA injections in double-stimulation experiments (Fig. 8A). Using double-barrelled microcapillaries, a stimulation site in the neuropile was first located at which stridulation could be reliably elicited by the injection of ACh or muscarine. GABA (10^{-3} mol l^{-1}) was then released at the same stimulation site during a bout of calling song by a pressure pulse to the GABA-containing barrel of the capillary. Stridulation stopped immediately, and struggling movements of the legs, thorax and abdomen occurred. These episodic movements ceased after approximately 2 s, and singing activity gradually resumed within the next few seconds. The immediate inhibition of ongoing singing behaviour by GABA was reliably demonstrated in four out of five crickets.

The effects of GABA indicate the existence of ionotropic GABA receptors within the region of the brain neuropile controlling stridulation. We therefore analysed the system in more detail by injecting picrotoxin (10^{-3} mol l^{-1}), which is

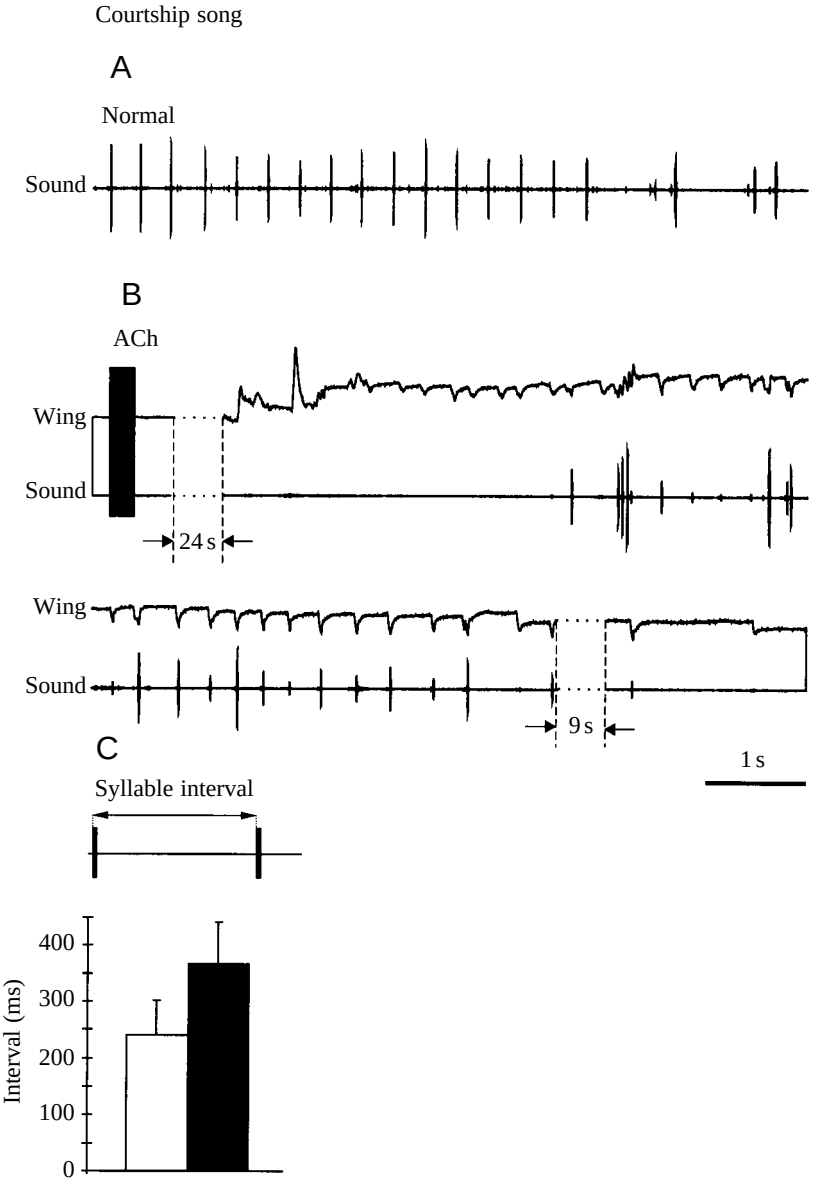

Fig. 6. (A) Recording of a normal courtship song. The chirps contain only a single syllable. (B) Courtship song elicited by microinjection of acetylcholine (ACh) into the frontal protocerebrum. (C) Comparison of the syllable interval of normal (open columns) and elicited (filled columns) courtship songs. Values are means ± S.D. Wing, movement of the right forewing; Sound, sound pattern of stridulation.

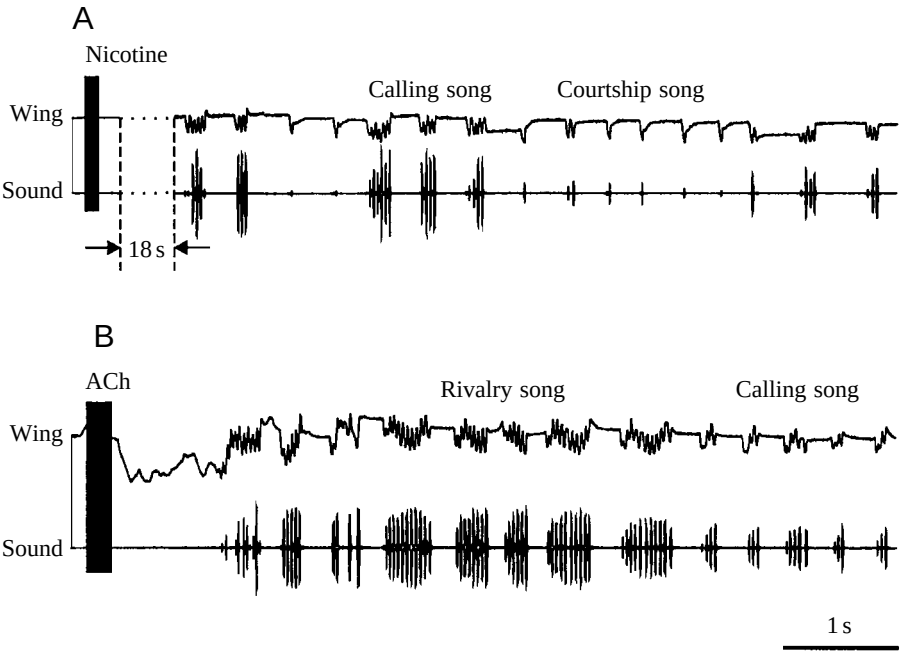

Fig. 7. Transitions between different song patterns in pharmacologically released sequences of stridulation. (A) Transition from calling song to courtship song after microinjection of nicotine. (B) Transition from rivalry song to calling song after injection of acetylcholine (ACh). In both cases, there is a rapid transition between the song patterns. Wing, movement of the right forewing; Sound, sound pattern of stridulation.

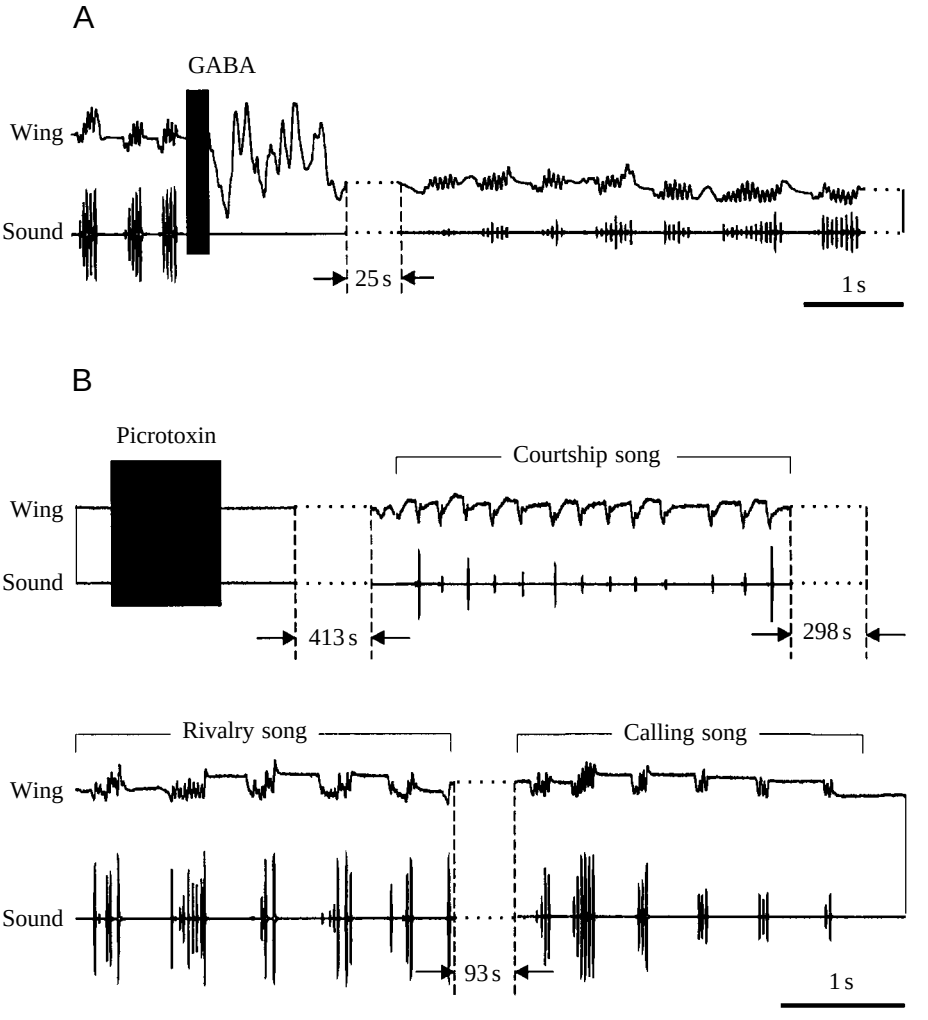

Fig. 8. Inhibition and disinhibition of sound production. (A) A continuous sequence of calling song stridulation was elicited by microinjection of acetylcholine (ACh). A subsequent injection of γ-aminobutyric acid (GABA) during stridulation immediately blocked the calling song activity. Singing activity started again after 25 s. (B) Disinhibition of sound production by microinjection of picrotoxin. After the injection, a variable enhanced motor activity started that contained sequences of the three different song patterns. The nonspecific motor activity between the bouts of singing is not presented. Traces in A and B are from different animals. Wing, movement of the right forewing; Sound, sound pattern of stridulation.

known to bind to GABA receptors in their activated state (Hosie et al., 1997). Relatively large doses of picrotoxin were administered to the central frontal brain areas to produce clear effects. The duration of the pressure pulse was increased tenfold compared with the other experiments. After the application of picrotoxin, it generally took several minutes before the crickets showed enhanced motor activity, which lasted for up to 20 min. The motor activity consisted of episodic body movements, but also contained sequences of singing behaviour (Fig. 8B). All three song patterns, calling song, courtship song and rivalry song, were performed. The songs, however, were not as regular and stable as those elicited by ACh. The chirp rate and wing movements during the syllables were rather variable, and silent stridulatory movements occurred. Motor activity changed abruptly between courtship song and other patterns, whereas gradual transitions occurred between rivalry song and calling song.

Stimulation sites within the brain

The injection sites were chosen to coincide with the branching pattern of descending interneurons controlling stridulation (Hedwig, 1996). In a series of experiments, we used double-barrelled capillaries to combine the application of ACh with the injection of fluorescent dextrans to mark the position of pharmacological injection sites at which calling song could be successfully elicited.

Detailed information about the stimulus sites was obtained by analysing serial sagittal sections of the brains (Fig. 9A,B). The sections showed that the tips of the microcapillaries were always located between the pedunculus and the dorsal (with reference to the embryonic neuroaxis) surface of the α-lobe of the mushroom bodies. This area contains a dense oval neuropile structure with fibres entering from lateral and median brain areas. The descending command neurons for stridulation have many dendritic arborizations within this oval area of neuropile B (B. Hedwig, unpublished observations). The effective pharmacological stimulation sites were all closely related to this neuropile structure (Fig. 9C) and thereby to the arborization pattern of the stridulatory command neurons. With respect to the frontal plane of the brain, all successful injection sites were located in an area around the base of the α-lobe (Fig. 9D).

Discussion

The microinjection of neuroactive substances into central neuropile areas is a powerful method for investigating the neurochemical properties of neuronal networks controlling

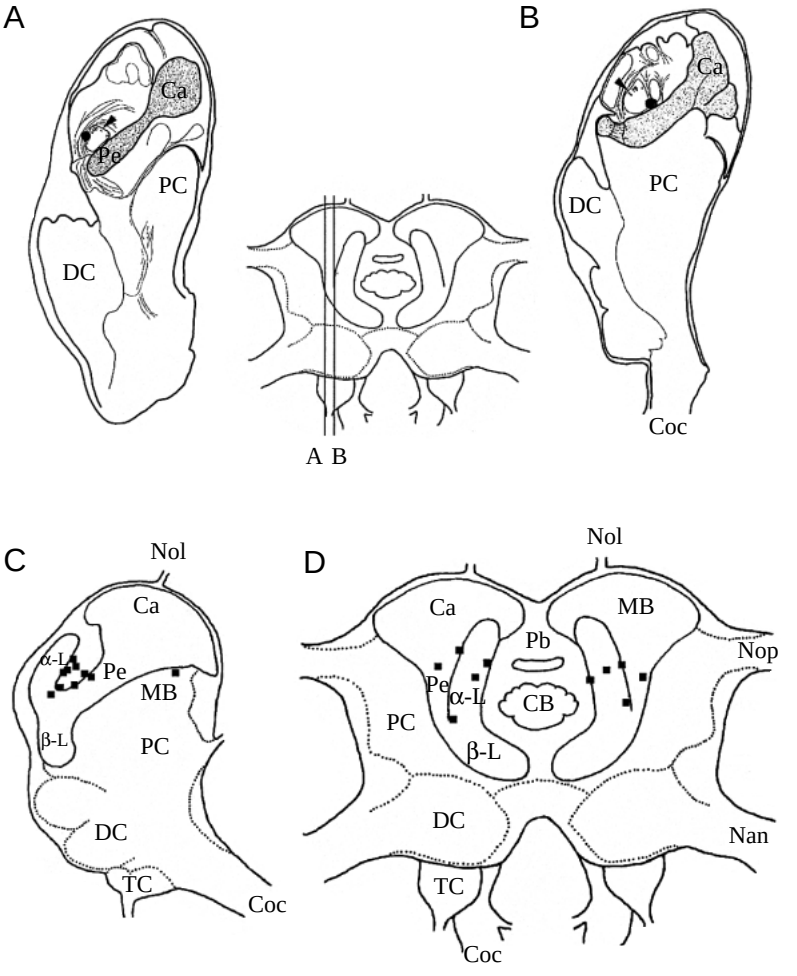

Fig. 9. Localisation of the pharmacological stimulation sites within the brain of *Gryllus bimaculatus*. (A,B) Identification of stimulus sites (indicated by a filled circle) in two different brains of *G. bimaculatus*. Note the oval-shaped neuropile structure (arrowhead) close to the anterior border of the pedunculus. The α-lobe lies in adjacent sections. (C) A parasagittal reconstruction of the brain demonstrates that all stimulation sites (filled squares) are positioned close to or in the neuropile between the α-lobe and the pedunculus. One stimulus site, however, was close to the dorsal surface of the brain. (D) Frontal view of the brain. The sites of microinjections are located in a restricted area around the base of the α-lobe. PC, protocerebrum; DC, deutocerebrum; TC, tritocerebrum; MB, mushroom body; Ca, calix; Pe, pedunculus; α-L, α-lobe; β-L, β-lobe; Pb, protocerebral bridge; CB, central body; Coc, circumoesophageal connective; Nop, optic nerve; Nan, antennal nerve; Nol, ocellar nerve.

behaviour. The data we present are a first step towards a neurochemical analysis of cricket stridulatory behaviour.

Pharmacologically released singing in crickets

Singing behaviour in crickets has been reliably elicited by mechanical brain lesions, by focal electrical stimulation within the protocerebrum (Huber, 1964) and by injection of neuroactive substances into the brain (Otto, 1978; this study). In all these experiments, the thoracic stridulatory machinery was left intact, and the brain was the only site of experimental operation. In the injection studies, minute differences in the injection sites and in the amounts of active substances administered are inevitable and may contribute to differences in the performance of the behaviour under study. Nevertheless, the microinjections of cholinergic neuroactive substances into the brain of male *G. bimaculatus* clearly demonstrate that all three species-specific song patterns and also the normal transitions between song types can be released. Chirp intervals during pharmacologically elicited calling song and rivalry song were significantly shorter than during normal songs but, as in *G. campestris* after ACh stimulation, the syllable intervals during pharmacologically elicited calling song, rivalry song and courtship song were significantly longer than during normal songs. This indicates an influence of the brain on the timing of chirp and syllable pattern generation, which is slightly modified during pharmacological stimulation (Otto, 1978).

We assume that the injected neuroactive substances had no direct pharmacological effect on the thoracic networks involved in the generation of the stridulatory motor pattern but that they activated the descending command neurons directly or *via* networks presynaptic to these neurons. This differs from experiments in which neuroactive substances were used to elicit 'fictive' motor behaviour. Neuroactive substances were administered directly to the motor networks in deafferented ganglia, and they activated central components of rhythmic motor behaviour such as walking (Ryckebusch and Laurent, 1993; Chrachri and Clarac, 1987; Büschges et al., 1995), flight (Sombati and Hoyle, 1984; Stevenson and Kutsch, 1987) and swimming (Wallen and Williams, 1984).

Injection sites and descending pathways

Successful injection sites for calling song were located in a restricted area in the frontal protocerebrum between the α-lobe and the pedunculus. The spread of the low-molecular-mass neuroactive substances from the release site into the surrounding neuropile probably occurred in a different manner from the spread of fluorescent dextrans, so the volume of neuropile influenced by the neuroactive substance cannot be determined precisely. However, the data on the injection sites are in good agreement with the results of electrical stimulation experiments (Huber, 1964; see Fig. 292a,b), which indicated that calling song stridulation can be elicited most reliably in this region of the protocerebral neuropile. The command neurons controlling stridulation have dendritic branches that arborize in an area between the α-lobe and the pedunculus (Hedwig, 1996; B. Hedwig, unpublished results). These neuroanatomical data support the assumption that the injection of neuroactive substances stimulated the descending interneurons either directly or indirectly *via* local presynaptic brain neurons. Since rivalry song and courtship song were elicited by injections into the same area as calling song, we assume that this part of the protocerebrum is involved in the control of all three song types. The neuroactive substance may have spread into the mushroom bodies following injection and, therefore, the data could also support the assumption of Huber (1960, 1964) that the mushroom bodies are involved in the control of stridulation.

The transitions between the song patterns are in agreement with the suggestion of Otto (1971) that two different descending pathways control rivalry/calling song and calling/courtship song. An alternative hypothesis is that three pathways, one for each song pattern, exist. The neurons controlling calling song may overlap spatially to some degree with the neurons controlling rivalry song and with the neurons controlling courtship song, but there is no overlap between the neurons controlling rivalry song and courtship song. Thus, focal stimulation can only elicit rivalry/calling song or calling/courtship song in combination and, because of the spatial separation of neurons, a combination of rivalry and courtship song cannot be evoked. This assumption is supported by data from the picrotoxin injections, which influenced larger regions of the central brain and always elicited all three song patterns.

Cholinergic effects on the stridulatory control system

Acetylcholine is a major excitatory transmitter within insect sensory and central neurons (Breer, 1987; Booth and Larsen, 1987; Homberg, 1994). The distribution of ACh-relevant enzymes has been mapped in the brain of the the honeybee (Kreissl and Bicker, 1989) and of *Drosophila melanogaster* (Buchner et al., 1986). The distribution of ACh receptors has been described in the brain of *D. melanogaster* (Blake et al., 1993) and of the cockroach *Periplaneta americana* (Orr et al., 1990, 1991), and the presence of muscarinic binding sites has been demonstrated in the terminal ganglion of *Acheta domesticus* (Meyer and Edwards, 1980). To date, the distribution of ACh and of its receptors has not been mapped in the cricket brain. A preliminary histochemical analysis of the distribution of acetycholinesterase did not indicate an enhanced presence of the enzyme in the area between the α-lobe and the pedunculus (Wenzel, 1998).

Currently, three different cholinergic receptor types (AChRs) have been described pharmacologically and electrophysiologically (for reviews, see Sattelle, 1980; Breer and Sattelle, 1987; Hannan and Hall, 1993; Trimmer, 1995). Nicotinic ACh receptors (nAChRs), ligand-coupled cation channels that can be activated by nicotinic ACh agonists, are abundant in the central nervous system (CNS). Muscarinic AChRs (mAChRs) make up only 10 % of AChRs; they can be activated by muscarinic ACh agonists and exist as at least two different types. There is evidence that mAChRs located

presynaptically function as negative-feedback autoreceptors and inhibit the release of ACh (Breer and Sattelle, 1987; Hue et al., 1989; Le Corronc et al., 1991). Postsynaptic mAChRs activate G-protein-mediated second-messenger responses and may modulate the excitability of neurons. If the postsynaptic nicotinic and muscarinic AChRs of a neuron are pharmacologically activated independently, they cause membrane depolarizations with different time courses. Nicotinic agonists elicit short, rapid depolarizations, whereas muscarinic ACh agonists cause slow, long-lasting depolarizing effects (Trimmer and Weeks, 1989; Benson, 1992; Bai et al., 1992; Le Corronc and Hue, 1993; Trimmer and Weeks, 1993; Bai and Sattelle, 1994; Baines and Bacon, 1994).

The neuroactive substances were applied in a different manner in neurophysiological experiments on single cells and in the intact brain of crickets. It is, therefore, difficult to compare the actual values of latency and duration of the responses. Nevertheless, the general time course of singing behaviour elicited by nicotine or muscarine matches the time course of membrane depolarizations triggered by nicotinic and muscarinic ACh receptors, respectively. The singing activity elicited by muscarine occurred after a significantly longer latency and lasted significantly longer than the singing activity elicited by nicotine. For ACh, we expected a mixed response in the crickets, with a latency corresponding to the nicotinic time course and a duration of singing corresponding to the muscarinic time course. The almost pure nicotinic response of the stridulatory system after ACh injection is unexpected, but is similar to the effects in grasshoppers (Heinrich et al., 1997). It may be due to the rather long exposure of the neurons to ACh. Prolonged ACh application is reported to reduce the response of a coxal motoneuron of the cockroach (David and Sattelle, 1990). The authors speculated that this may be caused by an increased internal Ca^{2+} concentration, which directly or indirectly inactivates the ACh receptors. Whether these assumptions also apply to the pharmacologically activated cephalic neurons remains to be analysed at a cellular level.

Sound production can also be elicited by microinjections of neuroactive substances into the brain of grasshoppers, squirrel monkeys and cats. In acridid grasshoppers, ACh and nicotinic and muscarinic agonists elicit stridulation when injected into the dorsal neuropile of the protocerebrum (Ocker et al., 1995; Heinrich et al., 1997). Nicotine and ACh release short sequences of stridulation (10–30 s) after a short latency (0.5 s), whereas muscarine elicits prolonged stridulatory sequences (120–180 s) after a long latency (5 s). In the grasshopper *Omocestus viridulus*, all three motor patterns of courtship behaviour can be released (Hedwig and Heinrich, 1997). Thus, the behavioural effects of cholinergic microinjections are similar in these orthopteran species. There is currently no indication of a neurochemical differentiation underlying the activation of stridulatory motor patterns, with different transmitters controlling specific motor patterns, as occurs in the squirrel monkey *Saimiri sciureus* (Jürgens and Lu, 1993a,b; Jürgens, 1994). Microinjections of glutamate agonists, acetylcholine agonists, histamine or excitatory amino acids into the periaquaeductal grey area, a midbrain relay station for vocal behaviour, elicits vocalization. In the squirrel monkey, however, specific vocal elements are preferentially elicited by particular substances. In cats, chemical stimulation of the periaquaeductal grey area with different transmitters (ACh, glutamate, aspartate) can release vocal behaviour (Hernandez-Peon et al., 1963; Bandler and Carrive, 1988). The more elaborate vocal behaviour of these vertebrates seems to be reflected in a higher degree of neurochemical differentiation.

Pharmacological inhibition of cricket song patterns

The early lesion experiments, which elicited long-lasting stridulation, and the finding that electrical brain stimulation blocked ongoing stridulation indicated the existence of inhibitory mechanisms within the brain controlling stridulation (Huber, 1955, 1964; Otto, 1971). Our data from GABA and picrotoxin injections support this evidence. GABA is a common inhibitory transmitter within the CNS that binds to ionotropic receptors. It elicits a rapid transient opening of Cl^- channels and thereby a hyperpolarization of the affected neuron (Sattelle, 1990; Hosie et al., 1997). GABAergic neurons are widely distributed within the CNS of insects (Homberg, 1994), where there is some evidence for different subtypes of GABA receptors (Sattelle, 1990; Hue, 1991; Anthony et al., 1993), although their spatial distribution within the CNS has not yet been mapped. The effects of GABA injections on singing behaviour indicate that the control system for stridulation in the brain of crickets is sensitive to this transmitter. These data are supported by the effects of picrotoxin, an alkaloid that is known to stabilize activated insect GABA receptors in an agonist-bound closed formation (Hosie et al., 1997), thus blocking GABA-mediated inhibitory postsynaptic potentials (Sattelle, 1990; Anthony et al., 1993). Since picrotoxin elicited motor activity combined with singing behaviour, we assume that picrotoxin caused a general disinhibition of descending motor commands that also allowed the song patterns to occur. Thus, either the descending command neurons for stridulation and/or their presynaptic neurons seem to be under constant inhibition from GABAergic neurons.

Very similar results were obtained in acridid grasshoppers (Heinrich et al., 1998) and in the squirrel monkey *Saimiri sciurus* (Jürgens and Lu, 1993a,b; Jürgens, 1994). Sound production elicited either pharmacologically or electrically is suppressed by the injection of GABA into the dorsal protocerebrum of grasshoppers and into the periaquaeductal grey area in the squirrel monkey. Further, injection of picrotoxin elicited a rather irregular stridulation in grasshoppers. Picrotoxin also facilitated the release of vocalization in the squirrel monkey and, additionally, elicited nonspecific types of calling. Thus, there is evidence for a general scheme of GABAergic control of sound production and vocalization both in orthopteran insects and in vertebrate species.

Future prospects

We cannot currently exclude the possibility that additional transmitters or neuromodulators are also involved in the control of cricket stridulation. In future experiments, microinjections of neuroactive substances will be combined with simultaneous electrical recordings of identified command neurons to analyse the pharmacological effects at the level of identified interneurons.

We thank N. Elsner for supporting of the project and our Cambridge colleagues T. Friedel, M. Wildman and J. Poulet for most valuable comments on the manuscript. The work was funded by the Georg-August University of Göttingen and by a Heisenberg Stipendium to B.H. (DFG He 2018/1-3).

References

Anthony, N. M., Harrison, J. B. and Sattelle, D. B. (1993). GABA receptor molecules of insects. In *Comparative Molecular Neurobiology* (ed. Y. Pichon), pp. 172–209. Basel, Switzerland: Birkhauser Verlag.

Bai, D., Erdbrugger, H., Breer, H. and Sattelle, D. B. (1992). Acetylcholine receptors of thoracic dorsal midline neurones in the cockroach *Periplaneta americana*. *Arch. Insect Biochem. Physiol.* **21**, 289–301.

Bai, D. and Sattelle, D. B. (1994). Muscarinic acetylcholine receptors on an identified motor neurone in the cockroach, *Periplaneta americana*. *Neurosci. Lett.* **175**, 161–165.

Baines, R. A. and Bacon, J. P. (1994). Pharmacological analysis of the cholinergic input to the locust VPLI neuron from an extraocular photoreceptor system. *J. Neurophysiol.* **72**, 2864–2874.

Bandler, R. (1982). Induction of 'rage' following microinjections of glutamate into midbrain but not hypothalamus of cats. *Neurosci. Lett.* **30**, 183–188.

Bandler, R. and Carrive, P. (1988). Integrated defence reaction elicited by excitatory amino acid microinjection in the midbrain periaqueductal grey region of the unrestrained cat. *Brain Res.* **439**, 95–106.

Benson, J. A. (1992). Electrophysiological pharmacology of the nicotinic and muscarinic cholinergic responses of isolated neuronal somata from locust thoracic ganglia. *J. Exp. Biol.* **170**, 203–233.

Blake, A. D., Anthony, N. M., Chen, H. H., Harrison, J. B., Nathanson, N. M. and Sattelle, D. B. (1993). *Drosophila* nervous system muscarinic acetylcholine receptor: Transient functional expression and localization by immunocytochemistry. *Mol. Pharmac.* **44**, 716–724.

Booth, M. and Larsen, J. R. (1987). Histochemistry of acetylcholinesterase in the insect brain. In *Arthropod Brain: its Evolution, Development, Structure and Functions* (ed. A. P. Gupta), pp. 439–456. New York: John Wiley.

Breer, H. (1987). Neurochemical aspects of cholinergic synapses in the insect brain. In *Arthropod Brain: its Evolution, Development, Structure and Functions* (ed. A. P. Gupta), pp. 415–437. New York: John Wiley.

Breer, H. and Sattelle, D. B. (1987). Molecular properties and functions of insect acetylcholine receptors. *J. Insect Physiol.* **33**, 771–790.

Buchner, E., Buchner, S., Crawford, G., Mason, W. T., Salvaterra, P. M. and Sattelle, D. B. (1986). Choline acetyltransferase-like immunoreactivity in the brain of *Drosophila melanogaster*. *Cell Tissue Res* **246**, 57–62.

Büschges, A., Schmitz, J. and Bässler, U. (1995). Rhythmic patterns in the thoracic nerve cord of the stick insect induced by pilocarpine. *J. Exp. Biol.* **198**, 435–456.

Chrachri, A. and Clarac, F. (1987). Induction of rhythmic activity in motoneurons of crayfish thoracic ganglia by cholinergic agonists. *Neurosci. Lett.* **77**, 49–54.

David, J. A. and Sattelle, D. B. (1990). Ionic bases of membrane potential and of acetylcholine-induced currents in the cell body of the cockroach fast coxal depressor motor neurone. *J. Exp. Biol.* **151**, 21–39.

Elsner, N. (1994). The search for the neural centers of cricket and grasshopper song. *Prog. Zool.* **39**, 167–193.

Fielden, A. (1960). Transmission through the last abdominal ganglion of the dragonfly nymph *Anax imperator*. *J. Exp. Biol.* **37**, 832–844.

Hannan, F. and Hall, L. M. (1993). Muscarinic acetylcholine receptors in invertebrates: Comparisons with homologous receptors from vertebrates. In *Comparative Molecular Neurobiology* (ed. Y. Pichon), pp. 98–140. Basel: Birkhäuser.

Hedwig, B. (1994). A cephalothoracic command system controls stridulation in the acridid grasshopper *Omocestus viridulus* L. *J. Neurophysiol.* **72**, 2015–2025.

Hedwig, B. (1996). A descending brain neuron elicits stridulation in the cricket *Gryllus bimaculatus* (de Geer). *Naturwissenschaften* **83**, 428–429.

Hedwig, B. and Heinrich, R. (1997). Identified descending brain neurons control different stridulatory motor patterns in an acridid grasshopper. *J. Comp. Physiol.* A **180**, 285–294.

Hedwig, B. and Knepper, M. (1992). NEUROLAB, a comprehensive program for the analysis of neurophysiological and behavioural data. *J. Neurosci. Meth.* **45**, 135–148.

Heinrich, R., Hedwig, B. and Elsner, N. (1997). Cholinergic activation of stridulatory behaviour in the grasshopper *Omocestus viridulus* (L.) *J. Exp. Biol.* **200**, 1327–1337.

Heinrich, R., Jacobs, K. and Lakes-Harlan, R. (1998). Tracing of a neuronal network in the locust by pressure injection of markers into a synaptic neuropile. *J Neurosci. Meth.* **80**, 81–89.

Hernandez-Peon, R., Chavez-Ibarra, G., Morgane, P. J. and Timo-Iaria, C. (1963). Limbic cholinergic pathways involved in sleep and emotional behaviour. *Exp. Neurol.* **8**, 93–111.

Homberg, U. (1994). Distribution of neurotransmitters in the insect brain. *Prog. Zool.* **40**, 1–88.

Hosie, A. M., Aronstein, K., Sattelle, D. B. and ffrench-Constant, R. H. (1997). Molecular biology of insect neuronal GABA receptors. *Trends Neurosci.* **20**, 578–583.

Huber, F. (1955). Sitz und Bedeutung nervöser Zentren für Instinkthandlungen beim Männchen von *Gryllus campestris* L. *Z. Tierpsychol.* **12**, 12–48.

Huber, F. (1960). Untersuchungen über die Funktion des Zentralnervensystems und insbesondere des Gehirnes bei der Fortbewegung und der Lauterzeugung der Grillen. *Z. vergl. Physiol.* **44**, 60–132.

Huber, F. (1964). The role of the central nervous system in Orthoptera during the co-ordination and control of stridulation. In *Acoustic Behaviour of Animals* (ed. R. G. Busnel), pp. 440–487. New York, Amsterdam, London: Elsevier.

Hue, B. (1991). Functional assay for GABA receptor subtypes of a cockroach giant interneurone. *Arch. Insect Biochem. Physiol.* **18**, 147–157.

Hue, B., Lapied, B. and Malecot, C. O. (1989). Do presynaptic

muscarinic receptors regulate acetylcholine release in the central nervous system of the cockroach *Periplaneta americana*? *J. Exp. Biol.* **142**, 447–451.

Jürgens, U. (1994). The role of the periaqueductal grey in vocal behaviour. *Behav. Brain Res.* **62**, 107–117.

Jürgens, U. and Lu, C. L. (1993a). Interactions between glutamate, GABA, acetylcholine and histamine in the periaqueductal gray's control of vocalization in the squirrel monkey. *Neurosci. Lett.* **152**, 5–8.

Jürgens, U. and Lu, C. L. (1993b). The effects of periaqueductally injected transmitter antagonists on forebrain-elicited vocalization in the squirrel monkey. *Eur. J. Neurosci.* **5**, 735–741.

Jürgens, U. and Richter, K. (1986). Glutamate-induced vocalization in the squirrel monkey. *Brain Res.* **373**, 349–358.

Knepper, M. and Hedwig, B. (1997). NEUROLAB, a PC-program for the processing of neurobiological data. *Comp. Meth. Prog. Biomed.* **52**, 75–77.

Koch, U. T., Elliott, C. J. H., Schäffner, K. H. and Kleindienst, U. (1988). The mechanics of stridulation of the cricket *Gryllus campestris*. *J. Comp. Physiol.* A **162**, 213–223.

Kreissl, S. and Bicker, G. (1989). Histochemistry of acetylcholinesterase and immunocytochemistry of an acetylcholine receptor-like antigen in the brain of the honeybee. *J. Comp. Neurol.* **286**, 71–84.

Kutsch, W. and Huber, F. (1989). Neural basis of song production. In *Cricket Behaviour and Neurobiology* (ed. F. Huber, T. E. Moore and W. Loher), pp. 262–309. Ithaca: Cornell University Press.

Le Corronc, H. and Hue, B. (1993). Pharmacological and electrophysiological characterization of a postsynaptic muscarinic receptor in the central nervous system of the cockroach. *J. Exp. Biol.* **181**, 257–278.

Le Corronc, H., Lapied, B. and Hue, B. (1991). M_2-like presynaptic receptors modulate acetylcholine release in the cockroach (*Periplaneta americana*) central nervous system. *J. Insect Physiol.* **37**, 647–652.

Loher, W. (1989). Temporal organization of reproductive behaviour. In *Cricket Behaviour and Neurobiology* (ed. F. Huber, T. E. Moore and W. Loher), pp. 83–113. Ithaca: Cornell University Press.

Meyer, M. R. and Edwards, J. S. (1980). Muscarinic cholinergic binding sites in an orthopteran central nervous system. *J. Neurobiol.* **11**, 215–219.

Ocker, W.-G., Hedwig, B. and Elsner, N. (1995). Application of putative neurotransmitters elicits and modulates stridulation in two species of acridid grasshoppers. *J. Exp. Biol.* **198**, 1701–1710.

Orr, G. L., Orr, N. and Hollingworth, R. M. (1990). Localization and pharmacological characterization of nicotinic-cholinergic binding sites in cockroach brain using α- and neuronal bungarotoxin. *Insect Biochem.* **20**, 557–566.

Orr, G. L., Orr, N. and Hollingworth, R. M. (1991). Distribution and pharmacological characterization of muscarinic-cholinergic receptors in the cockroach brain. *Arch. Insect Biochem. Physiol.* **16**, 107–122.

Otto, D. (1971). Untersuchungen zur zentralnervösen Kontrolle der Lauterzeugung von Grillen. *Z. vergl. Physiol.* **74**, 227–271.

Otto, D. (1978). Änderung von Gesangsparametern bei der Grille (*Gryllus campestris*) nach Injektion von Pharmaka ins Gehirn. *Verh. Dt. Zool. Ges*. **1978**, 245.

Ryckebusch, S. and Laurent, G. (1993). Rhythmic patterns evoked in the locust leg motor neurons by the muscarinic agonist pilocarpine. *J. Neurophysiol.* **69**, 1583–1595.

Sattelle, D. B. (1980). Acetylcholine receptors of insects. *Adv. Insect Physiol.* **15**, 215–315.

Sattelle, D. B. (1990). GABA receptors of insects. *Adv. Insect Physiol.* **22**, 1–113.

Sombati, S. and Hoyle, G. (1984). Generation of specific behaviour in a locust by local release into neuropile of the natural neuromodulator octopamine. *J. Neurobiol.* **15**, 481–506.

Stevenson, P. A. and Kutsch, W. (1987). A reconsideration of the central pattern generator concept for locust flight. *J. Comp. Physiol.* A **161**, 115–129.

Trimmer, B. A. (1995). Current excitement from insect muscarinic receptors. *Trends Neurosci.* **18**, 104–111.

Trimmer, B. and Weeks, J. C. (1989). Effects of nicotinic and muscarinic agents on an identified motoneurone and its direct afferent inputs in larval *Manduca sexta*. *J. Exp. Biol.* **144**, 303–337.

Trimmer, B. A. and Weeks, J. C. (1993). Muscarinic acetylcholine receptors modulate the excitability of an identified insect motoneuron. *J. Neurophysiol.* **69**, 1821–1836.

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

Wallen, P. and Williams, T. L. (1984). Fictive locomotion in the lamprey spinal cord *in vitro* compared with swimming in the intact and spinal animal. *J. Physiol., Lond.* **347**, 225–239.

Wenzel, B. (1998). Neuropharmakologische und histologische Untersuchungen zum Gesangsverhalten von Grillen. Diplomarbeit, Universität Göttingen. 89pp.

Wenzel, B., Elsner, N. and Hedwig, B. (1998). Microinjection of neuroactive substances into brain neuropile controls stridulation in the cricket *Gryllus bimaculatus* (de Geer). *Naturwissenschaften* **85**, 452–454.