BRAIN NEURONES INVOLVED IN THE CONTROL OF WALKING IN THE CRICKET GRYLLUS BIMACULATUS

BY HARTMUT BÖHM
Zoologisches Institut der Universität, Poppelsdorfer Schloß, 5300 Bonn 1, Germany

AND KLAUS SCHILDBERGER
Max-Planck-Institut für Verhaltensphysiologie, 8130 Seewiesen, Germany

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Summary

The responses of single brain neurones to artificial calling song, to moving striped patterns and to air puffs were recorded while tethered crickets were walking on a sphere in such a way that their intended orientation to the stimuli could be measured.

Local and descending brain neurones responsive to only one of the stimuli tested often encoded the directional information contained in the stimulus (e.g. the direction of the sound source or the direction of stripe movement). Brain neurones with little directional sensitivity responded with marked habituation to all stimuli, so that their responses primarily signalled changes in the overall stimulus situation.

The responses of some neurones were stronger during walking than when the cricket was standing still. In the case of one descending neurone, which increased its level of activity shortly before and during the walking phases, the mean spike rate was correlated with the forward velocity. By altering the discharge rate of another descending neurone, it was possible to elicit walking in the manner typical of crickets. Maintenance and control of walking by such 'command neurones' is discussed.

Introduction

The question of how animals select modes of behaviour adapted to their immediate circumstances (Roeder, 1970) has previously been approached at several levels, from the behaviour itself through neural networks to the individual neurone, particularly in invertebrates (Huber, 1988; Hennig, 1990). At an early stage it was demonstrated that, in the cricket brain, sensory information from the environment is processed to produce descending inhibitory or excitatory commands that trigger various forms of behaviour: walking, singing, courtship and fighting (Huber, 1955).

Key words: brain neurones, course control, Gryllus bimaculatus, orientation behaviour, command neurone.
Changes in the behaviour are observed when different stimuli – for instance, an attractive song and a visual pattern – are presented simultaneously (Stout et al. 1987). In crickets, a visual stimulus can alter the pattern of phonotactic walking (Weber et al. 1987; Weber, 1990). The changes are probably based on summation of the control commands resulting from the auditory and the visual stimuli (Böhm et al. 1991).

Neurones descending from the brain are thought to play a crucial role in the organization of such control commands (Rowell, 1989; Böhm and Schildberger, 1990). Only a few of these neurones have so far been identified (O’Shea et al. 1974; Richard et al. 1985; Bacon and Tyrer, 1978; Boyan and Williams, 1981; Strausfeld and Bassemir, 1985; Griss and Rowell, 1986; Tyrer et al. 1988). They constitute a group of neurones that either process auditory (Schildberger, 1985), visual (Milde, 1988), olfactory (Olberg, 1983) or other modalities (Bacon and Möhl, 1983) or are multimodal. Their role in controlling flight and walking has been investigated in the locust (Rowell and Reichert, 1986; Kien, 1983, 1990a, b; Kien and Altman, 1984).

Here we present an approach that permits the activity of single identifiable descending brain neurones to be recorded while the behaviour of the animal is analyzed. In these experiments, intracellular recordings of brain neurone activity in walking animals were made while stimuli of various modalities were presented simultaneously or in succession. Our goal was to clarify mechanisms by which diverse stimuli elicit, maintain and modify walking. Two aspects of course control at the level of the descending neurones have been examined: (i) is there a difference between the processing of sensory information during and in the absence of motor activity; and (ii) are specific parameters of walking, such as direction and velocity, controlled by individual neurones?

**Materials and methods**

Newly moulted adult female *Gryllus bimaculatus* de Geer were removed from a laboratory colony and kept singly at 24°C and 80% relative humidity in a day:night cycle of 12 h:12 h. The animals were used for experiments at the age of 2–4 weeks.

**Experimental apparatus**

The animals were attached to a holder so that the head and thorax were fixed, but the legs could move, and were then positioned on top of a hollow styrofoam sphere supported by an airstream. The legs, which were in contact with the sphere surface, rotated the sphere as the animal walked. This rotation about two orthogonal axes was recorded electronically by a camera containing a photodiode. From these data, sampled every 100 ms, the forward velocity ($v$, translational velocity measured in cm s$^{-1}$) and the turning velocity ($\omega$, rotational velocity in degrees s$^{-1}$) of the walk were measured independently (Schildberger and Hörner, 1988; Böhm et al. 1991).
Auditory stimuli

An artificial calling song (four syllables, each 20 ms in duration, separated by 20-ms intervals; chirp repetition rate 2 Hz; carrier frequency 5 kHz) was presented from two loudspeakers positioned 30 cm from the cricket at an azimuth of 30° to the left and right of the long axis of the body. Sound-reflecting objects in the vicinity made the sound field inhomogeneous. Sound intensities were adjusted so that the 5 kHz tones from the two loudspeakers were equally loud at the position of the animal.

Visual stimuli

A white, acoustically transparent curtain (20 cm high, 15 cm in diameter) was suspended as a cylinder around the styrofoam walking sphere, covering a visual field of 240° in azimuth. A special optical system (Scharstein, 1989) was used to project a striped pattern onto this screen. Normally the stripes were vertical, but they could also be made horizontal. The contrast frequency of the moving striped pattern (measured in Hz) was recorded with a photodiode. The visual field so stimulated extended 150° in azimuth, 60° vertically above and 10° below the horizon. The stripe width was 40°. There were no non-moving objects in the stimulated visual field.

Air-puff stimuli

Air puffs, the durations of which were controlled by a special magnetic valve, were directed through tubes towards the antennae or the cerci of the animal. The airstream was monitored by an anemometer and did not show any significant built-up pressure. The duration and intensity of the puffs were not systematically varied. As a rule the duration was 300 ms, with a velocity of 1 m s⁻¹ and a repetition rate of 1 Hz.

Electrophysiology

In 50 crickets, the activity of 22 local and 13 descending brain neurones was recorded and the neurones were then marked for histological identification. Because the body was in the normal walking position during the experiments, access to the brain was gained by opening the head capsule dorsally, between the compound eyes, and exposing the supraoesophageal ganglion by removing the overlying muscle and other tissue. The gut, antennal musculature and tracheal air supply were kept largely intact. The mandibular musculature was transected, and the brain was stabilized by two spoons. The lower spoon, pushed between the circumoesophageal connectives, served as the reference electrode. The other spoon had the shape of a ring (open in the centre), and was positioned on the upper surface of the brain.

Glass microelectrodes filled with 3% Lucifer Yellow were inserted into the brain from the dorsal surface and used to record from single neurones which were subsequently marked by injection of Lucifer Yellow. Conventional histological procedures were used to process the brain, and the marked neurones were
reconstructed from photographs of whole mounts and serial sections. The anatomical positions of the neurones in the brain were specified in all cases with respect to the long axis of the body. The electrophysiological data were stored on magnetic tape, together with the measurements of sphere rotation, and were subsequently evaluated using a computer.

Results

Anatomy and responses of brain neurones

Local brain neurones

Twenty-two of the neurones studied in the 50 crickets did not leave the protocerebrum. Their arborizations were extensive, reaching from visual areas such as the lobula and the posterior lateral region of the protocerebrum to posterior ventrolateral and ventromedial regions at the boundary between protocerebrum and deutocerebrum. Some of the local brain neurones arborized in areas also occupied by terminal branches of the ascending auditory neurones (see Schildberger, 1984b). None of the neurones found here had branches in the mushroom bodies or in the central complex.

Some of the neurones (N=13) responded only to a moving striped pattern, only to an auditory stimulus or only to air puffs, while others (N=9) responded to two or all of these. The responses during bouts of walking were usually different from those when the animal was standing still. During walking, additional action potentials often appeared.

One of these local neurones (Fig. 1A), with dendritic arborizations outside the known arborization regions of auditory neurones, responded to the calling song with a latency of about 25–30 ms (Fig. 1C inset). Air puffs also excited this neurone, producing a response of about the same intensity during both walking and standing. The responses of this neurone to calling song, however, were clearly different in the standing and walking animal (Fig. 1B,C). The ‘spontaneous’ activity of the cell in the standing cricket was low, and the response to calling song was phasic (Fig. 1C). Often only the first syllable elicited spikes, the response to subsequent syllables remaining below threshold. During walking, however, additional spikes were discharged, so that there was a response to each syllable, and only slight habituation was evident. Despite the increased spontaneous activity, the resolution of the sound pattern was quite clear. It appears that during walking the transfer characteristic of the neurone was improved for calling song only, because this ‘facilitation’ was not observed when the other effective stimulus, the air puff, was presented.

Descending neurones

The cell bodies of the 13 descending neurones (DIN) in this sample were situated in posterior parts of the protocerebrum, but their locations cannot be specified (e.g. with respect to particular cell clusters). Two classes of DIN can be
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Fig. 1. Local brain neurone. (A) Reconstruction from serial sections: frontal view. (B) Responses of the neurone to artificial calling song (above) and the rotational component of the walking of the animal recorded simultaneously (below). Each vertical row of dots represents (from top to bottom) the action potentials occurring during one chirp period, successive rows are aligned with the corresponding rotational component of the walk. Vertical broken lines mark a period of standing. (C) Responses of the neurone during standing (left) and walking (right) for three chirps (top) and as histogram for 100 chirps (bottom).

distinguished on the basis of the side of the nervous system on which the axon descends: some DINs have axons ipsilateral to the cell body (IDIN, see Figs 3, 5, 6, 7) and others have contralateral axons (CDIN, see Figs 2, 4).

Neurones with contralaterally descending axons, CDINs, were found to arborize on both sides of the brain. The dendritic arborizations of the IDINs were usually restricted to the ipsilateral brain hemisphere. The dendrites of neurones in both classes were located in posterior regions of the protocerebrum, often with
arborizations near the boundary between proto- and deutocerebrum. Occasionally, however, branching across the midline of the brain was observed. Branches in the ventromedial parts of the brain were most commonly those of CDINs.

The arborizations of the descending neurones were outside the mushroom bodies and the central complex and were also distinct from the known target regions of these glomerular structures (such as the lateral horn and the lateral accessory lobe). In general, the terminal branches were not particularly dense and the number of secondary and tertiary dendritic branches was low.

Some of the DINs (N=7) responded to only one of the stimulus modalities presented, even if different modalities were combined. These neurones were considered to be unimodal, even if it was not possible to test the three modalities in all combinations. The responses of unimodal neurones were characterized by only slight habituation and often exhibited directional specificity. For example, auditory stimuli or air puffs might elicit no response, while the response to a moving striped pattern was directionally selective. The CDIN presented in Fig. 2 gave only subthreshold responses to a striped pattern moving downwards or to the left, regardless of its contrast frequency. However, movement upwards or to the right elicited action potentials phase-coupled to the light–dark alternation of the pattern. Up to a contrast frequency of 5 Hz, a spike was discharged for almost every stripe. At higher contrast frequencies, the phase-coupling deteriorated...
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(action potentials were more often omitted) so that the response as a whole became weaker. The effect of raising the contrast frequency above 5 Hz was greater (that is, the response declined more rapidly) for movement to the right than for upward movement. Changing the movement direction to 45° also weakened the response; hence, the sensitivity of the neurone to vertical and horizontal motion cannot be explained simply by addition of inputs from lower-order neurones responding preferentially to vertical and to horizontal movement. We cannot explain the directional sensitivity of this neurone, because very little is known about the directional characteristics of neurones in the optic lobes in crickets (see Honnegger, 1980; Richard et al. 1985).

Another unimodal neurone (Fig. 3A) responded neither to moving stripes nor to air puffs, but only to auditory stimulation. Sound patterns with the species-specific temporal structure caused the neurone to respond more strongly than did other sound patterns (Fig. 3B); other sound patterns remained less effective even when their sound energy was higher (or lower) than that of the species-specific pattern. The intensity characteristics of the response to normal calling song were measured (Fig. 3C) and showed an effect of loudspeaker position at all intensities. For equivalent responses, there is a difference of about 10 dB between the intensities of stimulation at 30° to the right and 30° to the left of the animal. That is, this neurone exhibits a clear sensitivity to the direction of the incident sound.

Another unimodal CDIN responded only to tactile stimulation of the cerci. It discharged briefly each time a cercus was touched (Fig. 4), and a few seconds later a brief episode of walking occurred. The directions of these walks did not depend on which cercus had been stimulated.

About half (N=6) of the descending neurones studied here were responsive to more than one modality. Their responses were characterized by marked habituation, extreme variability and low sensitivity to the direction of the stimulus.

One of these neurones (Fig. 5A) responded to calling song, with no representation of its syllabic structure (Fig. 5A, top right). After a few chirps the response had almost vanished (Fig. 5B, left). This neurone also responded to a moving striped pattern. At first the response level was high, regardless of the direction of motion, and there was no phase-coupling to the light–dark alternation (Fig. 5A, middle right). A few seconds after stimulus onset this response stabilized at a low level (Fig. 5B, left), at which time phase-coupling became visible. The response of this neurone was not affected by the direction of movement of the grid. The response to air puffs habituated more slowly (Fig. 5A, bottom right, and Fig. 5B, left).

When the different modalities were presented together, in various combinations, presentation of each new, superimposed modality was effective, although the neurone had habituated to the preceding stimulation. The response to the added stimulus was initially at about the same level as would have been produced in the non-habituated state (Fig. 5B, right) and declined in the same way as the habituation curve for that modality. The individual responses were not simply added to one another. Activity of this neurone, therefore, did not reflect the

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modality of a stimulus or its direction, but signalled that the stimulus situation had changed. The neurone was most active when the animal was walking (Fig. 5C).

Because of the pronounced habituation observed with these neurones, it was impossible to be certain whether the responses differed in detail depending on whether the stimuli were presented during walking or when the animal was standing still.
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Many brain neurones changed their levels of activity when the animal was walking. The activity of two DINs was directly correlated with walking and seemed to control parameters of the walk. One of them, an IDIN (Fig. 6A) with its cell body situated dorsolaterally and with dendritic arborizations in the...
Fig. 5
Fig. 5. Ipsilaterally descending brain neurone (IDIN). (A) Reconstruction from serial sections (left) and responses to different stimulus modalities (right). (B) Habituation to sensory stimulation: response strength is given as a function of time after the start of the first stimulus. Stimuli were repeated at 2 Hz for the calling song, at 1 Hz for the air puff and at 12 Hz contrast frequency for the grating; stimuli were presented alone (left) or in combination (right), when they were superimposed on the preceding continuous stimulation at the times indicated. (C) Rotational velocity of the animal (top) and neuronal activity (middle) during continuous presentation of different stimuli (bottom).

posterior part of the median protocerebrum restricted to the border with the deutocerebrum, discharged at a higher rate 100–200 ms before walking commenced as well as during the walk (Fig. 6C). For walking velocities of 3–10 cm s\(^{-1}\) the discharge rate of the cell was linearly correlated with the translational velocity of the walk (Fig. 6E). There was no detectable correlation with the rotational component (Fig. 6D).

This cell responded only to the auditory stimulus. While the animal was standing still, the calling song elicited a sequence of excitation and inhibition, with the inhibition predominant (Fig. 6F, left). During walking each chirp of the calling song elicited a response consisting of 1–5 action potentials, superimposed upon the walking-correlated excitation of the neurone (Fig. 6C,F right). That is, while calling song was being presented to the walking animal, the mean activity level of the neurone rose and the translational velocity also increased significantly (P<0.01 t-test, Fig. 6B). During brief pauses (1–3 s) between relatively long periods of walking, the excitatory response to the sound persisted (Fig. 6C). The inhibitory influence of the sound stimulus predominated only during longer interruptions to walking.

Another example was also an IDIN with arborizations in the median posterior protocerebrum (Fig. 7A). This neurone gave weak, distinctly habituating responses to stimuli of all three modalities (Fig. 7B). The responses were not discernibly direction-specific. At the onset of walking the neurone increased its discharge rate. Moreover, when the discharge rate was raised by injection of depolarizing current into the cell, walking was elicited and persisted for as long as the discharge rate was kept above a frequency of about 30 Hz (Fig. 7C).

Hyperpolarization of the neurone during walking caused the animal to stop immediately (Fig. 7D). When the hyperpolarizing current was turned off, the neurone resumed its spike discharge, but the animal continued to stand still. It did not begin to walk again until the discharge rate had risen to a higher level (arrow, Fig. 7D).

Neither during spontaneous walking nor during a walk induced by neuronal depolarization was there a correlation between the discharge rate of the neurone and the rotational component of the walk. The correlation between discharge rate and translational velocity was not linear (Fig. 7F); that is, the animal walked when the neurone discharged at rates above approximately 20–30 Hz, but its velocity did not increase as the discharge rate became higher. Even when the discharge rate
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Fig. 6. Ipsilaterally descending brain neurone (IDIN). (A) Reconstruction from serial sections. (B) Comparison of the firing rate (right ordinate) and the translational velocity (left ordinate) with and without sound presentation. Data represent the means and standard deviations of 500 ms samples ($N=100$) of translational velocity and simultaneously recorded neuronal activity. (C) Sample recording from the neurone shown in A. Traces (from top to bottom): rotational velocity ($\omega$), translational velocity ($v$), instantaneous firing rate of the neurone, and a tracing of neuronal activity in the absence and in the presence of artificial calling song (90 dB SPL, 5 kHz presented at an angle of $30^\circ$ from the right). Bold arrows (below) mark increases in firing rate before episodes of walking start (arrows above). (D) Correlation between the firing rate of the neurone and the simultaneously recorded rotational velocity for 500 ms samples ($N=80$); positive values indicate intended turning to the right. The correlation is not significant; $y=x+10.3$, $r^2=0.008$, $P>0.25$. (E) Correlation between the firing rate and the translational velocity for 500 ms samples ($N=70$). The correlation is highly significant; $y=0.8x-0.2$, $r^2=0.67$, $P<0.001$. (F) Comparison of the neuronal response to artificial calling song during standing (left) and walking (right). Sample recording (top) and histogram (bottom) for 16 successive chirps.

was raised to 100 Hz by current injection, the walking velocity was not increased. In order to induce walking the firing rate of this neurone had to be elevated well above the firing rate seen during spontaneous walking (Fig. 7E).

Discussion

In the biotope, phonotaxis is influenced by stimuli of various modalities. Behavioural studies of cricket phonotaxis have revealed an interaction between auditory and visual information (Weber et al. 1981, 1987, Weber, 1990; Shuvalow et al. 1990; Bohm et al. 1991).

The first steps in the processing of auditory and visual inputs in the central nervous system take place in separate centres. That for auditory information is in the prothoracic ganglion, the output from which is transmitted to the brain by a few ascending neurones (Schildberger et al. 1989). Visual information is processed in the optic ganglia (lamina, medulla and lobula) before being sent to the brain (Honegger and Schürmann, 1975; Honegger, 1980; Labhardt et al. 1984; Labhardt, 1988; Zufall et al. 1989). Presumably, therefore, integration of diverse sensory information is accomplished by local brain interneurones that transfer the result of this processing to neurones descending from the brain.

A number of such local neurones have been described, some of them with purely auditory inputs and others with bimodal or multimodal inputs (Boyan, 1980, 1984; Schildberger, 1984a,b). The descending neurones have their cell body and at least one dendritic input region in the brain and an axon that descends through the ipsilateral or contralateral connective to the suboesophageal ganglion. The terminal branches of the neurones described here are still unknown, so it is not yet possible to associate them with cells involved in the escape locomotion of crickets (Hörner, 1989). The arborization regions in the brain have been investigated and found to correspond to those of similar neurones in other
orthopteran species (Williams, 1975; Griss and Rowell, 1986; Hensler, 1988). The analyses in these studies were usually limited to immobilized animals, and no attention was paid to motor activity.

In the present study of the cricket, however, the patterns of response of brain neurones to uni- and multimodal stimulation have been found to differ depending on whether the animal was standing or walking. The differences in signal processing and the role of individual neurones in walking seem to be crucial for the control of behaviour.

The response characteristics of unimodal DINs of the cricket are less variable than those of multimodal neurones. In contrast, the latter rarely convey directional information. In this respect they differ from the multimodal DINs of the locust, which are involved in the control of flight and do give direction-specific responses. On the whole, the DINs of flying insects respond to visual stimuli with relatively little variability (Rind, 1989; Milde, 1986; Fischer et al. 1990). The apparent difference between cricket and locust neurones may be because only a few DINs have been examined in the cricket. It is also conceivable, of course, that commands controlling walking – in contrast to those controlling flight – are generated not in the brain but at the thoracic level. A largely parallel mode of processing at higher levels would allow more flexible control in a complex stimulus situation.

The response characteristics and the transfer properties of the DINs vary for different combinations of stimuli. Hence, these neuronal qualities are not permanently fixed; indeed, they are also influenced by simultaneous motor activity. For instance, the sound pattern of the calling song is more accurately reflected in the neuronal discharge while the animal is walking than when it is standing still. In this way, the processing of auditory information in brain neurones could perhaps compensate for ‘interference’ produced in the ear by the walking movements (Schildberger et al. 1988). Such phenomena, known as gating, have also been observed in thoracic neurones during flight (Reichert and Rowell, 1985).

Our results suggest that some of these DINs participate in the control of
walking: activation of one DIN caused the resting animal to begin walking, and interrupting the discharge of this DIN brought the walking animal to a halt. This neurone thus meets the operational criteria for a 'command neurone' (Wiersma and Ikeda, 1964; Kupfermann and Weiss, 1978), and shows that a single neurone can initiate behaviour (Eaton and DiDomenico, 1985; DiDomenico and Eaton, 1988). However, for two reasons there must be at least one additional 'command neurone' that initiates walking. First, there should be a mirror-image partner cell in the other side of the brain and, second, the depolarization-induced firing rate, necessary to initiate walking, is higher than the firing rate of the neurone during 'spontaneous' walking. Moreover, the course of the walk induced by neuronal depolarization showed no preferred direction, which can only mean that the initiation of a walk and the control of its velocity and direction depend on several descending brain neurones which, operating in parallel, are responsible for separate elements of walking activity. This finding confirms that our understanding of complex behaviour will advance only when it becomes possible to subdivide the behaviour into its components, to record simultaneously the activity of the neurones involved, and to manipulate these cells individually and in combination.

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