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GATING OF SENSORY RESPONSES OF DESCENDING BRAIN NEURONES DURING WALKING IN CRICKETS

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Summary

Single descending brain neurones were recorded and stained intracellularly in the neck connectives of crickets while they walked upon a styrofoam ball under open-loop conditions. The animal's translational and rotational velocities were measured simultaneously, and various stimuli were used to investigate the neuronal response characteristics. Stimulation with a moving grating or an artificial calling song of 5 kHz induced optomotor behaviour and positive phonotaxis. An acoustic stimulus of 20 kHz elicited negative phonotaxis.

We report the first clear evidence for behaviourally dependent gating of sensory responses of identified descending brain neurones. Most descending cells only responded to visual stimuli or to an artificial calling song of 5 kHz while the animal was walking, indicating that the responses to these stimuli were gated by the walking activity of the animal. In contrast to this, responses to stimuli that elicit negative phonotaxis, such as acoustic stimuli of 20 kHz, were not gated. This indicates that the gating of sensory responses in these cells depends on the behavioural context of the stimulus.

From these findings, we conclude that significant information about the properties of sensory processing in higher-order neurones can only be gained from tests in behaviourally relevant paradigms. Important characteristics might otherwise be missed, thus leading to misinterpretations regarding their function.

Key words: insect, brain, descending neurones, response characteristics, behaviourally dependent gating, correlations with walking, cricket, *Gryllus bimaculatus*.

Introduction

Measuring electrophysiological events in animals behaving normally remains one of the most powerful techniques for unravelling the neural basis of behaviour. The strength of such investigations lies in uncovering neuronal events that are not found in immobilized animals. Most studies have focused on locomotory behaviour, and it is now well established in both vertebrates and invertebrates that the operation of many motor reflexes is modulated in an activity-dependent fashion (for reviews, see Pearson, 1995; Prochazka, 1989). In arthropods, afferent information from the chordotonal organs sited on a leg exhibits different effects depending on the step phase. When the animal is standing, reflexes are employed which resist a flexion of the leg, while in the active state ongoing movements are reinforced (stick insect, Driesang and Büschges, 1996; locust, Wolf and Burrows, 1995; crayfish, El Manira *et al.* 1991). In cats, similar rhythmic reflex reversals occur in the Ib afferent pathway (Gossard *et al.* 1994). In all cases, these modulations are thought to provide the basis for adapting leg movements to different requirements during walking (e.g. uphill or downhill) or standing. Furthermore, flexible reactions, e.g. to obstacles encountered during walking, are made possible (Duysens *et al.* 1990). In crickets, differences in neural events and muscle activity were found to depend on the animal's activity state. Schildberger *et al.* (1988) showed that prothoracic auditory interneurones (e.g. ON1) in walking crickets receive excitatory and inhibitory inputs which are not evident when the animal is stationary. For *Teleogryllus oceanicus*, Nolen and Hoy (1984) reported that the dorsal longitudinal muscles that are active during behavioural responses to ultrasound in flight are not activated in response to the same stimulus when the animal is at rest, although no difference in the firing of sensory interneurones could be detected. So far, however, activity-dependent modulations and gating have been described only for neurones closely related to, or even belonging to, locomotor networks.

Huber (1959, 1960) showed that the cricket brain was crucial for initiating various behaviour patterns and also for determining their quantitative aspects. This indicates that interactions between sensory processing and command activity take place in the brain. Approximately 200 pairs of descending neurones (Staudacher and Schildberger, 1995) connect the brain with the caudal motor centres of the ventral nerve cord and convey

information about the brain's decisions (see Hedwig, 1994). Various studies on these cells have not only revealed command neurones (Böhm and Schildberger, 1992; Hedwig, 1994), but have also given indications that distributed neural activity codes for behaviour (locust walking, Kien, 1990*a*,*b*; locust flight, Hensler, 1992*b*; fly, Gronenberg and Strausfeld, 1990). However, little is known about whether they have the same importance under different stimulus conditions during walking.

In the present study, stimuli of different significance to the insect were used to elicit different natural taxis behaviours, such as optomotor behaviour (Hassenstein, 1951), positive phonotaxis (Weber *et al.* 1981) and negative phonotaxis (Moiseff *et al.* 1978; Nolen and Hoy, 1986). The behaviour was quantified, while simultaneously recording from single descending brain neurones and testing them for uni- or multimodality and directionality. We measure the response characteristics of descending neurones and correlate their activity with the rotational velocity of the animal. Moreover, we describe a form of gating of sensory responses during cricket walking which occurs in neurones that are not themselves directly involved in locomotion. Our results show that the responses of identified descending brain neurones to specific sensory stimuli of different behavioural significance are differentially gated in a behaviourally dependent manner when the cricket exhibits almost natural walking. Preliminary accounts of this work have been published as abstracts (Staudacher and Schildberger, 1993, 1995) and discussed in a review (Schildberger, 1994).

Materials and methods

Experimental animals and preparation

The experimental animals, 2- to 4-week-old adult female *Gryllus bimaculatus* de Geer, were reared in a laboratory culture at a temperature of 22–24 °C, a relative humidity of 40–60 % and with a 12 h:12 h light:dark regime. They were acoustically isolated from males as final instars until used in the experiments.

The sides of the animal's prothorax and the back of the head were attached to a metal holder with a beeswax and resin mixture in such a way that the antennae, mouthparts, legs and abdomen were free to move. The prothorax and neck were then opened dorsally, the fat body and connective tissue removed and the gut glued to one side in order to expose the neck connectives.

Experimental apparatus

The experimental arrangement is shown schematically in Fig. 1. The animals were positioned so that they could walk on top of an air-cushioned hollow styrofoam ball (diameter 12 cm; mass 4.5 g) covered with small reflecting dots (diameter 2 mm). A camera illuminated a field (diameter 3 cm) on the ball's equator in front of the animal with infrared light and detected the reflections of the dots (Weber *et al.* 1981). The positions of the dots within this field were measured separately for the *x*- and *y*-directions at a frequency of 100 Hz. A microprocessor transformed the measured dot positions on-line into analogue voltage values representing the distance moved by the dots within one measuring period (10 ms). Movements of the dots along the *x*-axis represent rotational movements of the ball; movements in the *y*-direction represent translational movements (Schildberger and Hörner, 1988). The running average for ten consecutive periods was calculated from these data and transformed into translational ($cm\,s^{-1}$) and rotational ($°\,s^{-1}$) velocities (Böhm *et al.* 1991) to give the intended walking speed and direction of the cricket.

Two loudspeakers were positioned to the left and right in front of the animal at an azimuth of 50 °. The distance between the speakers and the animal was 35 cm. The artificial calling song consisted of two chirps per second, each chirp containing four syllables. These had a duration of 20 ms, including rise and fall times of 2 ms, and were separated by a pause of 20 ms. Frequencies of 5 and 20 kHz were used with intensities of 80–90 dB SPL (±2 dB). A movable pattern of vertical black-and-white bars was projected onto a curtain surrounding the animal using a device developed by Scharstein (1989). The visual field extended 144 ° in azimuth and 65 ° above and 44 ° below the animal. The contrast of the stripes was 0.87, and their width in front of the animal 25 °. The contrast frequencies used lay between 0.5 and 11.0 Hz. The movement of the grating was monitored by a phototransistor placed at the curtain.

Electrophysiology

Intracellular recordings were made from axons of descending brain neurones in the exposed neck connectives. Microelectrodes were pulled from thick-walled glass capillaries (Clark 100G GF). The tips were filled with either 3 % Lucifer Yellow or 5 % Neurobiotin, while the shaft was filled with 0.1 mol l^{-1} lithium chloride or 0.1 mol l^{-1} potassium acetate, respectively. These electrodes had tip resistances between 60 and 100 MΩ. The connectives were mechanically stabilized by an underlying silver platform and an overlying metal ring. The silver platform also served as the indifferent electrode. After recording and iontophoretic dye injection, the animal was placed in a humidity chamber for 15–24 h at 4 °C to allow diffusion of the dye. The nervous system was then prefixed (4 % formaldehyde in Millonig's buffer, pH 7.4) within the animal and dissected as a whole chain of ganglia (from the terminal ganglion to the brain). After further fixation (2 h at room temperature), the chains of ganglia were treated in different ways depending on the dye used.

Anatomical techniques

Lucifer-Yellow-stained cells were processed with an antibody against the dye according to the peroxidase–antiperoxidase method (Taghert *et al.* 1982). Neurobiotin-stained cells were visualized using the avidin–biotin method (Horikawa and Armstrong, 1988). Diaminobenzidine served as the chromogen. Cells were reconstructed from serial sections or drawn from wholemount preparations using the *camera lucida* technique. Descriptions of their morphology refer to the embryonic neuroaxis. Cells were termed ipsilateral (contralateral) descending neurones when their axon left the brain on the side ipsilateral

(contralateral) to the pericaryon. Soma clusters were named according to a system developed on the basis of that given by Rosentreter and Schürmann (1982).

Data evaluation

Physiological data were stored on magnetic tape (Racal Store 7DS). These data were later digitized and, in a first step, evaluated using the SuperScope II program running on a Macintosh IIcx. Data were then transformed into velocity values, times of action potential occurrence and the number of action potentials per period in Microsoft Excel 4.0 for Macintosh. Dot plots, post-stimulus-time histograms, scatter diagrams, *t*-tests and correlation analyses were performed in StatView 4.0 for Macintosh.

Analyses of correlations between neural activity and mean translational or rotational velocity of the animal were carried out as follows. For 42 stimulus periods, the number of action potentials (n_{AP}), and the mean translational (V_{Tra}, $cm\,s^{-1}$) and rotational (V_{Rot}, $°\,s^{-1}$) velocities were evaluated. Each series of values was represented by a separate column (n_{AP}, V_{Rot}, V_{Tra}) in a spreadsheet. The first cell of each column contained the value measured in the first period. Paired values of n_{AP} and V_{Rot} or V_{Tra} were plotted in a bivariate scatter diagram that also gave a regression line (same period; StatView 4.0). Pearson's correlation coefficient (r) was calculated for these pairs of values and tested against zero using a *t*-test (StatView 4.0). Correlation coefficients greater than 0.5 and P values of 0.0002 or below were considered as statistically significant. To analyse correlations between neural activity and V_{Rot} or V_{Tra} values for the following (or previous) period, data for V_{Rot} and V_{Tra} were shifted by one cell. Depending on the direction of shift, the first (second) n_{AP} value was paired with the second (first) V_{Rot} and V_{Tra} value. These data were plotted in the same way as described above. Pearson's correlation coefficient (r) was also calculated for these shifted pairs of values and tested against zero using a *t*-test (paired or unpaired when appropriate; StatView 4.0).

Results

There are at least 200 ipsi- and contralateral brain neurones descending to the thoracic ganglia. Their pericarya are located in different clusters in the brain. This paper focuses on two of these neurones to exemplify our finding that many descending cells respond to sensory stimuli only when the animal is walking and that this activity-dependent gating of sensory responses is dependent on the behavioural context of the stimulus.

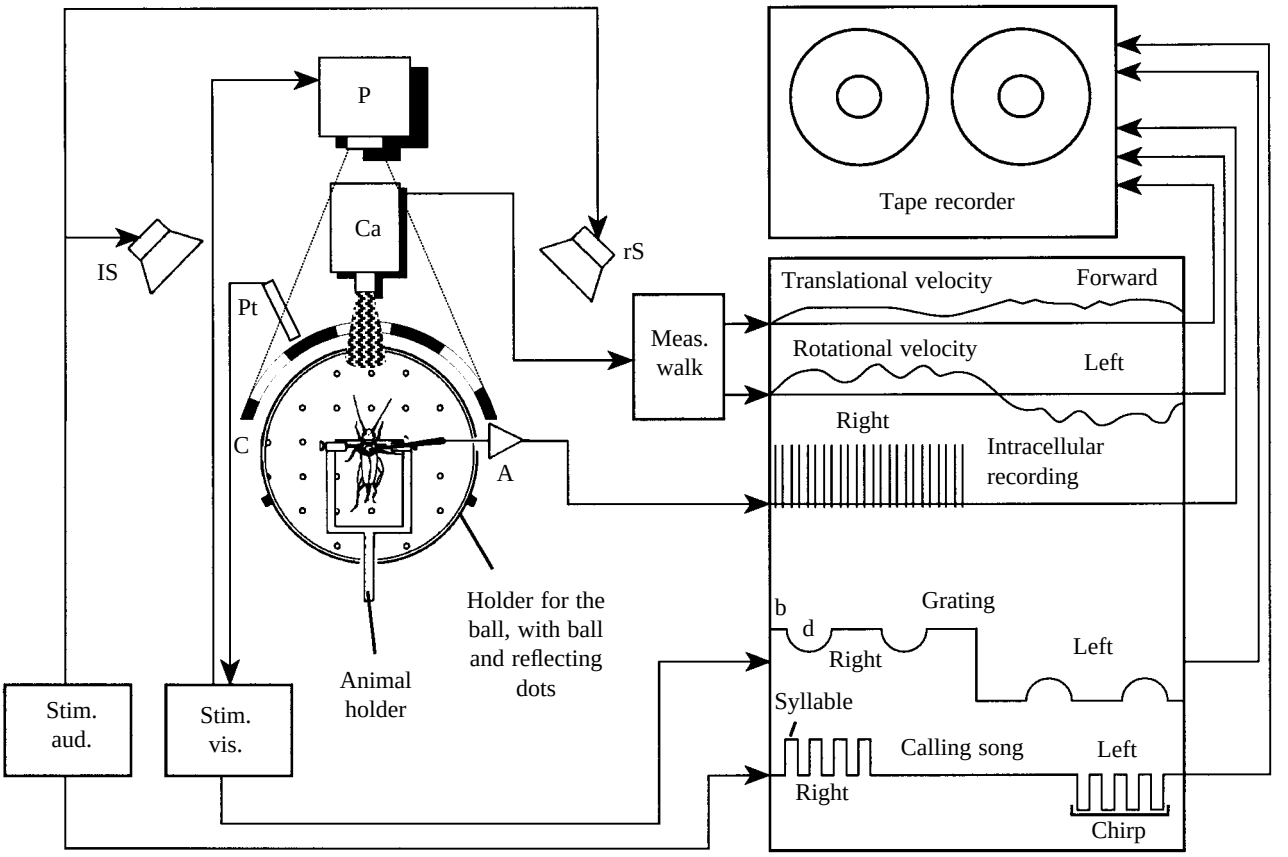


Fig. 1. Diagram of the experimental arrangement with all the components for stimulus generation, registration and coding. A, amplifier; C, curtain for projection of the grating; Ca, camera; b, bright; d, dark; lS, rS, left, right speaker; Meas. walk., unit for measurement of the walking parameters; P, projection unit for visual stimuli; Pt, phototransistor; Stim., stimulus generators for auditory (aud.) and visual (vis.) stimulation (modified after Schildberger and Hörner, 1988).

The first cell we descibe can be regarded as newly identified in crickets; it was recorded and stained ten times in different animals. The cell body of this contralateral descending neurone is located in cluster *c2*; we therefore call it DBNc2-1 (*d*escending *b*rain *n*eurone of cluster *c2* number *1*). This first cell responds to visual stimulation. The second part of this paper deals with data (nine recordings and subsequent stainings of the projections up to the brain) from a morphologically and physiologically quite homogeneous group of cells that have their cell bodies in the ipsilateral cluster *i5* (at least 19 neurones; Staudacher and Schildberger, 1995). Since we are not yet able to distinguish them individually, they are called *i5*-type cells and are regarded as characterized neurones. While they primarily respond to acoustic stimuli, some also respond weakly to the moving grating.

DBNc2-1, an identified visual neurone

Morphology

The large cell body (diameter approximately 40 μm in diameter) of the DBNc2-1 neurone (Fig. 2A) lies in the dorsal cluster *c2*. The primary neurite describes a latero-ventral-median curve towards the medial protocerebrum. This neurite branches at a depth of approximately 100 μm dorsal to the central complex (asterisks in Fig. 2A,B). The main ipsilateral arborization zone is located at the same depth. From here, one prominent large branch runs deep into the lateral ocellar root (Fig. 2A,B). The axon aims dorsally and turns medially to cross the midline in the dorsal commissure XVI (DC XVI; for the locust, see Boyan *et al.* 1993). Near the border with the deutocerebrum, the cell gives off some branches, at a depth of approximately 150 μm dorsal to the central complex, which reach into the non-glomerular neuropile of the dorsal lateral protocerebrum (Fig. 2A). The axon then descends *via* the circumoesophageal connective contralateral to the cell body. In the suboesophageal ganglion and the thoracic ganglia, the axon runs in the medial dorsal tract (MDT; for the locust, see Tyrer and Gregory, 1982). The arborizations are located dorsally and are exclusively ipsilateral (Fig. 2A). The stain always ended in the metathoracic ganglion. The DBNc2-1 -neurone is probably homologous to the locust 'DNC' (Griss and Rowell, 1986) or the 'O2' neurone (Williams, 1975).

Response characteristics: walking versus *standing*

In the quiescent, unstimulated animal, the DBNc2-1 neurone remained silent. When the animal walked spontaneously and without stimulation, the cell fired some action potentials, but no patterned activity occurred (Fig. 3A). Auditory stimulation elicited no response in this neurone. However, the activity pattern of the neurone changed quite dramatically when the cell was optically stimulated with the grating while the animal was walking. Fig. 3B shows an example of the response of the DBNc2-1 cell to the moving grating while the animal exhibits optomotor behaviour as shown by the intention to turn to the side of the movement of the grating. This neurone responded with 1–6 action potentials to each black/white change (Fig. 3B and inset) up to the maximal frequency tested (11 Hz). The short bursts of action potentials in the DBNc2-1 cells upon visual stimulation depended neither on the direction of pattern

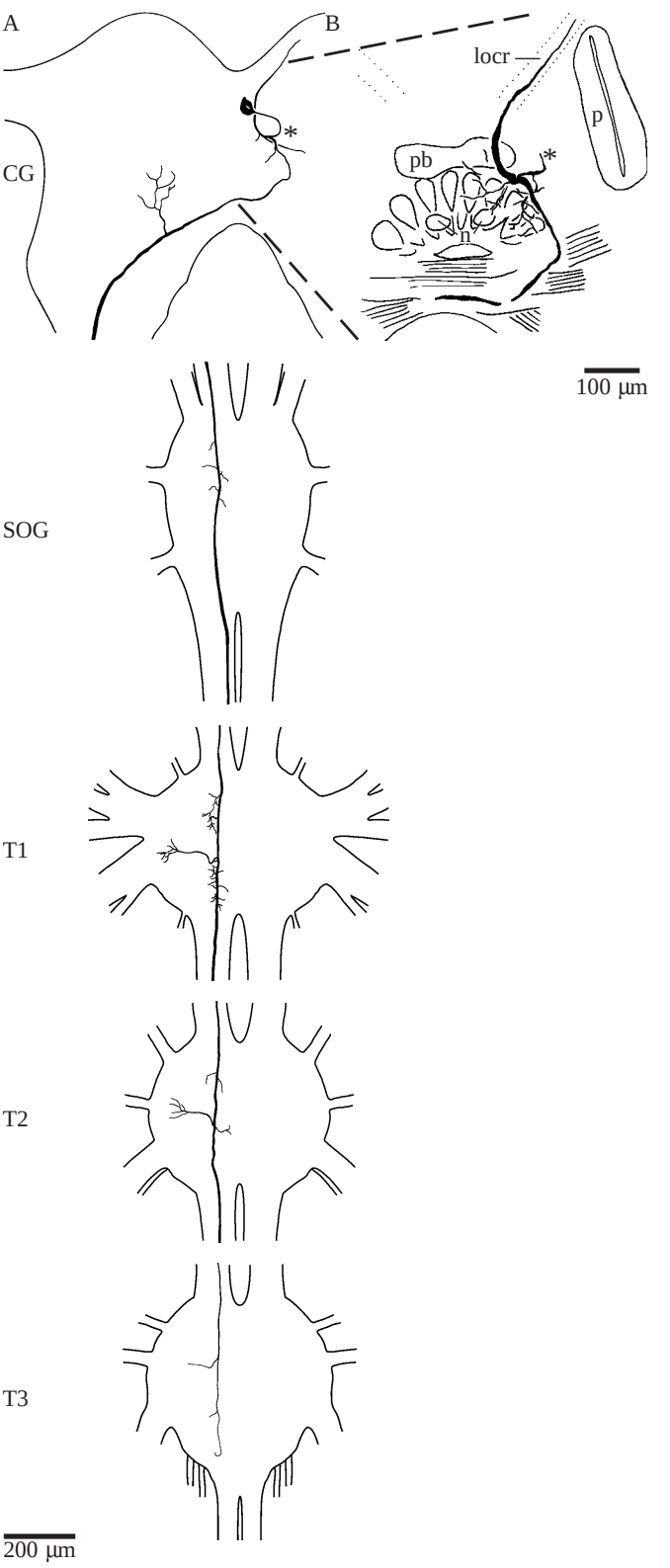


Fig. 2. (A,B) Morphology of the DBNc2-1 cell. (A) Wholemount drawing of the DBNc2-1 cell in the brain (CG), the suboesophageal ganglion (SOG) and the three thoracic ganglia (T1, T2, T3). (B) Reconstruction of the protocerebral arborizations of another DBNc2-1 cell based on four serial sections (thickness 40 μm). locr, lateral ocellar root; n, noduli; p, peduncle; pb, protocerebral bridge; asterisk, primary neurite.

movement nor on the walking direction of the animal (Fig. 3B and inset). However, when the animal stopped walking, the cell no longer responded to the moving grating. At the beginning of the recording shown in Fig. 3C, the animal was walking and the cell responded. When the animal stopped walking, the neural response to the black/white changes ceased while the animal was standing (middle part of Fig. 3C). This response decrement is different from habituation, since changing the direction of grating movement failed to evoke a response when the animal was stationary. When the animal resumed walking, the cell responded again (last part of Fig. 3C). In contrast to this, the cell responded to the lights being turned off with a burst of action potentials regardless of whether the animal was walking or standing. Interestingly, the cell's response to the moving grating did not cease immediately when the animals (N=9) stopped walking, but after a variable time of 1–4 stimulus periods (one stimulus period is 750 ms). In contrast, when the animals (N=9) started to walk again, the cells responded immediately to the black/white changes (Fig. 3C). One out of nine DBNc2-1 cells recorded showed only a weakening of its responses to the moving grating and not a complete cessation of activity when the animal stopped walking. Another of these nine cells only responded to the grating after 3.5 min had elapsed, although the animal walked all the time. However, its response characteristics did not differ from those of the other DBNc2-1 neurones.

No significant linear correlations between neural activity, as represented by the number of action potentials per stimulus period, and either the mean translational or rotational walking velocity within a period were found for any stimulus situation tested (criteria r>0.5, $P\leqslant$0.0002; compare *i5* cells, below).

i5 cells, a characterized group of auditory neurones

Morphology

At least 19 very similar ipsilateral descending neurones have

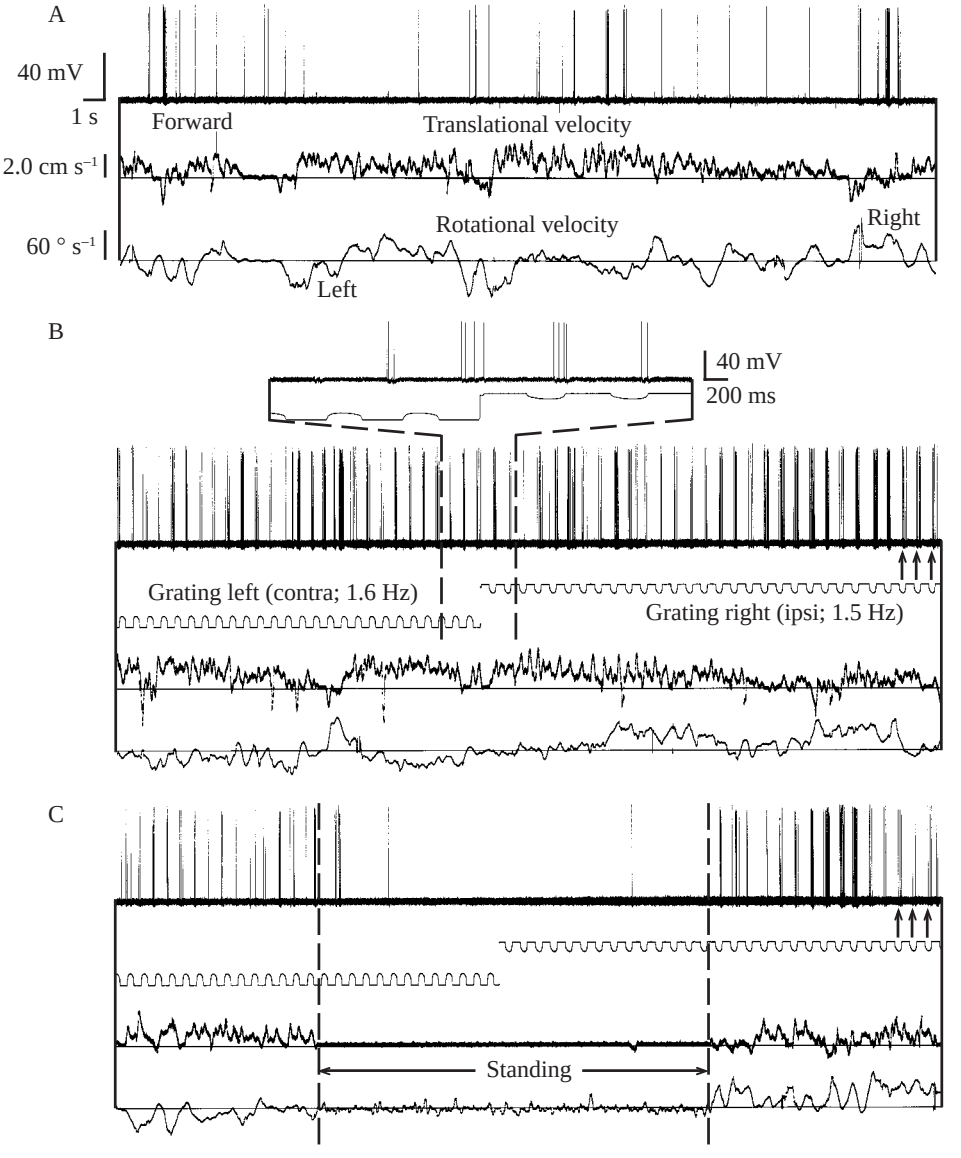


Fig. 3. (A–C) Response characteristics of the DBNc2-1 cell. (A) First trace, intracellular recording; second trace, translational velocity (cm s^{-1}); third trace, rotational velocity (° s^{-1}). (B,C) First trace, intracellular recording; second trace, visual stimulus, third trace, translational velocity (cm s^{-1}); fourth trace, rotational velocity (° s^{-1}). Calibration bars in A are valid for all parts of the figure, except the inset in B. All examples stem from one recording. (A) No stimulation, while the animal is walking. (B) Stimulation with a moving grating (left or right, contrast frequency 1.6 and 1.5 Hz respectively) while the animal is walking. Inset: expanded intracellular record. (C) The same situation as in B, but the animal stopped walking during the middle of the sequence. The regular peaks in the translational velocity (third trace) appear to represent single steps of the walking animal. These deflections are not correlated with the movement of the grating or with neuronal activity; they also occur in the unstimulated but walking animal (A).

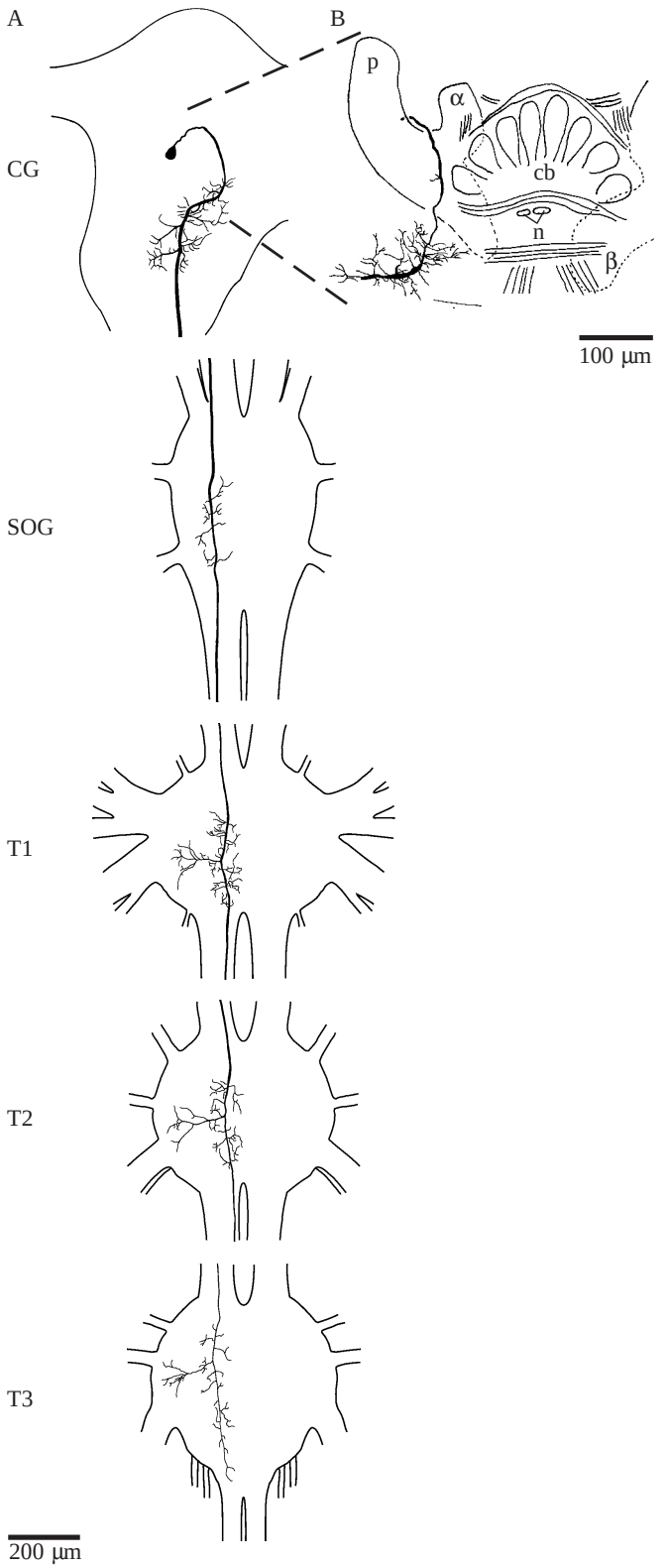


Fig. 4. (A,B) Morphology of the *i5*-type cell. (A) The arborizations of an *i5*-type cell in the brain (CG) were reconstructed from serial sections (thickness 20 μm). The arborizations in the other ganglia (SOG, T1, T2, T3) were drawn from whole mounts. (B) Reconstruction of the central arborizations of another *i5*-type cell based on four serial sections (thickness 40 μm). α, alpha lobe; β, beta lobe; cb, central body; n, noduli; p, peduncle.

their perikaryon in cluster *i5*, which is located in the lateral ventral soma rind of the protocerebrum. Fig. 4A shows a reconstruction of one *i5*-type cell. The central arborizations of another *i5* neurone are reconstructed at higher magnification in Fig. 4B. From the ventro-lateral soma, the primary neurite of the *i5*-type cell runs dorsally in a medial curve. After passing between the ramification of the alpha-lobe and the peduncle of the mushroom bodies, the neurite turns posteriorly at the depth of the central complex (Fig. 4B). Here, the main arborizations of the *i5*-type cell arise in the medio-lateral protocerebrum, posterior to the central complex. However, none of the branches enters the central complex. After bending posteriorly, the axon gives off some further branches in the dorsal non-glomerular deutocerebrum, before it descends through the circumoesophageal connective ipsilateral to the soma. The axon runs and arborizes exclusively ipsilaterally in the dorsal part of the suboesophageal and thoracic ganglia. Four basic subtypes of *i5* cells can be anatomically distinguished by the presence or absence of branches in the suboesophageal ganglion and on the basis of whether or not they descend further to the abdominal ganglia. Cells from cluster *i5* are homologous to locust LG cells (Williams, 1975) and the *i5* cells in *Acheta domesticus* (Rosentreter and Schürmann, 1982). The previously described IDBN cell (*Gryllus bimaculatus*; Boyan and Williams, 1981) and DBIN2 neurone (*Teleogryllus oceanicus*; Brodfuehrer and Hoy, 1990) probably also belong to the *i5* cluster.

Response characteristics: 5 kHz stimuli

The responses of *i5*-type cells to an artificial calling song with a carrier frequency of 5 kHz are shown in Fig. 5. In the first part of Fig. 5A, the animal was stimulated at 5 kHz 80 dB SPL from the side ipsilateral to the cell body of the recorded neurone (right speaker). The animal walked throughout most of the sequence and intended to turn towards the speaker. The cell responded to the ipsilaterally presented artificial song by producing at least one action potential for the first syllable of each chirp. For four chirps, this auditory response is shown at a higher temporal resolution in the inset of Fig. 5A. Most of the *i5*-type cells 'copied' every syllable of the song (Fig. 5C,D), while some only 'copied' the first syllable or the whole chirp. Dot plots (Fig. 5C) and post-stimulus-time histograms (Fig. 5D) both show that action potentials also appear that are not stimulus-correlated. These are probably due to noise introduced by leg movement during walking (Schildberger *et al.* 1988). As the second part of Fig. 5A shows, the *i5* cells did not respond to the acoustic stimulus when it was presented from the side contralateral to the cell body, even though the animal walked and intended to turn towards the active speaker (left speaker; Fig. 5A). Only one *i5*-type neurone responded, although very weakly, to a contralateral acoustic stimulus of 5 kHz 90 dB SPL. A similar situation is shown in Fig. 5B for the same neurone. Here, the animal walked while it was stimulated from the ipsilateral (right) and contralateral (left) side (first part of Fig. 5B). In the last part of this recording, the animal stood still while it was stimulated contra- and ipsilaterally. As in the DBNc2-1 neurone, the response of the

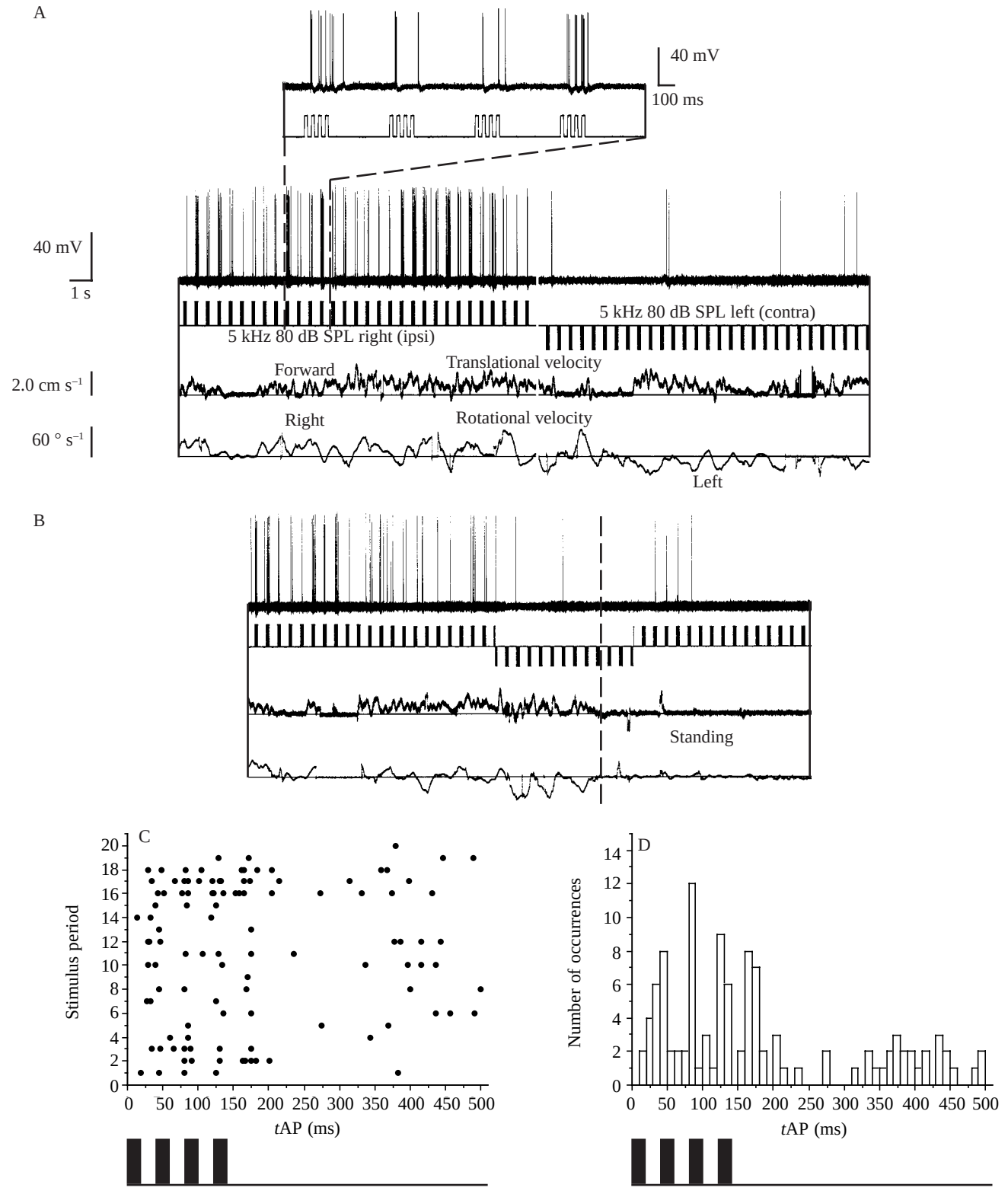


Fig. 5. Responses of *i5*-type neurones to an artificial calling song at 5 kHz. Recordings A and B are for the same cell. Second trace, acoustic stimulation (each black bar represents one chirp), other traces as in Fig. 3. Calibration bars are valid for both examples, except for the inset in A. (A) Acoustic stimulation, both ipsi- and contralateral, 5 kHz 80 dB SPL, while the animal is walking. Inset: expanded intracellular recording. (B) Stimulation with ipsi- and contralateral calling song, 5 kHz 80 dB SPL, with the animal standing in the last part. (C) Dot plot and (D) post-stimulus-time histogram for another *i5*-cell stimulated ipsilaterally with the calling song (5 kHz, 90 dB SPL) while the animal was walking. Both graphs contain data from 20 consecutive stimulus periods. One stimulus period (1 chirp = 4 syllables) is depicted schematically below the diagrams. Graphs (B,C) and stimulus period have the same time scale. t_{AP}, time of occurrence of an action potential. Bin width 10 ms.

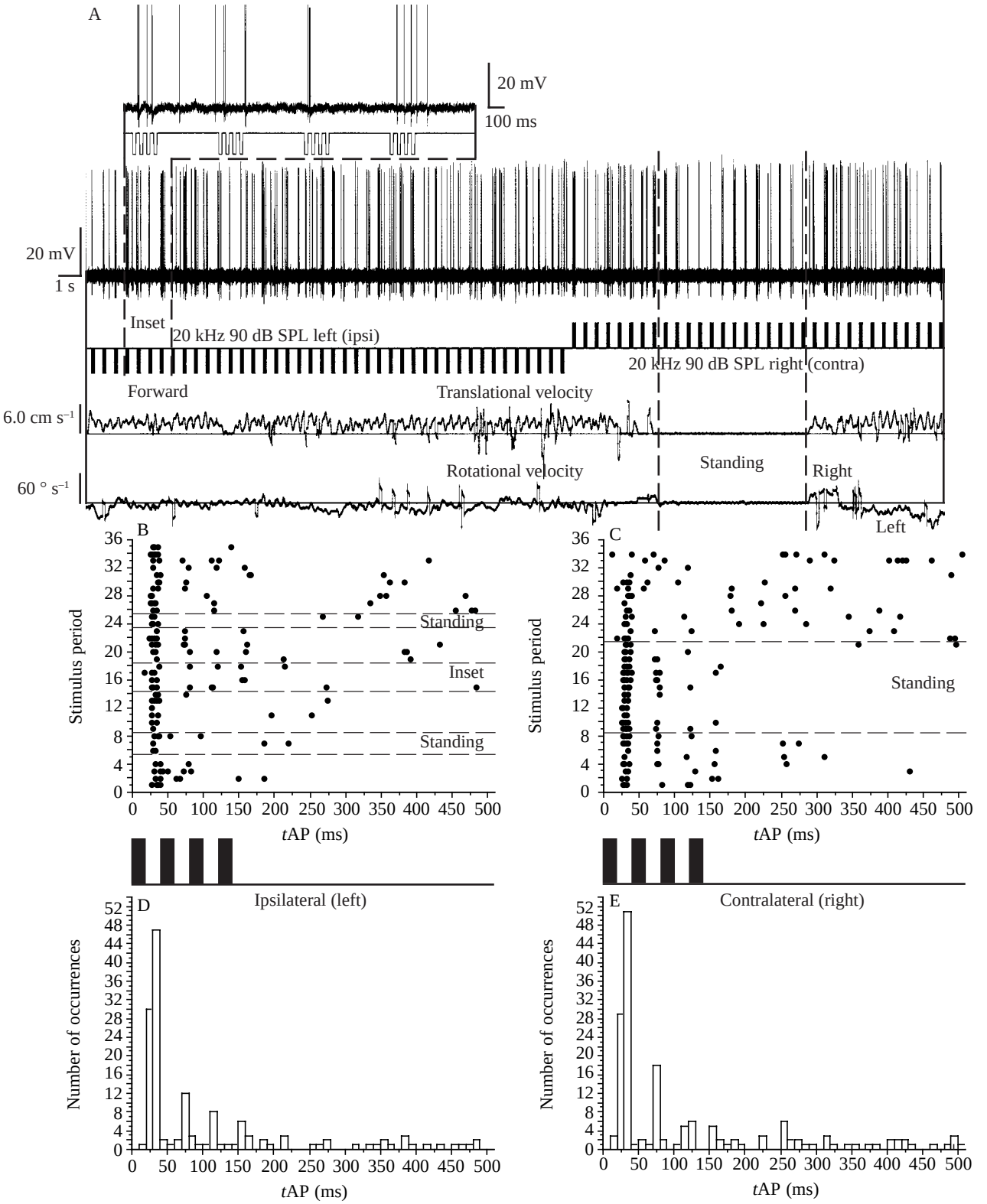


Fig. 6. Responses of an *i5*-type neurone to stimulation with an artificial calling song at 20 kHz. Recording and diagrams are for the same cell. Traces as in Fig. 5. (A) Acoustic stimulation, both ipsi- and contralateral, 20 kHz 90 dB SPL, while the animal is walking or standing. Inset: expanded intracellular recording. Calibrations are different from the compressed traces. (B,C) Dot plots and (D,E) post-stimulus-time histograms for this cell, stimulated ipsilaterally (B,D) and contralaterally (C,D) with the calling song (20 kHz, 90 dB SPL). Stimulus periods shown in A at higher temporal resolution are labelled 'inset' in B. Stimulus periods occurring while the animal was standing are labelled 'standing' in B and C. Both graphs contain data from 36 consecutive stimulus periods. Graphs (B–E) and stimulus period have the same time scale. t_{AP}, time of occurrence of an action potential. Bin width 10 ms.

cell depended on the animal's activity. The cell did not respond to the artificial calling song from the ipsilateral (right) side while the animal stood still. However, the first four chirps elicited a response of one action potential. As found for the DBNc2-1 cell (Fig. 3C), the *i5* cells immediately responded to the calling song when the animal started to walk.

Response characteristics: 20 kHz stimuli

When, as shown in Fig. 6A, the animal was presented with an acoustic stimulus that had the same temporal pattern as the calling song but a carrier frequency of 20 kHz (intensity 90 dB SPL), the cell responded to it regardless of the side from which it was presented. As the inset of Fig. 6A shows at higher temporal resolution, the first syllable of the stimulus was 'copied' with high fidelity, while the others were not always represented. This is also shown in the dot plots (Fig. 6B,C) and post-stimulus-time histograms (Fig. 6D,E). As in the previous example, the neurone fired not only in response to the acoustic stimulus but also in response to noise introduced by leg movements. Therefore, its overall activity seems to be higher during walking than during standing. As for stimulation at 5 kHz, there were differences in the way that the temporal pattern of the acoustic stimulus was represented. Some cells 'copied' all the syllables, others only the first two or only the chirp pattern.

When the animal was walking, all the *i5*-type cells tested responded to the ultrasound stimulus regardless of the direction from which it was presented. In contrast to the stimulus at 5 kHz, the neurones responded to the ultrasound stimulus even when the animal was stationary. As the dot plots in Fig. 6B,C show, the strength of the neural response to the first syllable remains quantitatively unchanged regardless of whether the animal is walking or standing (Fig. 6B,C, standing). In the standing animal, however, the response is more apparent, since no walking-induced neural activity appears (Fig. 6A, standing). Thus, in contrast to the response to 5 kHz, the response to ultrasound did not depend on the walking activity of the animal. Moreover, for ultrasound, there was no change in the number of action potentials in the responses of the walking or standing animal.

Comparison of different stimulus situations

A quantitative comparison of the mean neural activity during different stimulus and behavioural situations is shown in Fig. 7. Each column represents the mean neural activity within 17 (or four periods for the far right-hand column) periods of 510 ms, which is equivalent to one chirp period. When the animal was walking spontaneously, i.e. no experimentally controlled stimulus was presented, neural activity was significantly higher (P=0.0007) than during standing. While the animal was walking, an ipsilateral presentation of the artificial calling song (5 kHz 90 dB SPL) induced significantly greater (P=0.0002) neural activity than a contralateral presentation. Furthermore, activity elicited by ipsilateral presentation of the 5 kHz stimulus to the walking animal was significantly greater than activity elicited by ipsi- and contralateral presentations of the same stimulus while the animal was standing (P<0.0001). These findings differ in two ways from the results obtained for stimulation with ultrasound (20 kHz 90 dB SPL). First, the mean neural activities elicited by ipsi- and contralateral ultrasound stimulation do not differ. Second, no significant differences between neural activity in the walking and the standing animal were detected.

Correlations between neural activity and rotational velocity in i5-type neurones

5 kHz ipsilateral

As described above for the DBNc2-1 cell and *i5*-type neurones, there is a link between the animal's behaviour and

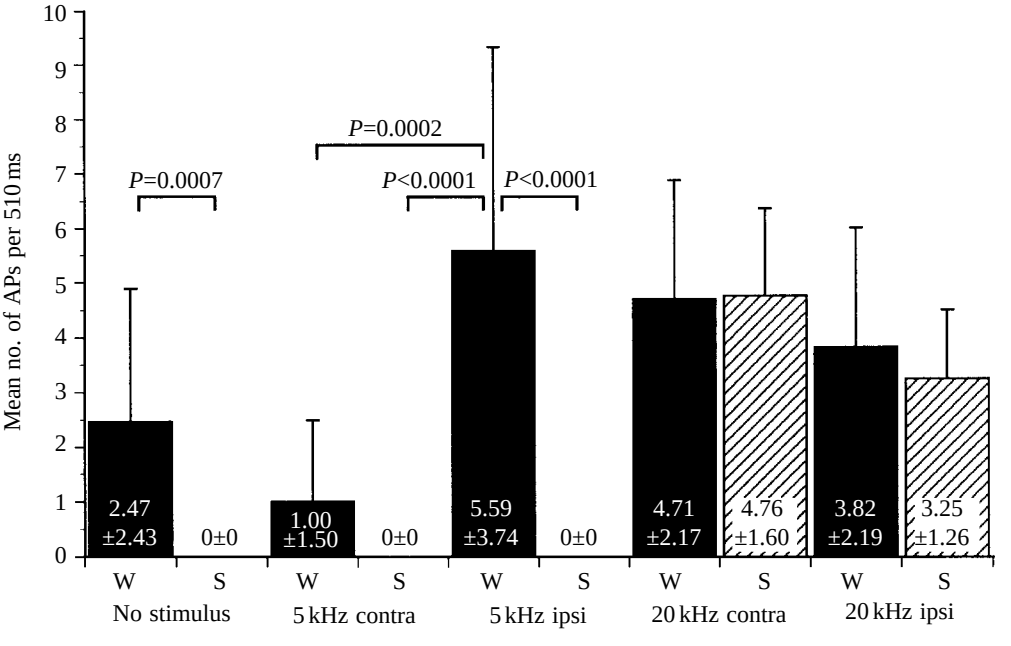
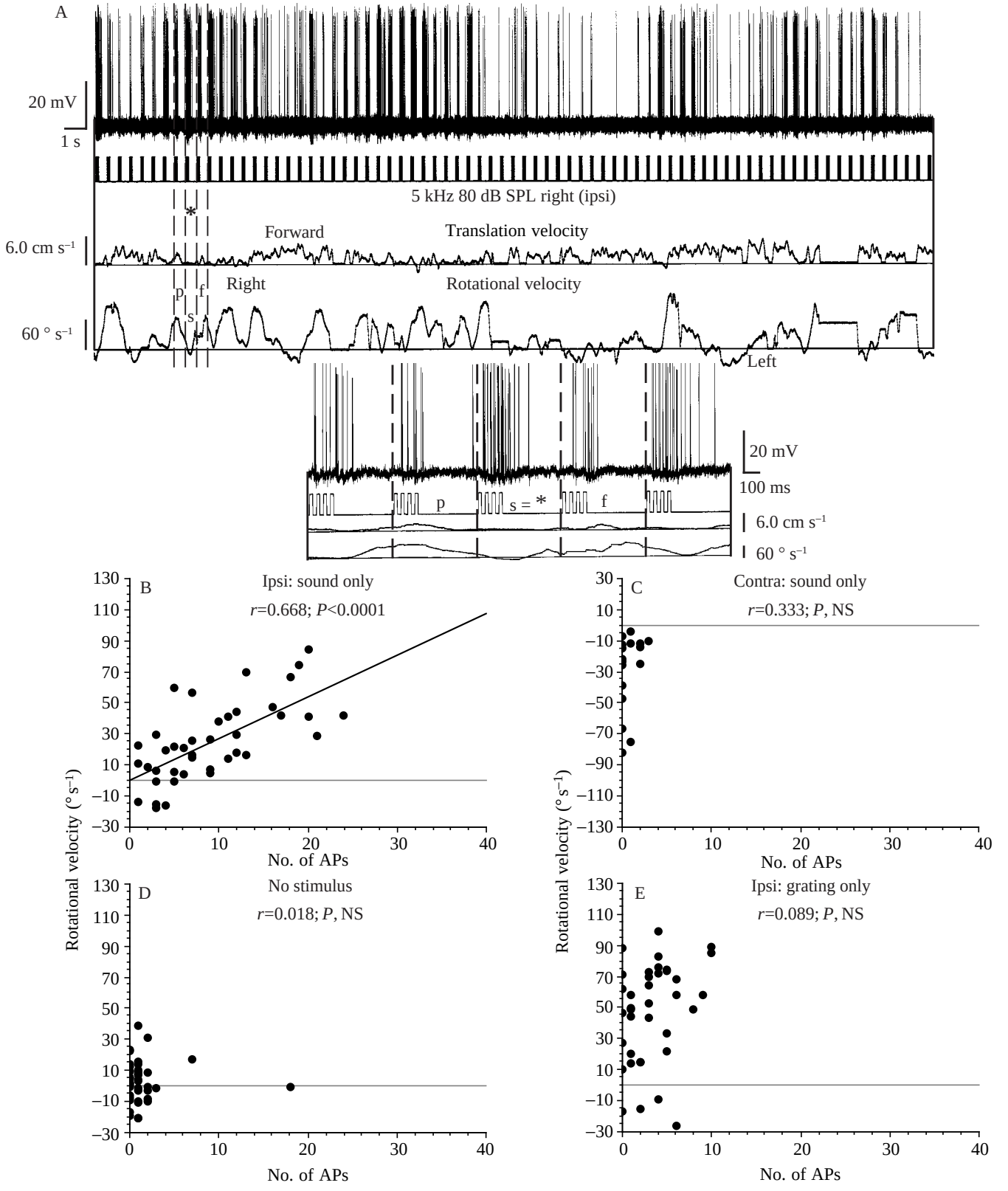


Fig. 7. Comparison of the responses when the animal is walking (W, filled bars) and stationary (S, hatched bars) of a single *i5*-type cell to the artificial calling song presented from either ipsilateral (ipsi) or contralateral (contra) at the natural carrier frequency (5 kHz, 90 dB SPL) and at ultrasonic frequency (20 kHz, 90 dB SPL). The evaluations for each stimulus/behaviour combination are based on 17 periods (duration of each period 510 ms), except the last (S 20 kHz ipsi), for which only four periods were available. The error bars give the standard deviations. Means and standard deviations are given at the bases of the columns. Statistically significant differences are marked by parentheses, with the P values noted above. APs, action potentials.

neuronal activity, since responses to certain stimuli only occur in the walking animal. Moreover, there is also a correlation between the auditory responses of *i5*-type cells and the rotational velocity of the animals. An example for this is shown in the recording in Fig. 8A. Here, the animal was mostly walking forward and intended to turn to the right. Since the intended turns are directed towards the active speaker, which presents a phonotactically attractive stimulus (right; ipsilateral; 5 kHz



80 dB SPL), this behavioural response is regarded as positive phonotaxis. At the same time, the *i5*-type cell responded to the acoustic stimulus. These responses to the acoustic stimulus were strong when the animal intended to turn towards the active speaker (right, ipsilateral), while they were weak or absent when the animal intended to turn away from the speaker.

For a quantitative analysis, the number of action potentials was counted for 42 consecutive stimulus periods (510 ms). Simultaneously, the mean translational and rotational velocities occurring during the same periods were calculated. To test whether the neural activity, i.e. the response to the acoustic stimulus, of the cell within one stimulus period (for example, the asterisk in Fig. 8A and the inset) was correlated significantly with the mean rotational velocity of the previous, the same or the following stimulus period (p, s, f: as indicated in Fig. 8A and the inset), correlograms were plotted for each of these combinations, and Pearson's correlation coefficients (r) were calculated and tested for their significance (t-test; P). This analysis revealed no significant linear correlations between neural activity and the mean rotational velocity within the same (s: r=0.426, P not significant) or previous (p: r=0.189, P not significant) stimulus period. However, the number of action potentials within one period was positively correlated to the mean rotational velocity in the next period (f: r=0.668, P<0.0001; Fig. 8B, y=0.438+2.684x). Here, the cell's activity varied between 1 and 24 action potentials per period (abscissa), while the animal's mean rotational velocities during the following period were between −17 and +85 °s^{-1} (ordinate). With increasing numbers of action potentials per period, the mean rotational velocities also increased. The activity within one period is plotted against the mean rotational velocity of the next period (f). This means that changes in neural activity take place one period before the mean rotational velocity alters. Thus, the animal turns more rapidly towards the sound source when the *i5*-type neurone fires more action potentials in response to the auditory stimulus.

Other stimulus conditions

In order to determine whether significant correlations are found for other stimulus conditions, the following analyses were made (Fig. 8C–E). During contralateral stimulation with 5 kHz 80 dB SPL, the animal intended to turn towards the speaker with similar rotational velocities to those during ipsilateral stimulation. In contrast, only a few action potentials occurred and these were not correlated to the rotational velocity of the animal (Fig. 8C). When no stimulus was presented, the animal's rotational velocities were distributed nearly homogeneously between +15 and −15 °s^{-1} (Fig. 8D). In this situation, the cell only fired a few action potentials. When stimulated with the grating only (turning right, ipsilaterally), the animal's rotational velocities were quite high (up to 100 °s^{-1}; Fig. 8E). Furthermore, the animal intended to turn with the direction of the moving grating, i.e. it showed optomotor behaviour. The *i5* neurone fired, but its neural activity did not exceed 10 action potentials per period (Fig. 8E). In none of these cases, however, could a significant linear correlation between neural activity and rotational velocity be detected.

For seven out of nine recorded and stained *i5* cells, significant linear correlations ($0.528 \leqslant r \leqslant 0.721$ and $P \leqslant 0.0002$) were found with the walking parameters of the animal. Most of these correlations occurred with rotational velocity and especially with the rotational velocity of the following period. This means that changes in the neuronal activity of many *i5* neurones occur before alterations in behaviour are seen. Moreover, no coupling was found between translational and rotational velocities since, in these cases, the correlations for translational and rotational velocity should both be higher than 0.5. This indicates that the neural representations of these parameters are separated.

Fig. 8. Correlation between the activity of an *i5*-type neurone and the rotational velocity of the walking animal. Recordings and graphs are for the same cell. (A) Recording for ipsilateral acoustic stimulation, 5 kHz 80 dB SPL, during walking. Traces are as in Fig. 5. The inset shows five stimulus periods at a higher temporal resolution. (B–E) Correllograms for the number of action potentials (APs) per stimulus period (510 ms) *versus* mean rotational velocity of the following (f) stimulus period. The graphs contain data from 42 consecutive stimulus periods, except C, for which only 17 periods were available. (B) Correllogram for ipsilateral stimulation with an artificial calling song at 5 kHz and the rotational velocity of the following period. (C) Correllogram for contralateral stimulation with 5 kHz 80 dB SPL. (D) Correlogram for no stimulation. (E) Correlogram for ipsilateral stimulation with a moving grating (1.5 Hz) alone. Significance level used, $P \leqslant 0.0002$. f, p, s, mean rotational velocity of the following, the previous or the same stimulus period (Fig. 8A); NS, not significant; asterisk, neural activity in one stimulus period (in A).

Discussion

Sensory processing and descending cells

The descending neurones investigated here respond either to visual or to auditory stimuli, and some *i5* cells respond to both. Moreover, different cells exhibit different characteristics with respect to directionality, sensitivity and gating of responses to one modality (Staudacher and Schildberger, 1993), thus indicating parallel descending pathways. When stimulated with 5 kHz at 80–90 dB SPL under free-field conditions, auditory receptors (Boyd and Lewis, 1983) and thoracic first-order interneurones (Stabel *et al.* 1989; Horseman and Huber, 1994) respond to sound presented from both the ipsi- and contralateral sides. In contrast, the *i5*-type cells in the brain only responded to ipsilateral sound presentation. Furthermore, the responses of *i5* cells to 20 kHz (80–90 dB SPL) did not differ quantitatively when sound was presented from the side ipsi- or contralateral to the soma. The same finding was reported for DBIN2 cell (*Teleogryllus oceanicus*; Brodfuehrer and Hoy, 1990), a presumed homologue of the *i5* cells. This is not only different from their characteristics at 5 kHz, but also from the directionality of prothoracic auditory neurones. In the latter, contralateral stimuli of the same intensity also elicit responses, but the responses are quantitatively weaker. The assumption of different auditory thresholds for 5 and 20 kHz might explain why 5 kHz stimuli are answered only when they

are presented ipsilaterally. However, it would not explain why the responses to 20 kHz are quantitatively the same regardless of the side from which the stimulus is presented. Therefore, these findings indicate that further auditory processing takes place between the ascending primary interneurones and the descending cells. Whether contralateral auditory connections, e.g. *via* the LBN-e or LBN-i cells (*Teleogryllus*; Brodfuehrer and Hoy, 1990), which also arborize in the dorsal lateral protocerebrum, are involved in forming the response characteristics of *i5* cells needs to be investigated.

The data presented in the present paper for the DBNc2-1 neurone are based on ten separate recordings and stainings. Since we do not know the connectivity of the DBNc2-1 neurone and cannot detect graded potentials in these axonal recordings, we are unable to explain two exceptional observations. However, these examples seem somewhat similar to the 'lapses' Roeder (1970) described in moth auditory brain neurones or the changes in the firing pattern of locust suboesophageal interneurones reported by Kien and Altman (1984).

Correlations with behaviour

Seven out of nine recorded *i5* neurones showed clear linear correlations between their response strength and sensory stimuli (e.g. auditory) and the animal's rotational or translational velocity. These highly significant correlations mostly occur for the rotational velocity of the animal and therefore indicate that the activity of these cells might influence the rotational component of the animal's walking behaviour. This is supported by the finding that activity changes in many of these neurones precede alterations in the rotational/translational velocity, thus indicating that these cells could determine the strength of the turning tendency. Furthermore, since significant correlations for translational and rotational velocity do not occur simultaneously, this indicates a neuronal separation of these walking parameters in the *i5*-type cells.

Most, although not all, *i5* cells show significant correlations for only one stimulus situation (see Fig. 8). This may be interpreted as a hint that turning tendency may be shaped by distributed neural activity (Kien, 1983; Georgopolos *et al.* 1986; Gronenberg and Strausfeld, 1990; Hensler, 1992*b*). This idea is supported by the finding that the DNC neurone, a presumed homologue of DBNc2-1, is important in flying (Rowell and Reichert, 1986; Hensler, 1992*a*), but not in walking locusts, although it is active. If the *i5* cells were to influence turning tendency *via* population coding (Georgopolos *et al.* 1986), i.e. neuronal assembly (Laurent, 1996), changes in rotational velocity should be minor if the activity of only one individual of this group is manipulated experimentally. This should be seen in a test for sufficiency and necessity (Hedwig, 1994; Kupfermann and Weiss, 1978) and might provide further evidence for the *i5*-cells acting as a neuronal assembly.

Behaviourally dependent gating

In the present paper, we describe behaviourally dependent gating in DBNc2-1 descending brain neurones and *i5*-type cells. Furthermore, in the *i5* cells, differential gating is found, i.e. gating depends on the carrier frequency of the stimulus. This finding may explain the results of an earlier report of Boyan and Williams (1981). Using immobilized animals, they found that IDBN responded to 15 kHz but not to 5 kHz stimulation. Brodfuehrer and Hoy (*Teleogryllus*; 1990) have shown that the response of DBIN2 to ultrasound is quantitatively the same during rest and flight. This lack of gating for ultrasound is similar to the situation in the presumably homologous *i5* cells in standing and walking animals (see Fig. 7). However, the DBIN2 cell seems not to respond to 5 kHz stimulation during flight (Brodfuehrer and Hoy, 1990). If this really were the case, it would indicate that gating in *i5*-type cells depends not only on the frequency of the auditory stimulus but also on the type of locomotor behaviour the animal exhibits.

A modulation of responses to sensory stimuli has been found in many other invertebrate and vertebrate systems. However, in contrast to the results presented in the present paper, most of these modulations are confined to mechanosensory responses closely related to the animal's locomotor systems (for reviews, see Pearson, 1995; Prochazka, 1989). In grasshoppers, the auditory response of a thoracic interneurone, the G-neurone, has been shown to be suppressed by a presumed central mechanism during stridulation (Wolf and von Helversen, 1986). This probably serves to prevent saturation in the auditory system. To elicit jumping in locusts, proprioceptive input from the hindleg has to occur simultaneously with activity in cells descending to the metathoracic ganglion (Steeves and Pearson, 1982; Robertson and Pearson, 1985). This indicates that the influence of descending neurones is limited by a gating mechanism in a way that depends on the behavioural context. In the stick insect, it was shown that the same afferent information from the femoral chordotonal organ leads to different responses in the premotor pathway depending on the activity state of the animal. In the inactive animal, a resistance reflex is employed which changes to an assistance reflex during walking (see Bässler, 1993; Driesang and Büschges, 1996). These modulations occur in neurones closely related to the locomotor network or even belonging to the central pattern generator (Driesang and Büschges, 1996). In locusts, both presynaptic inhibition of afferents from the femoral chordotonal organ (Burrows and Matheson, 1994) and inhibition *via* central neurones (Wolf and Burrows, 1995) are found to play a role in rhythmic reflex modulation during walking. Similar mechanisms are found in the crayfish walking system (El Manira and Clarac, 1991; El Manira *et al.* 1991; Skorupski and Sillar, 1986). In humans, cutaneous reflexes elicited by stimulation of the sural nerve are modulated in a phase-dependent manner (Duysens *et al.* 1990). During locomotion in cats, the Ib afferent pathway is also modulated rhythmically (Gossard *et al.* 1994; Pearson and Collins, 1993). Here, as in the stick insect, unidentified polysynaptic inhibitory pathways are opened or strengthened during walking (stick insect, Driesang and Büschges, 1996; cat, Gossard *et al.* 1994; Pearson and Collins, 1993). The only other example we know of, where gating of responses to sensory stimuli occurs on a level not directly coupled with locomotor behaviour, as shown in the present paper, was reported by Schuller (1979). He described

neurones in the bat inferior colliculus which only responded to acoustic stimuli during the animal's own vocalization. The underlying mechanism, however, remains unclear.

Since we did not record in the brain, no synaptic potentials are available for a direct evaluation with regard to possible mechanisms for differential behaviourally dependent gating in descending neurones. However, the findings of others (Boyan and Williams, 1981; Brodfuehrer and Hoy, 1990) in addition to our own results at least indicate possibilities for underlying mechanisms. Behaviourally dependent gating seems to differ from facilitation, as described for ultrasound-sensitive cells in flying crickets, since this only leads to stronger sensory responses during flight (Brodfuehrer and Hoy, 1989). It could, however, be argued that the inputs to the *i5* cells were subthreshold until walking commenced. If this were the case, the findings that 20 kHz stimuli are not gated and that the responses are not quantitatively different during walking and standing indicate that different mechanisms may operate on the 5 kHz and ultrasound pathways which converge on the *i5* cells. Furthermore, the mechanism underlying differential gating is unlikely to involve a general reduction of the membrane potential of the neurone during rest since, if this were the case, quantitative changes should occur in the ultrasound response. But this is not the case in the *i5* cells. A close look at the 5 kHz data presented for homologous neurones in other insects (Boyan and Williams, 1981, Fig. 1C; Brodfuehrer and Hoy, 1990, Fig. 3C) shows inhibitory potentials with latencies of approximately 30 ms. Although the authors do not explicitly comment on this, it might indicate direct inhibition of the responses of these cells to 5 kHz stimuli while the animal is not active. In other systems, modulations of responses to afferent information are brought into effect by combinations of pre- and postsynaptic mechanisms and even by opening additional pathways (see above). Recordings in the integrating segment of *i5* cells should provide insight into the mechanisms underlying differential gating.

In systems closely related to locomotion, rhythmic modulations of mechanosensory information can be explained by the need to adjust the reflexes to the ongoing behaviour without losing flexibility in response to unpredictable situations (for reviews, see Pearson, 1995; Prochazka, 1989). Since ultrasound mimics the cries of bats (Moiseff *et al.* 1978), which are predators, it should always lead to escape behaviour, i.e. negative phonotaxis. Thus, 'categorial differences' (Hoy, 1989) might be the reason why the response to ultrasound is not gated like the response to the moving grating and to the calling song with the conspecific carrier frequency.

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