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PHYSIOLOGICAL CHARACTERISATION OF ANTENNAL MECHANOSENSORY DESCENDING INTERNEURONS IN AN INSECT (*GRYLLUS BIMACULATUS*, *GRYLLUS CAMPESTRIS*) BRAIN

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Summary

We investigated five different descending brain interneurons with dendritic arborizations in the deutocerebrum in the crickets *Gryllus bimaculatus* and *G. campestris*. These interneurones convey specific antennal mechanosensory information to the ventral nerve cord and all responded to forced antennal movements. These interneurones coded for velocity and showed preferences for distinct sectors of the total range of antennal movements. Their axons descended into the posterior connective either ipsilateral or contalateral to the cell body. Electrical stimulation of sensory nerves indicated that the interneurons received input from different afferents of the two antennal base segments. One interneuron had a particularly large axon with a conduction velocity of 4.4 m s^{-1}. This was the only one of the five interneurons that also received visual input. Its activity was reduced during voluntary antennal movements. The reduction in activity occurred even after de-efferentation of the antenna, indicating that it had a central origin. Although we do not have experimental evidence for behavioural roles for the descending antennal mechanosensory interneurons, the properties described here suggest an involvement in the perception of objects in the path of the cricket.

Key words: insect, cricket, descending brain interneurone, antenna, mechanoreception, vision, efference copy, *Gryllus bimaculatus*, *Gryllus campestris*.

Introduction

Antennae are important multimodal sense organs in insects (e.g. Fuldalewizc-Niemczyk and Rosciszewska, 1973; Schaller, 1978; Stengl et al., 1990). Unlike many other sense organs, antennae can be actively moved relative to the body. In particular, insects with antennae longer than one body length, such as crickets, have antennae that are precisely targeted at objects around the animal (Honegger, 1981). During walking, the antennae move continuously to scan the terrain ahead of the insect (Horseman et al., 1997; Dürr, 1999) and serve as mobile multimodal sensors of the environment around the insect.

Antennal movements are controlled by mechanosensory afferents located at the first two antennal segments, the scape and the pedicel (Kammerer and Honegger, 1988). The maintenance of antennal movements and postures, however, requires a connection between the brain and the suboesophageal ganglion (Horseman et al., 1997). Antennal mechanosensory afferents also participate in flight control (Gewecke, 1974) and gravity perception (Horn and Bischoff, 1983), and their stimulation may elicit evasive behaviour (Burdohan and Comer, 1990; Stierle et al., 1994; Burdohan and Comer, 1996). These various functions require a connection between antennal mechanoreceptors and the motor centres of the thoracic ganglia. Since the branches of antennal mechanoreceptive afferents are confined to the deutocerebrum and the suboesophageal ganglion (Suzuki, 1975; Bräunig et al., 1983; Rospars, 1988; Honegger et al., 1990; Staudacher and Schildberger, 1999), intersegmental interneurons must necessarily relay the output of these antennal mechanosensory afferents to the thoracic ganglia.

Our experiments were aimed at characterising the sensory physiology of descending brain interneurons that receive inputs from antennal mechanosensory afferents. We recorded intracellularly in the deutocerebrum from such interneurons, which were activated during forced antennal movements. Single-cell staining revealed the dendritic arborizations in the deutocerebrum and the positions of the somata in the dorsal protocerebrum. The interneurons belong to a population of approximately 200 descending brain interneurons that have been characterised morphologically (Staudacher, 1998).

Some of the data presented in this study have been published in abstract form (Gebhardt and Honegger, 1997).

Materials and methods

Experimental animals

Experiments were performed on adult *Gryllus bimaculatus* De Geer of either sex obtained from our laboratory culture and on *Gryllus campestris* L. caught near Munich, Bavaria, Germany.

Preparation and electrophysiology

The electrophysiological methods used in this study followed standard protocols for intracellular recording and staining with Lucifer Yellow or hexammine cobaltic chloride (Stewart, 1978; Brogan and Pitman, 1981). Electrode resistance ranged from 40 to 80 MΩ (borosilicate capillaries, Clark Biomedical Instruments). Briefly, the cricket was fixed dorsal side up on a cork holder, and the brain was exposed by removing the frontal cuticle of the head together with the underlying adipose tissue. A silver platform was used to stabilise the brain and served as a reference electrode. Penetration of the ventral sheath of the brain by the microcapillaries was facilitated by the application of collagenase (one crystal, type IV, Sigma) for 45–60 s. In all experiments, the interneurons were impaled with the microelectrode in their deutocerebral dendrites. The activity of antennal motoneurons was recorded with a pair of hook electrodes implanted under nerve N4B, which contains the axons of one adductor motoneuron and of three abductor motoneurons, or under N4, which contains the axons of nine antennal motoneurons (Honegger et al., 1990). In total, 60 recordings were made from descending antennal mechanosensory interneurons. All experiments were carried out at an ambient temperature of 20–22 °C.

Stimulation

The interneurons were stimulated by forced deflections of the antenna. One antenna was cut at the tenth flagellar segment, and a minuten pin was inserted a few segments deep into the remaining stump. The first antennal joint was immobilised by gluing the scape to the head capsule. We focused on the second antennal joint between the scape and the pedicel because horizontal antennal movements are naturally executed at this joint and its organisation is simpler than that of the head–scape joint. The latter includes five muscles and 10 motoneurons compared with two muscles and seven motoneurons controlling the scape–pedicel joint. The minuten pin with the attached flagellum was deflected using a magnet (0.5 mm×0.5 mm×1.0 mm) fixed to a servo-motor (Megatron type 26-2) controlled by a custom-built feedback waveform generator. ‘Ramp-and-hold’ stimuli were delivered with different ramp velocities, angular amplitudes and holding times that were within the range of natural antennal movements. Controlled visual stimuli (looming disks, moving grids and dots) were generated on a PC and displayed on a computer monitor (refresh rate 72 Hz) centred in front of one compound eye of the cricket. The monitor screen subtended 45 °×59 ° at the eye. Care was taken to align the screen parallel to the eye to avoid distortions in the angular size and velocity of the patterns displayed. To characterise the gradient of visual sensitivity of interneuron DBNi1-2, stationary black dots subtending 8 ° were displayed on a white background. Dots were displayed at the centre and at the four corners of the screen for 400 ms in random sequences for each trial.

In experiments on antennal–mechanosensory inputs to the interneurons, both optic nerves were cut to prevent unwanted visual stimulation by the moving magnet. Other stimuli tested were puffs of odour to the antenna (e.g. acetic acid, sage extract, lavender extract, tobacco smoke), hissing and other sounds and touching different parts of the body of the cricket.

Antennal sensory nerves were electrically stimulated by pairs of hook electrodes insulated with petroleum jelly. Stimulation pulses were 0.5 ms in length and ranged between 1 and 6 V.

Data analysis

Recordings together with stimulus monitors were taped (Racal Store D7) and later digitised (CED 1401, Cambridge Electronic Design Ltd) and analysed off-line on a PC using Spike2 software (Cambridge Electronic Design Ltd). The responses of the interneurons to antennal stimulation were evaluated only if no antennal motor activity occurred.

The strength of synaptic activity in interneuron DBNi1-2 was quantified by calculating the integrals of the membrane potential over a period of 200 ms. Antennal motoneuron spikes were counted over the same intervals to quantify antennal motor activity. The intervals were chosen either from the onsets of motoneuron bursts or from episodes without any motor activity. An interval of 200 ms was chosen because it corresponded to the minimum duration of spontaneous bursts of antennal motor activity. Before calculating integrals, the recordings were digitally low-pass-filtered using default Spike2 digital filter functions to remove action potentials (–3 dB at 281 Hz). Data from different animals were separately normalised to maximum spike counts or to maximum integrals of synaptic potentials for each experiment and then pooled. Non-parametric statistical tests were performed using WinStat software (Kalmia Co. Inc.).

Results

We describe the morphology and sensory physiology of the descending interneurons DBNi1-2, DBNi2-1, DBNc1-2, DBNc2-2 and DBNc2-3 (Fig. 1) identified morphologically by Staudacher (Staudacher, 1998) according to the location of their cell bodies in distinct soma clusters in the rind of the protocerebrum. The terminology for these interneurons was coined by Staudacher (Staudacher, 1998). For instance, DBNi1-2 is the second descending brain neuron of soma cluster 1 with an axon descending ipsilateral to the soma. All neurons with their axons in the contralateral connective carry the label ‘DBNc’. A similar terminology for descending cephalic interneurons in grasshoppers, active during stridulation, was used by Hedwig (Hedwig, 1986). All the interneurons described in this study have a characteristic dendrite in the non-glomerular deutocerebrum, thus differentiating them from other descending interneurons. We

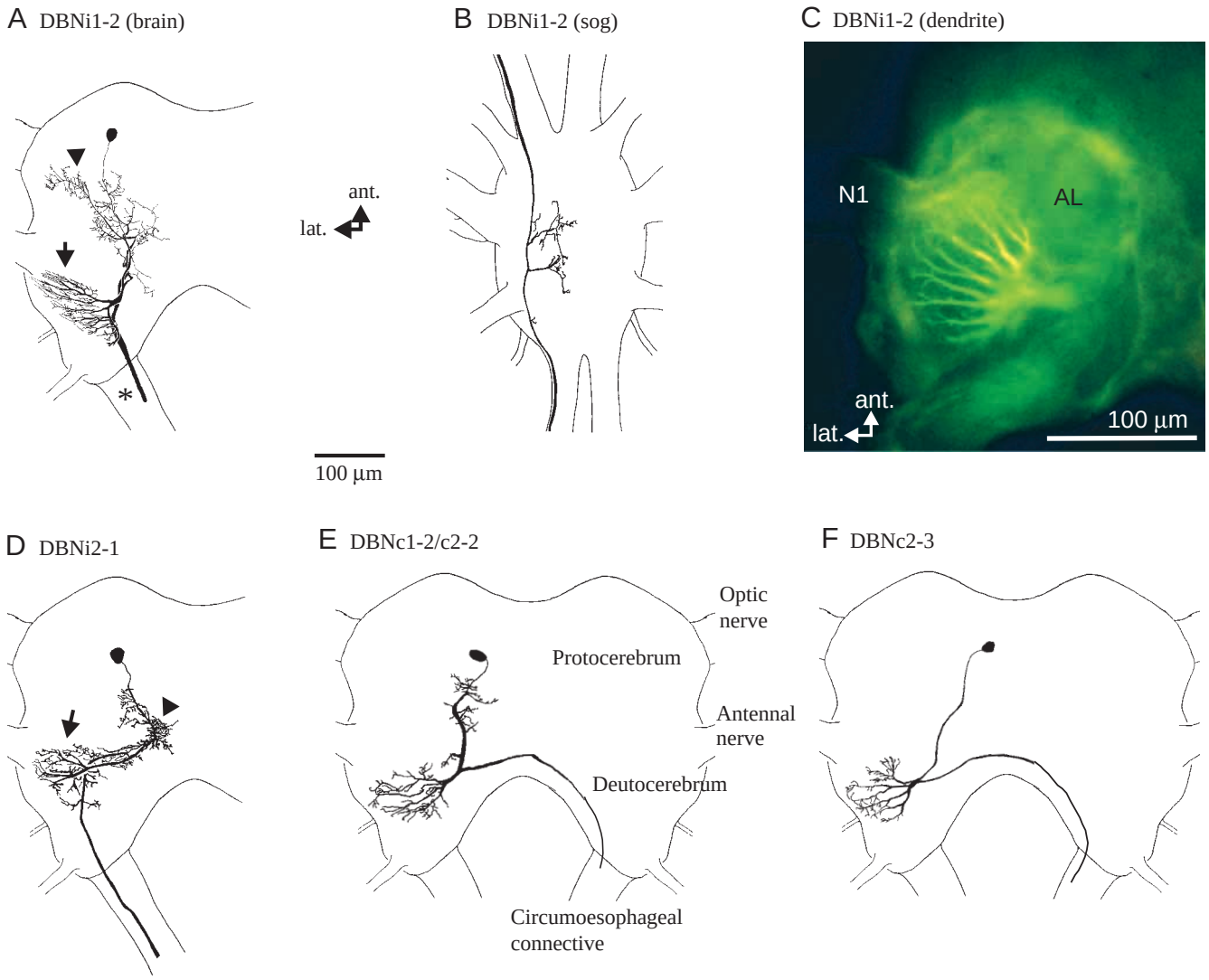

Fig. 1. (A–F) Morphology of the descending interneurons that receive antennal mechanosensory input. (A) DBNi1-2 is characterised by its large dendrite (arrow), which ramifies extensively in the ventral antennal mechanosensory part of the deutocerebrum. Another dendrite extends anteriorly into the lateral protocerebrum (arrowhead). The axon (asterisk) projects into the ipsilateral connective. ant., anterior; lat., lateral. (B) Sparse projections of DBNi1-2 in the suboesophageal ganglion (sog). (C) Fan-like projections of DBNi1-2 in a typical intracellular fill (Lucifer Yellow). AL, antennal lobe; N1, flagellar nerve 1. (D) DBNi2-1 ramifies extensively in the deutocerebrum (arrow) dorsal to DBNi1-2. Dense arborizations are present in the medial protocerebrum (arrowhead). (E) The DBNc1-2/c2-2 interneuron descends into the connective contralateral to the soma. The single main dendrite is in the ispilateral deutocerebrum. (F) The contralaterally descending DBNc2-3 interneuron is characterised by a primary neurite devoid of any branchings and a fan-like dendrite in the deutocerebrum. All diagrams are in ventral view.

could reliably identify interneurons DBNi1-2, DBNi2-1 and DBNc2-3 on the basis of their morphology. We do not, however, distinguish between DBNc1-2 and DBNc2-2, both of which showed very similar morphologies and physiological responses. In addition, they do not meet the identification criteria of Staudacher (Staudacher, 1998) in our preparations. They will therefore be referred to as 'DBNc1-2/c2-2'.

Morphology

The axons of the interneurons under consideration project into the circumoesophageal connectives either ipsilateral (DBNi1-2, DBNi2-1) or contralateral (DBNc1-2/c2-2, DBNc2-3) to the cell bodies (Fig. 1A,D–F). All interneurons have their major dendrite in the lateral deutocerebrum. They differ, however, in the shape and in the ventro-dorsal extent of their branchings in this neuropile. While DBNi2-1 ramifies in the dorsalmost layers of the deutocerebrum, the branchings of DBNc1-2/c2-2 are more medial. The dendrites of DBNi1-2 and DBNc2-3 are positioned more ventrally.

DBNi1-2 projects with regularly spaced fan-like branches (Fig. 1C) running close to the ventral surface of the brain into a mechanosensory neuropile of the deutocerebrum, where flagellar afferents project (vfa; Staudacher and Schildberger, 1999). This regular branching pattern of the dendrite suggests

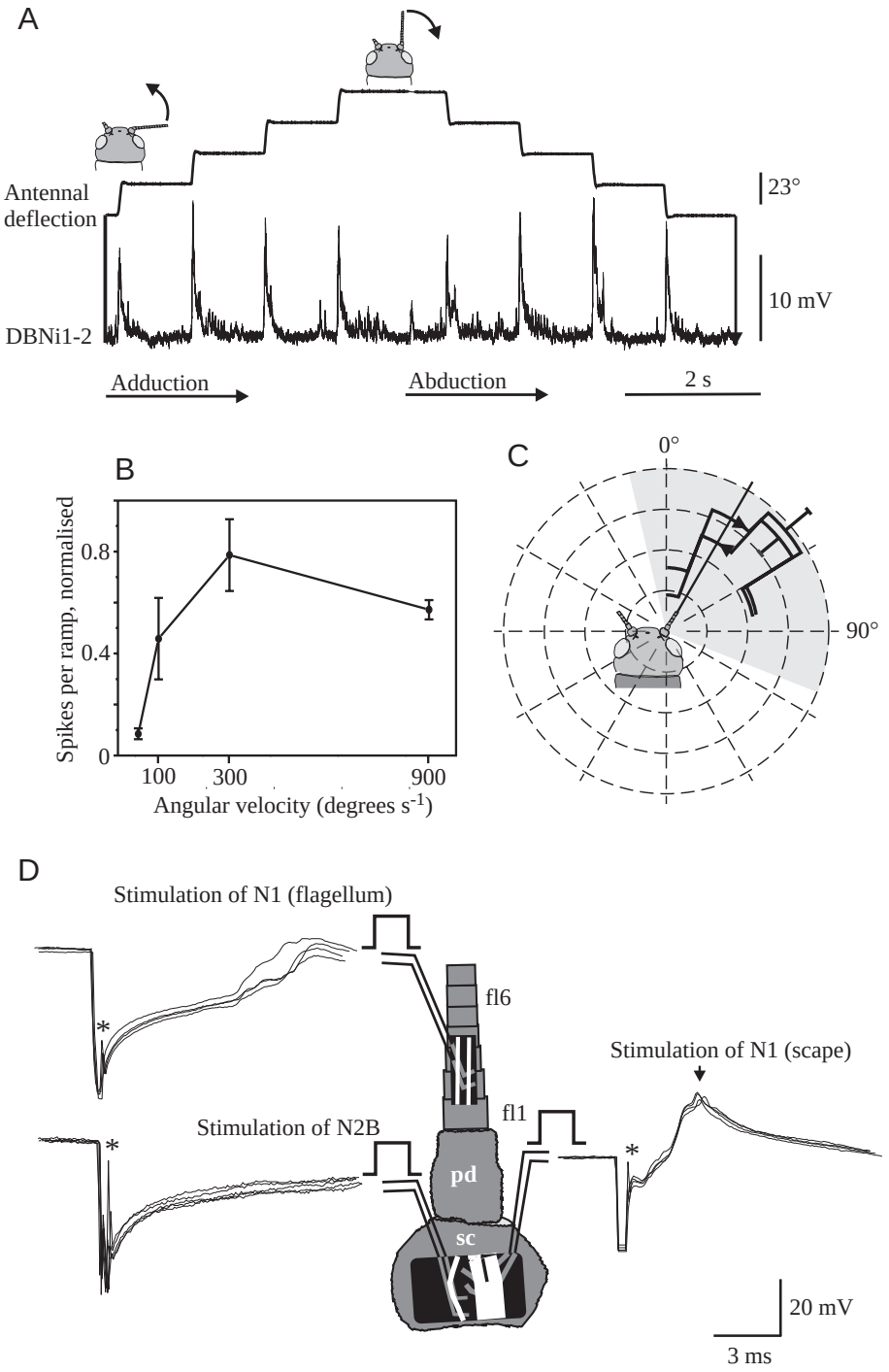

Fig. 2. (A–D) Response characteristics of DBNi1-2 evoked by forced deflections of the second antennal joint between the scape and pedicel. (A) Compound excitatory postsynaptic potentials (EPSPs) elicited by imposed movements with ramps of amplitude 23° at an angular velocity of 300°s^{-1} during both adduction and abduction of the antenna. The positions of the antenna at the extreme positions of the deflection are indicated as insets. (B) Correlation between the increase in spike frequency and movement velocity up to 900°s^{-1} during abduction and adduction between 0 and 100°. Values are means ± S.E.M. of normalised data for 3–7 stimulus periods in five animals. (C) Directional sensitivity to imposed deflections of the scape–pedicel joint (range 0–80°; four steps of 20° each; 300°s^{-1} as in A). Curves indicate relative mean spike counts (± S.E.M.) per ramp (*N*=7 animals). The curves are discontinuous every 20°, corresponding to the pauses between two ramps at a constant angular position. Arrows indicate the direction of movement (the outer curves are for movements towards the back of the insect; abduction); the solid line at 30° indicates the antennal resting position. The radius of circles represents the percentage of the maximum response (outer circle, 100%). The shaded sector indicates the range of naturally occurring movements of the scape–pedicel joint. (D) Electrical stimulation of afferent antennal nerves during intracellular recording from DBNi1-2. The shortest latencies (2 ms) resulted from stimulation of N1 in the scape (the arrow indicates spikes). Electrical stimulation of the flagellar N1 resulted only in EPSPs of small amplitude and long latency (7 ms). Stimulation of N2B failed to elicit any responses. The downward shift in the intracellular trace after stimulation is due to the microelectrode capacitance and was also seen when the electrode tip was extracellular. sc, scape; pd, pedicel; fl1, first flagellar segment; fl6, sixth flagellar segment. Asterisks indicate transient stimulation artefacts. Each recording consists of five superimposed sweeps.

a topological ordering of the surrounding sensory neuropile. An additional dendrite emerges from the primary neurite and extends anteriorly into the dorsolateral protocerebrum (Fig. 1A). The axon of DBNi1-2 is 16–20 μm in diameter and descends dorsally in the ipsilateral connective. The axon gives rise to a small region of branches in the maxillar segment of the suboesophageal ganglion (Fig. 1B) before extending at least as far as the mesothoracic ganglion (one successful staining). The branching pattern of DBNi1-2 in the thoracic ganglia is not known.

Physiology of DBNi1-2

Antennal mechanosensory inputs to DBNi1-2

Intracellular recordings were made from the deutocerebral dendrite of DBNi1-2 in all experiments. They revealed summed excitatory postsynaptic potentials (EPSPs) in response to imposed deflections of the ipsilateral scape–pedicel joint (Fig. 2A). Deflections of the contralateral joint had no effect on the activity of the interneurons. In all specimens, EPSPs were reliably elicited by each deflection and could reach amplitudes of up to 40 mV. Spiking responses consisted of

phasic bursts and followed the onsets of deflections with a latency of 7.7–8.8 ms.

One variable influencing the strength of spiking responses was the angular velocity of antennal deflections, which was tested in the range 30–900 °s^{-1}. Deflections with velocities of 30 °s^{-1} were already above threshold for the generation of EPSPs. In five experiments, spiking responses increased at approximately 2 spikes 100 °s^{-1} between 90 and 300 °s^{-1} and decreased above 300 °s^{-1} (Fig. 2B). A second variable influencing spike production was the antennal position before a single deflection step. The range of antennal positions tested was 0 ° (antenna in the forward-pointing position) to 80 ° lateral. DBNi1-2 responded best to deflections of the antenna at angles between 40 and 60 °. This is slightly lateral to the antennal resting position of approximately 30 ° (Fig. 2C; two-tailed Friedman test, P=0.018, N=7). At a given angle, however, the response of DBNi1-2 did not depend on the direction of movement (arrowheads in Fig. 2C); adductions and abductions were equally efficient (two-tailed Wilcoxon test, P=0.12, N=7).

The spike conduction velocity of DBNi1-2 was 4.4 m s^{-1} as measured by intracellular recordings close to the axon origin combined with extracellular recordings from the ipsilateral neck connective.

Responses of DBNi1-2 to electrical stimulation of antennal sensory nerves

Three pairs of stimulating electrodes were placed under different antennal nerves to identify the origin of the postsynaptic potentials following antennal stimulation (for nerve terminology, see Honegger et al., 1990). First, one pair of electrodes was attached to both branches of nerve 1 (N1) in the third/fourth flagellar segment to stimulate flagellar afferents. Second, the afferents of the scapal chordotonal organ and a scapal hair plate in nerve 2B (N2B; H.-W. Honegger, unpublished data) were stimulated. Third, hook electrodes under both branches of N1 in the distal scape allowed pedicellar and flagellar afferents to be stimulated. One experiment on DBNi1-2 (Fig. 2D) clearly demonstrates that two sources of antennal sensory input to DBNi1-2 existed. Stimulation of flagellar N1 elicited EPSPs with an amplitude of approximately 23–26 mV in DBNi1-2 with a latency of 7 ms, whereas stimulation of the scapal N1 evoked EPSPs with an amplitude of approximately 30 mV and a latency of 2 ms. Each of the latter EPSPs gave rise reliably to one spike (arrow in Fig. 2D). Stimulation of N2B had no effect on DBNi1-2. The mechanoreceptive organ most effectively exciting DBNi1-2 was, therefore, located in the pedicel, with inputs from the flagellum being weaker and possibly polysynaptic.

Visual inputs to DBNi1-2

Of the five interneurons investigated, only DBNi1-2 was activated by visual stimulation of the ipsilateral compound eye. There is no evidence for inputs from the ocelli and the contralateral compound eye. All visual responses of DBNi1-2 consisted either of spikes and EPSPs smaller than the EPSPs evoked by antennal mechanosensory stimuli or of spikes alone (see Discussion). Severance of the ipsilateral optic stalk between the medulla and lobula abolished all visual responses in DBNi1-2. Toggling a computer monitor between black and white elicited bursts of spikes (Fig. 3A). In general, 'Light-off' responses were more effective in triggering spikes than 'Light-on' responses (N=4 crickets, two-tailed Wilcoxon test, P=0.033). In addition, small moving, high-contrast stimuli such as a black dot (subtending a visual angle of 8 °) moving on a white background and looming black disks on a white background increasing at 7 °s^{-1} were effective in triggering spikes. The visual responses of DBNi1-2 were subject to fast habituation in all experiments. To reveal the spatial organisation of the visual input to DBNi1-2, stationary black dots were presented for 400 ms on a white background in the centre and at the corners of the computer screen, which was centred at the eye. The strongest spike responses per dot displayed occurred when dots were presented at the outer corner of the dorso-anterior quadrant of the computer screen,

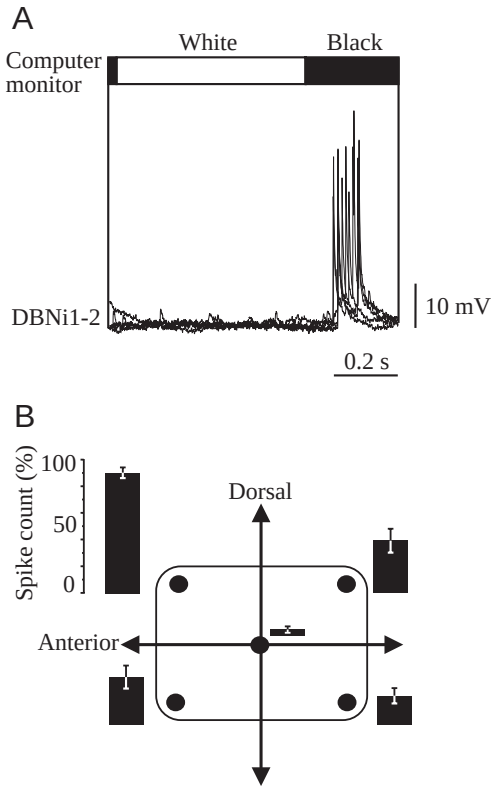

Fig. 3. (A,B) Response of DBNi1-2 to visual stimuli. (A) Light-off stimuli elicited bursts of spikes. Upper trace, stimulus monitor; lower trace, five superimposed sweeps of the intracellular recording. (B) Mapping of responsiveness to visual input. Sensitivity to the presentation of stationary black dots subtending a visual angle of 8 ° changes over the receptive field of DBNi1-2. Columns represent relative means of the spike counts (± S.E.M., standardised for each experiment, N=5). DBNi1-2 responded best to dots in the dorso-anterior corner of the computer screen. Note that the the size of the screen did not allow the full size of the receptive field of this neuron to be probed (see Materials and methods).

i.e. approximately 37° dorso-anterior of the centre of the eye (see Materials and methods for more details). Minimum responses were elicited by dots in the centre of the screen (Friedman test, P=0.031, Fig. 3B).

Antennal motor-activity-dependent modulation of synaptic activity to DBNi1-2

DBNi1-2 clearly received strong excitation from afferents of the second antennal joint between the scape and pedicel. How does DBNi1-2 respond to active antennal movements that are likely to excite these afferents ? In a tethered cricket walking on a stationary ball (Kammerer and Honegger, 1988), bursts of motoneuron spikes were recorded extracellularly from a pure antennal motor nerve (N4B; Honegger et al., 1990; Fig. 4A) that drove antennal movements with large amplitudes and velocities. Additional short bursts of motor activity were closely coupled to the respiratory rhythm and did not result in visible antennal movements. There was a clear negative correlation between motor activity and synaptic activity in DBNi1-2. Postsynaptic potentials had amplitudes of less than than 5 mV during episodes of longer-lasting motor activity and during some of the bursts related to respiration. EPSPs with an amplitude of 20 mV and spikes occurred during intervals between motor activity. This decrease in the amplitude of postsynaptic potentials during motor activity also occurred during antennal stimulation (Fig. 4B). Deflections of the scape–pedicel joint elicited EPSPs in DBNi1-2 and sometimes also produced bursts in N4B, probably reflecting resistance reflexes. During motoneuron bursts, the amplitudes of EPSPs and the frequency of small-amplitude postsynaptic potentials were clearly reduced compared with stimuli without concurrent motor activity (broken bar in Fig. 4B).

To quantify the relationship between synaptic potential amplitude and motor activity, Spearman rank correlation analyses of DBNi1-2 membrane potential integrals (see Materials and methods) and antennal motoneuron spike counts were carried out in five experiments (Table 1). The correlation coefficient (r) ranged from –0.57 to –0.69 (experiments 1–4 in Table 1), indicating that the amplitudes of EPSPs in DBNi1-2 decreased with increasing motor activity. The motor spike count was the only variable found to be correlated with the size

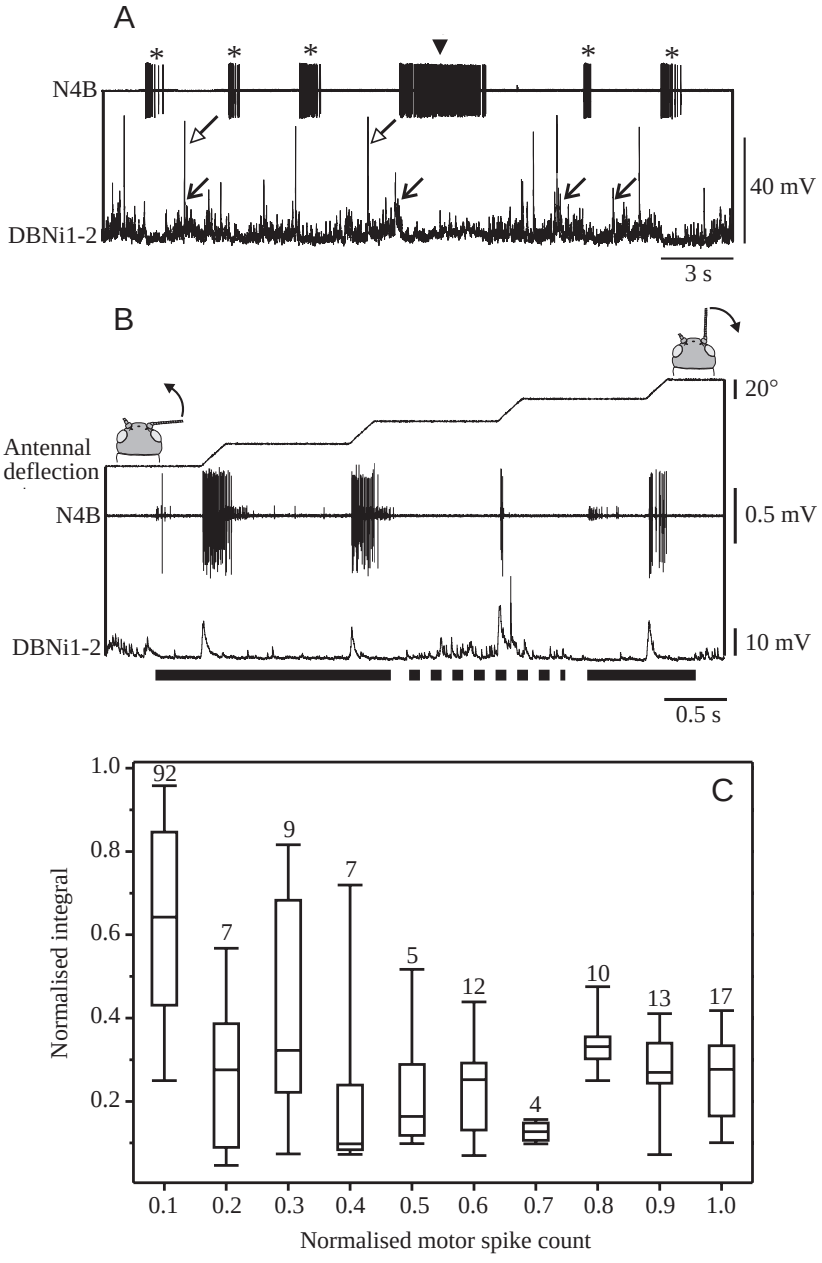

Fig. 4. (A–C) Synaptic activity of DBNi1-2 during motor activity. (A) Intracellular recording of DBNi1-2 (lower trace, hyperpolarised with –2 nA) and extracellular recording of antennal motoneurons from N4B (upper trace) as a measure of antennal motor activity. Movements of the antenna were not registered. Large excitatory synaptic potentials (arrows) and action potentials (open arrows) are absent during a spontaneous motor neuron burst (arrowhead) and during respiratory-related bursts (asterisks). (B) During forced antennal movements, excitatory postsynaptic potential (EPSP) frequency was also reduced in the presence of high levels of antennal motor activity. Solid bars represent episodes of low synaptic activity in DBNi1-2 and the dashed bar represents episodes of high activity. (C) Box-and-whisker plot showing the median, the second and third quartiles and the fifth and ninety-fifth percentiles of normalised integrals of 176 spontaneous EPSPs plotted against normalised motor spike count in motor nerve N4B from four crickets (see Materials and methods). Large synaptic potentials did not occur in the presence of high motor spike counts. The integral values were binned into classes with a width of 0.1 relative motor spike count units. The maximum integral values decrease with increasing spike count (Spearman rank correlation coefficient r=–0.85, P<0.001). Numbers indicate numbers of samples in each class.

Table 1. *Summary of the experiments analysed for the motor-activity-dependent suppression of synaptic inputs to DBNi1-2*

	Experiment				
	1	2	3	4	5
Number of intervals analysed	37	60	35	55	63
Spearman correlation coefficient, *r*	–0.57	–0.69	–0.62	–0.67	–0.58
Probability of error	<0.001	<0.001	<0.001	<0.001	<0.001
Antennal sensory–motor loop	Closed	Closed	Closed	Closed	Open

of the integrals. The resting potential immediately before the onset of EPSPs was not correlated with the size of the integrals.

The Spearman rank correlation coefficient for the pooled data from experiments 1–4 in Table 1 is –0.64 (P<0.001, N=176). During periods of low antennal motor activity, both large and small synaptic inputs to DBNi1-2 were measured, resulting in considerable variance in the data shown in Fig. 4C. However, the gradual decline in synaptic inputs is clearly revealed when the data are grouped into classes. The maximum integral values decline gradually with increasing motor spike count (Spearman r=–0.85, P<0.001).

These experiments were performed under closed loop-conditions with all antennal nerves intact. In one experiment, all the nerves that contain antennal motoneurons (N2, N3, N4) were transected (Table 1, experiment 5), rendering the treated antenna completely motionless and thereby preventing peripheral sensory feedback. This open-loop condition still results in a negative correlation coefficient for the relationship between motoneuron activity and synaptic inputs to DBNi1-2 (r=–0.58), indicating a central origin for the motor-activity-dependent modulation of synaptic inputs to DBNi1-2.

The antennal motor activity did not seem to have an effect on visually elicited activity. One experiment showed that the strength of spiking responses to ‘Light-on’ and ‘Light-off’ stimuli during antennal motor activity did not differ from the strength of responses during its absence (N=16; Mann–Whitney U-test, P=0.45 and P=0.47, respectively).

Physiology of DBNi2-1, DBNc1-2/c2-2 and DBNc2-3

Recording from DBNi2-1 was more difficult than recording from DBNi1-2, probably because of the dorsal position of the deutocerebral dendrite. The recordings from DBNi2-1 yielded only spiking responses and no large compound EPSPs upon forced deflections of the ipsilateral scape–pedicel joint (Fig. 5A). One specimen responded preferentially to adductions between 80 and 67 ° with a phasic–tonic response of an average of 23 spikes per ramp (Fig. 5B, one experiment, N=11 stimulus periods, 90 ° s^{-1}). A second DBNi2-1 spiked more phasically in response to comparable stimuli; this specimen responded to abductions in an angular range between 23 and 90 ° (data not shown). This indicates that a single interneuron may respond differently to identical stimuli in different animals. Experiments on the origin of the antennal mechanosensory input to DBNi2-1 and on antennal motor-activity-dependent suppression of its synaptic activity were not performed.

Recordings from DBNc1-2/c2-2 interneurons were made in 14 animals. They responded to step-like forced deflections of the ipsilateral scape–pedicel joint with compound EPSPs and spikes (Fig. 5C) in the angular range 0–100 ° at angular velocities of 30–900 ° s^{-1}. Adductions were significantly more effective than were abductions in stimulating DBNc1-2/c2-2 (Fig. 5D). Electrical stimulation of N2B, which contains the axons of the chordotonal organ in the scape and of two scapal hairplates (H.-W. Honegger, unpublished data), elicited spikes at a mean latency of 2.5 ms (Fig. 6A). In two experiments on DBNc1-2/c2-2, no indications were found that the synaptic activity was suppressed by antennal motor activity, as in DBNi1-2.

Two DBNc2-3 interneurons resembled DBNi1-2 and DBNc1-2/c2-2 in that forced deflections of the ipsilateral scape–pedicel joint resulted in compound EPSPs and bursts of spikes in one specimen (Fig. 5E) and EPSPs alone in another specimen (not shown). The spiking response was best at lateral positions between 80 and 100 ° for abductions; the amplitudes of EPSPs in the second specimen were independent of angular position and of the direction of the ramps. One experiment identical to that shown in Fig. 2D indicated that mechanosensory afferents located in the pedicel must excite DBNc2-3 (Fig. 6B).

None of the four interneurons was activated by other olfactory, acoustic or mechanical stimuli to parts of the body other than the antennae, as tested under the conditions of our experiments.

Discussion

We show that, in the cricket, large identified descending brain interneurons compute specific antennal mechanosensory information and convey it posteriorly. Additional visual inputs converge onto one of the five interneurons. In crickets, only the tritocerebral commissure giant has been shown to receive antennal mechanosensory input (TCG; Bacon and Tyrer, 1978; Bacon, 1980). In the locust, the descending movement detector (DCMD) neuron receives inputs from the antenna, and it has been been suggested that these contribute to the periodic non-responsiveness of the DCMD (Rowell and O’Shea, 1980).

Antennal mechanosensory inputs

All interneurons responded reliably to forced deflections of the second antennal joint, which allows horizontal movements of the antennae. In this study, only forced deflections were used, although we are currently investigating the response properties of the neurons during voluntary antennal movements. Electrical stimulation of antennal sensory nerves

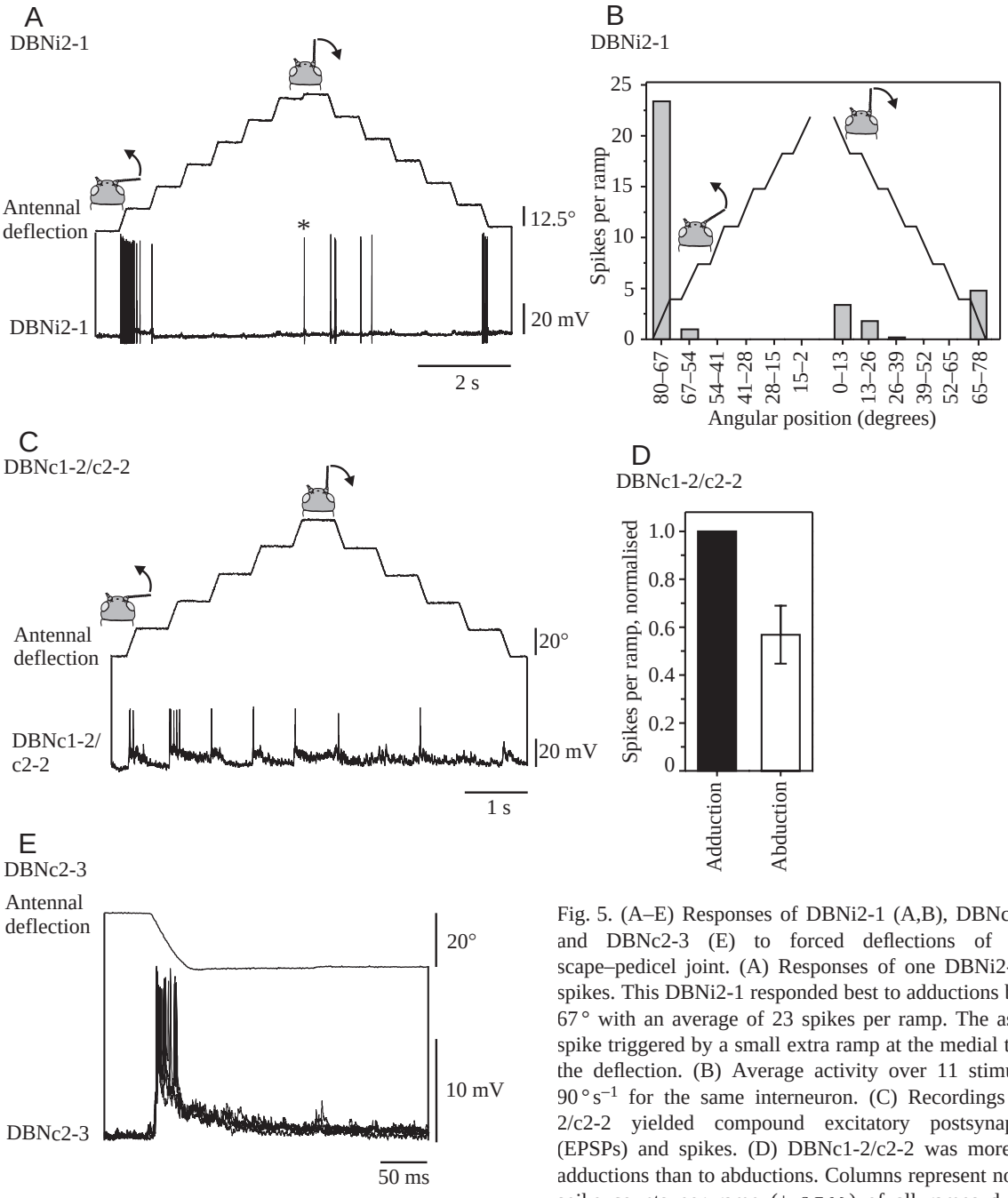

Fig. 5. (A–E) Responses of DBNi2-1 (A,B), DBNc1-2/c2-2 (C,D) and DBNc2-3 (E) to forced deflections of the ipsilateral scape–pedicel joint. (A) Responses of one DBNi2-1 consisted of spikes. This DBNi2-1 responded best to adductions between 80 and 67 ° with an average of 23 spikes per ramp. The asterisk marks a spike triggered by a small extra ramp at the medial turning point of the deflection. (B) Average activity over 11 stimulus periods at 90 ° s^{-1} for the same interneuron. (C) Recordings from DBNc1-2/c2-2 yielded compound excitatory postsynaptic potentials (EPSPs) and spikes. (D) DBNc1-2/c2-2 was more responsive to adductions than to abductions. Columns represent normalised mean spike counts per ramp (± S.E.M.) of all ramps during adduction *versus* abduction in five animals (N=23 stimulus periods at 90 ° s^{-1}; Wilcoxon test P=0.03). (E) Compound EPSPs and bursts of spikes were triggered in one DBNc2-3 by abductions between 80 and 100 ° at 300 ° s^{-1} (five sweeps superimposed). Other angular positions were less effective in stimulating this example of DBNc2-3.

demonstrated that DBNi1-2 and DBNc2-3 were excited by afferents from the pedicel, whereas DBNc1-2/c2-2 were excited by afferents from the scape. This is consistent with morphological data showing that DBNi1-2 and DBNc2-3 have their input dendrites close to each other in the ventral deutocerebrum, which receives projections of flagellar afferents (vfa; Staudacher and Schildberger, 1999). Stimulation of flagellar afferents evoked only long-latency and small-amplitude responses in DBNi1-2, indicating several layers of intercalated interneurons. In comparison, the latencies of the EPSPs following electrical stimulation of N1 in the distal scape for DBNi1-2 and DBNc2-3 ranged from 1.0 to 2.0 ms (EPSPs) and from 1.9 to 3.6 ms (spikes), suggesting that only a few synaptic layers exist between the afferents and the interneurons. The deutocerebral dendrites of DBNc1-2/c2-2 branch more dorsally than those of DBNi1-2 and DBNc2-3 and in an area where the proprioceptors of the antennal base terminate (Bräunig et al., 1983; Honegger et al., 1990;

Staudacher and Schildberger, 1999). It is likely that afferents of the scapal chordotonal organ excite DBNc1-2/c2-2. A contribution from other scapal mechanosensory afferents, however, cannot be excluded because N2B contains the axons of receptors in the scapal bristles. The origin of the mechanoreceptive input to DBNi2-1 remains unknown.

Staudacher (Staudacher, 1998) has described seven descending interneurons with dendritic arborizations in the mechanosensory area of the deutocerebrum. We have recorded from five of the seven interneurons, which seem to represent specific lines each coding for a set of variables of antennal movement and/or position. While DBNc1-2/c2-2 appears to code preferentially for movement direction, DBNi2-1 and DBNc2-3 code for extreme antennal positions. Interneuron DBNi1-2 is special in representing movements at more medial antennal positions. It also appears to be the only one of the descending interneurons to show motor-activity-dependent suppression and to receive visual input. The remaining two interneurons, whose physiologies are so far unknown, may complement this information and support a finer resolution of the whole angular range of the scape–pedicel joint.

Although only a few recordings have been made from the interneurons DBNi2-1 and DBNc2-3, it is apparent that their response properties can differ between animals. Thus, the activity of the interneurons may be altered by modulatory effects as has been shown, for instance, for the direction-specific antennal response in honeybees, which is modulated by octopamine and serotonin in an antagonistic way, probably *via* the underlying visual interneurons (Erber and Kloppenburg, 1995; Kloppenburg and Erber, 1995). Morphologically identical 'twin' interneurons with different response properties might also explain this interindividual variability. Such interneurons, however, were not reported by Staudacher (Staudacher, 1998).

DBNi1-2: visual responses

The fact that DBNi1-2 is the only one of the five antennal mechanosensory interneurons to receive visual input coincides with its morphology. It is the only one of the five interneurons with a dendritic arborization in the lateral protocerebrum. In contrast to the antennal mechanosensory responses, in addition to spikes, only small synaptic potentials could be recorded from DBNi1-2 during visual stimulation. This probably reflects a long electrotonic conduction time between the site of the visual inputs and the recording site, leading to attenuation of EPSPs. The geometric distance between the electrode and the protocerebral dendrite, the putative site of visual inputs (arrowhead in Fig. 1A), was approximately 300 μm.

The basic properties of the visual responses of DBNi1-2 resemble those of the locust DCMD (Rowell, 1971). Both interneurons respond to novel stimuli, such as small, erratically moving, high-contrast objects. These stimuli cause fast habituation in the DCMD (Rowell, 1971) and of DBNi1-2. Recent results demonstrate that the DCMD functions as a detector of approaching objects (Rind and Simmons, 1992; Hatsopoulos et al., 1995; Gabbiani et al., 1999; Rind and Simmons, 1999). Since looming discs with a linear rate of increase of $7°\,s^{-1}$ in radius were used in the present study, it is not known whether DBNi1-2 might similarly work as an 'approach detector'.

Our results showed that DBNi1-2 was not evenly responsive to stimuli throughout its visual receptive field, although only a small area of the total visual field was probed. Our experimental arrangement did not allow us to investigate the limits of the receptive field. The spatial response pattern of DBNi1-2 is likely to derive from a retinotopic source, as found,

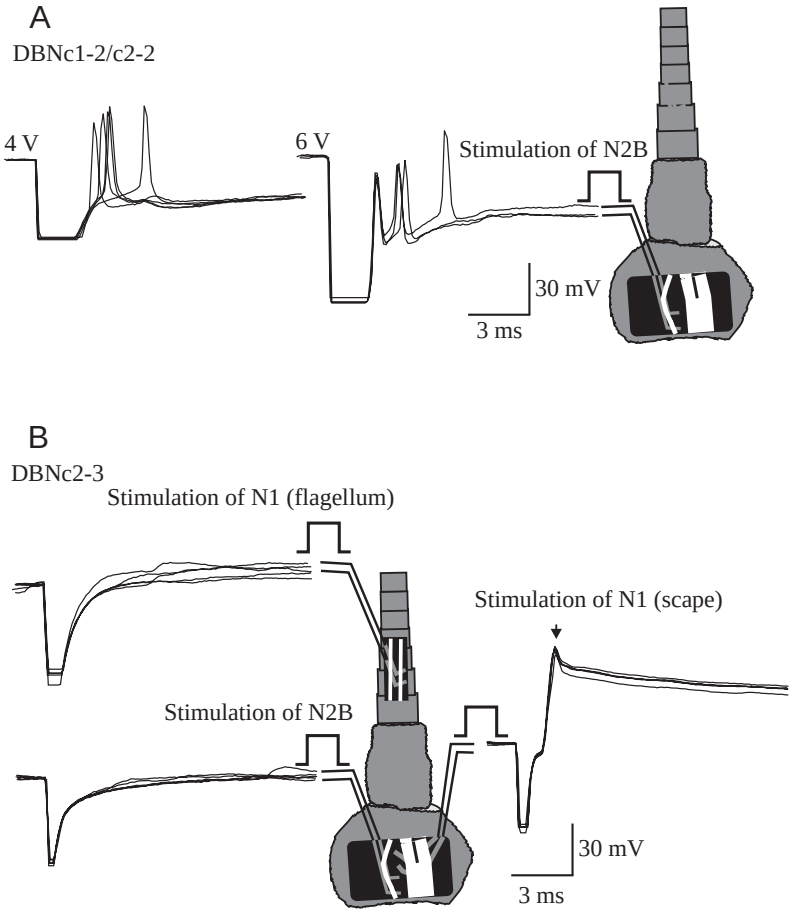

Fig. 6. (A,B) Electrical stimulation of afferent antennal nerves during intracellular recordings from DBNc1-2/c2-2 (A) and DBNc2-3 (B). (A) Stimulation of N2B (carrying the axons from the scapal chordotonal organ) elicited spikes in DBNc1-2/c2-2 at latencies of approximately 2.5 ms. Increasing the stimulus strength from 4 to 6 V resulted in an increase in spike count per stimulus and better synchronisation of the first spike. (B) DBNc2-3 received strongest inputs (spikes, marked by an arrow, and excitatory postsynaptic potentials) upon electrical stimulation of N1 in the distal scape, suggesting input from pedicellar proprioceptors. Stimulation of N1 in the third/fourth flagellar segment and of N2B were not effective.

for instance, in the fly male-specific visual neurons (Strausfeld, 1991; Gilbert and Strausfeld, 1991).

DBNi2-1: antennal motor-activity-dependent modulation of synaptic activity

During episodes of active antennal movements, DBNi1-2 shows a suppression of its antennal mechanosensory synaptic activity (Fig. 4). This suppression does not depend on the presence of sensory feedback and, as our results indicate, may thus be generated centrally. The strength of this suppression gradually increases as the strength of the motor activity increases, i.e. large EPSPs are absent during strong antennal motor activity. Although the graded relationship is masked by small EPSPs occurring either spontaneously or during weak antennal motor activity, the decline is visible in the trend of the upper 95 % quartile values in Fig. 4C. There are several potential mechanisms that would result in a reduction in the amplitude of the synaptic potentials in DBNi1-2. Hyperpolarising inhibitory synaptic potentials could superimpose on depolarising synaptic potentials, thus reducing the amplitude of the latter. This mechanism can probably be excluded since hyperpolarising potentials were never observed in DBNi1-2 and the amplitudes of EPSPs were independent of the membrane potential before the EPSPs. Conductance changes in the dendritic membrane of DBNi1-2 could contribute to the reduction in EPSP amplitudes by shunting the membrane. Currently, no experimental evidence is available to support or reject this possibility. The terminals of neurons presynaptic to DBNi1-2 could have been the target of presynaptic inhibition, as demonstrated for the sensory terminals of, for example, locust leg afferents (Burrows and Laurent, 1993; Burrows and Matheson, 1994) or cercal hair afferents (Boyan, 1988). Only the antennal mechanosensory input, not the visual input, was suppressed. This suggests that this suppression occurred in neurons presynaptic to the deutocerebral dendrite of DBNi1-2.

Motor-activity-dependent modulation of sensory information has been observed in several systems (e.g. Zaretsky and Rowell, 1979; Bell, 1981; Bell, 1982; Camhi and Nolen, 1981; Guthrie, et al., 1983; Paul, 1989; Robert and Rowell, 1992; Hjelmstad et al., 1996; Wolf and Burrows, 1995) and is generally considered to aid the central nervous discrimination of self-induced and external sensory feedback. Two reports (von Holst and Mittelstaedt, 1950; Sperry, 1950) independently proposed a central nervous copy of a motor command (‘efference copy’, von Holst and Mittelstaedt, 1950; ‘corollary discharge’, Sperry, 1950) to counterbalance self-induced sensory feedback. If such a mechanism were effective in DBNi1-2, external stimuli to the antenna should be represented by the interneuron irrespective of motor-activity-dependent suppression. It is not yet known, however, whether the responsiveness to external antennal mechanosensory stimuli is affected by motor-activity-dependent suppression. Experiments are currently being carried out to address this question.

Functional considerations

What conclusions concerning the behavioural function of the descending interneuron system emerge from our results? First, the high spike conduction velocity of DBNi1-2 is comparable with velocities found in fibres of similar calibre, e.g. the locust giant interneurons (Boyan and Ball, 1989). This suggests that speed is an important variable, at least for DBNi1-2. A spike initiated in the brain and travelling at $4\,m\,s^{-1}$ would reach the thoracic ganglia within 2 ms in an adult cricket. Second, all five antennal descending interneurons seem to code for a portion of the antennal space. DBNi1-2 appears to act as a direction-independent, but position-sensitive, detector of deflections of the antenna slightly lateral to its resting position of 30 °. We are currently investigating whether the maximum spatial sensitivity of the antennal mechanosensory response may match that of the visual response.

It is known that antennal signals are necessary for obstacle avoidance during locomotion in the potato-beetle (Pelletier and McLeod, 1994). Furthermore, mechanical stimulation of a cockroach antenna can trigger escape turns (Burdohan and Comer, 1990; Stierle et al., 1994; Burdohan and Comer, 1996; Ye and Comer, 1996). In our experiments, motor actions were never elicited by injections of depolarising currents into DBNi1-2. It is likely that the descending antennal interneurons participate in the control of the fast motor programmes of the legs, such as obstacle-induced correctional turns during fast walking, escape movements or flight, by priming thoracic motor networks for subsequent activity in response to an aversive stimulus.

In crickets, the activity of several descending neurons correlates with the rotational or the translational velocity of walking (Böhm and Schildberger, 1992; Staudacher and Schildberger, 1998); one interneuron even proved to be sufficient and necessary for the maintenance of walking (Böhm and Schildberger, 1992). None of the interneurons investigated in the present study represents such a type. Experiments are currently being carried out to test whether DBNi1-2 is activated during free walking or during encounters with obstacles, conspecifics or predators.

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