Encoding of near-range spatial information by descending interneurons in the stick insect antennal mechanosensory pathway

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Ache JM, Dürr V. Encoding of near-range spatial information by descending interneurons in the stick insect antennal mechanosensory pathway. J Neurophysiol 110: 2099–2112, 2013. First published August 7, 2013; doi:10.1152/jn.00281.2013.—Much like mammals use their whiskers, insects use their antennae for tactile near-range orientation during locomotion. Stick insects rapidly transfer spatial information about antennal touch location to the front legs, allowing for aimed reach-to-grasp movements. This adaptive behavior requires a spatial coordinate transformation from “antennal contact space” to “leg posture space.” Therefore, a neural pathway must convey proprioceptive and tactile information about antennal posture and contact site to thoracic motor networks. Here we analyze proprioceptive encoding properties of descending interneurons (DINs) that convey information about antennal posture and movement to the thoracic ganglia. On the basis of response properties of 110 DINs to imposed movement of the distal antennal joint, we distinguish five functional DIN groups according to their sensitivity to three parameters: movement direction, movement velocity, and antennal joint angle. These groups are simple position-sensitive DINs, which signal the antennal joint angle; dynamic position-sensitive DINs, which signal the joint angle with strong dependence on movement; unspecific movement-sensitive DINs, which signal movement but not the velocity, position, or direction of movement; and ON- and OFF-type velocity-sensitive DINs. The activity of the latter two groups is increased/attenuated during antennal movement, with the spike rate increasing/decreasing linearly with antennal joint angle velocity. Some movement-sensitive DINs convey spikes to the thorax within 11 ms, suggesting a rapid, direct pathway from antennal mechanosensory to thoracic motor networks. We discuss how the population of DINs could provide the neural basis for the intersegmental spatial coordinate transfer between a touch sensor of the head and thoracic motor networks.

tactile sensing; interneurons; coordination; proprioception; ensemble coding; antenna

Both mammals and arthropods use actively movable tactile sensors, such as whiskers or antennae, to sample the adjacent space for near-range orientation (see for review Diamond et al. 2008; Prescott et al. 2011; Staudacher et al. 2005). In insects and crustaceans, antennae play an important role in the control of adaptive locomotion. This ranges from course control (e.g., Camhi and Johnson 1999; Cowan et al. 2006; Okada and Toh 2000) to tactile spatial localization (e.g., Zeil et al. 1985), exploration (e.g., Dürr et al. 2001; Harley et al. 2009), and negotiation of obstacles (e.g., Schütz and Dürr 2011; see also review by Staudacher et al. 2005). To exploit antennal information in adaptive behavior, the nervous system needs to process spatial and temporal features from various sensor signals. These include exteroceptive tactile hairs on the flagellum and a number of proprioceptors located at the antennal joints.

Two antennal mechanosensory pathways connecting the antennal periphery to motor control networks have been discussed. First, antennal mechanosensory signals are forwarded with relatively long delays to the central complex of the protocerebrum (Harley and Ritzmann 2010; Ritzmann et al. 2008), where they could contribute to the selection of appropriate locomotor patterns. Second, there is a rapid, direct neural pathway involving descending interneurons (DINs) that may convey spikes to the thoracic ganglia within <20 ms after tactile stimulation. This pathway is direct in that it connects the input region of antennal afferents in the deutocerebrum and subesophageal ganglion (SOG) to neuropiles in the thoracic ganglia (e.g., Burdohan and Comer 1996; Gebhardt and Hon-egger 2001; Schöneich et al. 2011; Staudacher 1998), which control leg movements (Burrows 1996; Büschges and Gruhn 2007; Büschges et al. 2008). Here we used the stick insect Carausius morosus (de Sinéty 1901) for characterizing a large population of antennal mechanosensory DINs that mediate antennal posture and movement to thoracic ganglia. These neurons constitute an important component of the fast, direct pathway involved in descending motor control.

The function of the antennal mechanosensory system in the control of motor behavior has been studied most thoroughly in cockroaches and stick insects. Members of both taxa may carry long, thin antennae that are used for near-range orientation during walking (Dürr et al. 2001; Krause et al. 2013; Okada and Toh 2004), searching (Dürr 2001; Okada and Toh 2004), and climbing (Okada and Toh 2006; Harley et al. 2009; Krause and Dürr 2012).

In the stick insect C. morosus, antennal contact can induce goal-directed front leg movements (Schütz and Dürr 2011). When stick insects approached a vertical rod in the absence of visual cues, antennal contact led to retargeting of the ipsilateral front leg toward the contact site (if the leg was in swing) or to an aimed reaching movement (if the leg was in stance). These aimed movements require a coordinate transformation from antennal contact location to leg posture. Retargeting of ongoing swing movements may begin within 40 ms after antennal contact. The brevity of this delay suggests that the rapid, direct antennal mechanosensory pathway is involved. DINs should therefore transmit short-latency information about antennal contact location to thoracic motor networks in stick insects.

In C. morosus, the antennae are as long as the front legs, so that antennal contact locations are potentially within reach of a leg. Moreover, all stick insects have antennae with two simple hinge joints (Mujagic et al. 2007). Every antennal pointing
direction is therefore defined by a unique set of two joint angles (Krause and Dürr 2004). During searching movements, the flagellum maintains its straight shape (Dürr et al. 2001), meaning that the coding of antennal contact distance does not require the consideration of antennal bending. As a consequence, the transformation of antennal contact location coordinates into foot position coordinates requires a 3-to-N mapping. That is, the combination of two antennal joint angles and contact distance along the flagellum needs to be mapped to the appropriate set of N degrees of freedom of the leg joints.

The two antennal joint angles correspond to the proximal head-scape (HS) joint and the next distal scape-pedicel (SP) joint. Both joint axes are slanted (Dürr et al. 2001). The HS joint moves the antenna dorso-laterally or ventro-medially, whereas the SP joint moves the antenna dorso-medially or ventro-laterally. Movements of both joints are monitored by proprioceptive hair fields, located directly at the joints (Krause et al. 2013). Such antennal hair fields accurately encode the antennal joint angle in cockroaches (Okada and Toh 2001). Ablation of these hair fields impaired the ability of cockroaches to turn toward an object after antennal contact (Okada and Toh 2000). Hence, antennal postural information, derived from the activity of antennal hair field afferents, is involved in the descending control of locomotion. Antenna hair fields are therefore likely candidates for supplying the required postural information for the coordinate transfer between antennae and front legs.

As a first step to understanding the fast coordinate transfer from antennae to legs, this study is focused on DINs that convey information about antennal joint posture and movement to the thorax. More specifically, we confine this study to proprioceptive information about the distal SP joint.

Mechanosensory information from the antennae is mediated to thoracic motor networks via the neck connectives, which contain all axons projecting from the brain to the thorax and vice versa. Median numbers of 92 ipsilateral and 55 contralateral descending brain interneurons were found in the cricket (Staudacher 1998). In the stick insect, median numbers of 176 pairs of DINs with somata in the brain and 183 DINs with somata in the SOG were found (J. Goldammer, personal communication). While both studies did not reveal how many of these neurons convey mechanosensory information, they clearly showed that the neck connectives constitute a bottle-neck between brain/SOG and thoracic neurons. In principle, all descending neurons are candidates for mediating antennal mechanosensory information to the thorax.

Earlier studies describing DINs physiologically focused on the detailed characterization of individually identified DINs. Thus two giant DINs responding to antennal contact and air puffs delivered to the antennae were identified in the cockroach (Burdohan and Comer 1996; Ye and Comer 1996). Schöneich et al. (2011) identified six DINs responding to antennal touch stimuli in the cricket. With respect to antennal mechanosensory information transfer, these studies showed that flagellar contact and bending were mediated to the thorax via direct, fast-mediating pathways. Gebhardt and Honegger (2001) identified five DINs responding to imposed SP joint movement in the cricket. One DIN was sensitive to the velocity of antennal movement, and another DIN was position sensitive.

Such characterizations of individual neurons are particularly helpful for understanding the contributions of specific neurons to the descending information. They also permit analyses of the input and output regions of identified neurons, and thus help to unravel the wiring of a particular neural network. However, we currently lack a quantitative description of how different parameters of antennal movement are calculated and what kind of antennal mechanosensory information DINs mediate to thoracic ganglia. For example, there is little information about the degree to which antennal posture and movement velocity are encoded separately. From a computational viewpoint, this is essential for understanding what thoracic networks can “infer” about what is happening at the antenna. In this study, we are trying to estimate the information provided by the population of DINs by characterizing individual response properties of a large sample of 110 DINs while imposing movements of the SP joint. Therefore, rather than identifying a small number of DINs individually, we first focused on measuring what kind of information the population of DINs mediates. We show that the DINs can be divided into five functional subgroups, mediating precise information about antennal posture and movement to thoracic neural networks.

MATERIALS AND METHODS

Animals and preparation. Adult female stick insects (C. morosus) with intact antennae from a colony bred at Bielefeld University were firmly fixed dorsal side up with dental glue (Protemp, 3M ESPE, Neuss, Germany). All legs and the left antenna were removed. The HS joint of the right antenna and the stump of the left antenna were fixed with dental glue. The flagellum of the right antenna was ablated between the 4th and 6th proximal annuli, where the flagellum is stiff (Dirks and Dürr 2011; Fig. 1A). The thoracic cuticle was opened up along the dorsal midline, from the pro- to the metanotum, and fixed laterally. The gut was kept intact but was lifted out of the thorax and fixed laterally outside the animal. This gave access to the underlying nervous tissue. The body cavity was filled with stick insect saline solution (Bässler 1977; Weidler and Diecke 1969). Loose fat tissue covering the ventral nerve cord was removed, revealing the prothoracic ganglion and the connectives. The head capsule was opened posteriorly, rendering both neck connectives and the prothoracic ganglion accessible for electrophysiological recordings. The main trachea connected to the prothoracic ganglion and surrounding fat body tissue were removed. The lateral nerves innervating the front legs were cut close to the ganglion. A small, wax-coated steel platform was positioned underneath the prothoracic ganglion, and the tissue surrounding the ganglion was incised with a pair of fine scissors. The tissue was then spread and fixed laterally to the ganglion in five positions with fine cactus spines. Protease (Pronase; Merck, Darmstadt, Germany) was applied to the neck connectives and the anterior part of the prothoracic ganglion, close to the insertion sites of the neck connectives, for 40–60 s to facilitate impalement with sharp glass microelectrodes. All experiments were carried out at room temperature between 23 and 25°C.

Stimulation. The tip of a metal minuten pin was inserted into the proximal part of the right flagellum (Fig. 1A). With the HS joint fixed, the antenna could be moved about the SP joint by use of a contact-free magnetic stimulator (Gebhardt and Honegger 2001; Fig. 1A). Since the SP joint axis is rotated by 28° with respect to the horizontal plane (Krause and Dürr 2004), the orientation of the stimulator was slanted such that the rotation axes of joint and stimulator were aligned in parallel. Thus the stimulator moved the flagellum and the pedicel from ventro-lateral to dorso-medial joint angles and back. The stimulus was a staircase of ramp-and-hold deflections of the SP joint. The ramp velocity, number of steps, and interval durations could be varied. Usually, the antenna was held in the ventro-lateral extreme position at the beginning of a stimulus sequence (Fig. 1A, ventral antennal
Control experiments were performed to test whether the stimulation was indeed restricted to the SP joint. We used a 100-Hz digital video camera (Basler A602f-2, Basler, Ahrensburg, Germany) equipped with a macroobjective (TechSpec VZM 200, Edmund Optics, Barrington, NJ) to monitor scape, pedicel, and flagellum while running the stimulus protocol. At all stimulus velocities, the scape did not move relative to the head and the flagellum did not move relative to the pedicel. All movement during stimulation was therefore restricted to the SP joint. Although the stimulus was well suited to stimulate antennal hair fields, we cannot exclude costimulation of other pedicellar or flagellar mechanoreceptors, e.g., campaniform sensilla, in the form of cuticular strain. We mention and discuss this wherever relevant.

All experiments were performed either in the dark or with blindfolded animals (eyes covered with black paint). This suppressed potential visual stimulation of multimodal DINs. The terms “ipsilateral” and “contralateral” are used with reference to the stimulated antenna (i.e., ipsilateral connective is right, contralateral connective is left). Averaged data were obtained from staircases with four ramps, as shown in Fig. 1B. The first two of these ramps are referred to as levation and the last two as depression of the antenna. Note, however, that levation was always accompanied by adduction and depression by abduction of the antenna (see Dürr et al. 2001). We did not average data across recordings.

Recordings. Whole nerve recordings were obtained en passant from one or both neck connectives with unipolar tungsten hook electrodes connected to preamplifiers (MA-101; Electronics work shop, University of Cologne, Germany; total amplification: 2,000X).

Thin-walled borosilicate glass microelectrodes (GB100TF-10, Science Products) filled with either 1 M LiCl (shaft) and 5% Lucifer yellow (Sigma-Aldrich, Taukirchen, Germany; tip) or 1 M KCl (shaft) and 4% Neurobint tracer (Vector Labs, Burlingame, CA; tip) with resistances between 14 and 60 MΩ were used for intracellular recording of axons. Low-resistance electrodes were used when targeting a contralateral giant DIN. Recording sites were located in the anterior neuropile of the prothoracic ganglion, close to the insertion sites of the connectives, or in the connectives themselves (Fig. 1C). A synchronous, whole nerve recording of the connective ipsilateral to the intracellular recording site was always acquired. Intracellular signals were amplified with a BA-03X amplifier (NPI, Tamm, Germany). All reference electrodes were placed in the saline in the metathoracic body cavity. Data were recorded at 6,500 and 12,000 Hz with a Micro1401 A/D converter and Spike2 data acquisition software (version 7.01, Cambridge Electronic Design, Cambridge, UK). The recording quality depended somewhat on the DIN type, with the action potential amplitude typically varying between 15 and 80 mV. Recordings lasted up to 90 min, and recording times typically ranged from 5 to 60 min. In most experiments, several DINs were recorded subsequently and only the last DIN recorded was injected with a tracer. We did not obtain stainings of DINs from all groups, and most stainings were restricted to the prothoracic ganglion and the SOG. We therefore focus on a description of the most important response types to quantify the information transfer between antennae and thorax, rather than a morphological description of the DINs.

Once a neuron was impaled by the electrode, the antenna was touched and moved with a fine paintbrush (Fig. 1C). If the neuron responded, the stimulator was brought near the antenna and a protocol of different staircases was used to determine the response properties of the neuron. In some cases, neurons responded strongly to the mechanical stimulation of the flagellum but not to the subsequent protocols of SP joint movement. Figure 1C shows a representative recording of such a touch-sensitive DIN. As our touch stimuli were delivered manually with a paintbrush, we did not characterize these DINs in a quantitative manner. Nevertheless, it is worth noting their existence. In the context of spatial coding of antennal contact location, touch-sensitive DINs may serve a function complementary to the posture- and movement-sensitive DINs on which this study focuses.
After each experiment, intracellular spikes were used to calculate spike-triggered averages of the neck connective recording(s). For all DINs presented in this report, the resulting spike-triggered average only ever revealed one spike in one connective (either ipsi- or contralateral with respect to the stimulated antenna). This extracellular spike preceded the intracellular spike in all cases. All DINs were therefore physiologically descending, i.e., conveying spikes from the head to the thorax.

Data analysis. After acquisition with Spike2, data were resampled at 5,000 Hz and exported to MATLAB (version 7.9; MathWorks, Natick, MA), where they were analyzed and plotted with custom-written scripts. Where graphs contain error bars, they show mean ± standard deviation.

Several parameters were used to quantify the response properties of DINs. Three of them are presented in this report. First, we quantified the spike activity during plateau periods of the stimulus, i.e., when the antenna was held still. For this, the spike frequency during an 800-ms window, starting at least 50 ms after the last ramp and ending at least 100 ms before the next ramp, was calculated for all sweeps of the same staircase (typically 4). The plateaus at the ventro-lateral extreme position (−52°), the resting position (0°), and the dorso-medial extreme position (+52°) were used for this analysis (Fig. 1, A and B). Second, the number of spikes that occurred during each ramp was divided by the ramp duration. This yielded the mean spike frequency during the ramps. Third, we measured the coincident joint angle for each spike recorded during stimulation, independent of whether it occurred on a plateau or on a ramp. Histograms were generated in which the number of spikes was plotted against the joint angle in bins of 5°. For comparison of histograms between stimulus conditions, the number of spikes was normalized to the duration the antenna spent in each joint angle bin. This compensated for differences in joint angle velocity and duration of hold periods. Data were pooled across subsequent identical staircases.

RESULTS

The main objective of this study was to characterize the response properties of antennal mechanosensory DINs and to determine what kind of proprioceptive information these neurons convey to the thoracic ganglia. The recording site was located in the neck connective, between the SOG and the prothoracic ganglion. All DIN groups described in this study therefore potentially contributed information about antennal posture and movement to neural networks of the prothoracic ganglion.

On the basis of our analysis of 110 recordings from DINs, a large variety of mechanoreceptive response properties were sampled. In the following, we provide a representative overview of five groups of antennal mechanosensory DINs. All of these mediate information about the SP joint angle and movement to thoracic ganglia. The groups are: 1) simple position-sensitive, 2) dynamic position-sensitive, 3) unspecific movement-sensitive, 4) ON-type velocity-sensitive, and 5) OFF-type velocity-sensitive DINs. Most DINs were sensitive to more than a single parameter of SP joint stimulation (i.e., movement velocity, direction, and position). Neurons of all groups descended on either side of the nervous system, through the ipsi- and contralateral connective (with respect to the stimulated antenna). Table 1 shows the number of recordings obtained from each DIN group, separated into ipsilateral and contralateral DINs. DINs from all groups were reliably recorded in different animals. Note that Table 1 does not necessarily reflect the relative number of DINs, as the sampling was likely biased toward DINs with larger axons. In the following, each of the five DIN groups is characterized with regard to their functional relevance, using representative examples.

Simple position-sensitive DINs. Simple position-sensitive DINs effectively signal the SP joint angle, as their spike rate was increased within a certain joint angular range (Fig. 2).

The representative simple position-sensitive DIN shown in Fig. 2A spiked almost exclusively at ventro-lateral joint angles. Antennal movement itself had only a minor effect on the DIN response, as the spike rate did not change markedly in response to the ramp onset (Fig. 2A). The angular distribution of all spikes occurring during staircases with different velocities showed that the DIN was much more likely to spike at ventral joint angles (Fig. 2B). Only few spikes were elicited when the SP joint angle was dorsal from the resting position (i.e., 0°), and the spike frequency increased at increasingly ventral SP joint angles (Fig. 2B). The spike distributions depended on the movement direction in that they were narrower and showed a stronger increase toward ventral joint angles during depression than during levation (Fig. 2B, compare left and right columns).

The spike distribution also depended on the movement velocity: the slower the movement, the wider was the range in which the DIN responded during depression of the antenna. However, the increase toward more extreme joint angles remained (Fig. 2B, compare rows). Low numbers of additional spikes fired during antennal movement also resulted in an apparent velocity sensitivity (Fig. 2C; see also Fig. 6). This velocity sensitivity differed strongly from that of truly velocity-sensitive DINs. For example, the mean spike frequency during ramp 1 only doubled and the variance increased drastically when the velocity was increased from 12°/s to 400°/s (Fig. 2C). In contrast, the spike frequency of ON-type velocity-sensitive DINs increased by a factor of ~50 and the variance remained constant over the same velocity range (for comparison, see Fig. 7).

The simple position-sensitive DIN in Fig. 2, A–C, exhibited relatively low spike frequencies at all SP joint angles, with peak frequencies in the range of 15 Hz. This was typical for simple position-sensitive DINs (Fig. 2D).

The population of simple position-sensitive DINs showed a simple form of range fractionation. Figure 2D shows five examples of the mean spike frequency of different DINs during

<table>
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<th>Simple Position</th>
<th>Dynamic Position</th>
<th>Unspecific Movement</th>
<th>ON-Type Velocity</th>
<th>OFF-Type Velocity</th>
<th>Other</th>
<th>Total</th>
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<td>Ipsi/contra</td>
<td>13/1</td>
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<td>Σ</td>
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The number of recordings from ipsilateral/contralateral descending interneurons (DINs) per group are given, along with the total number of recordings (Σ).
the plateau periods. Each of the DINs showed an increased spike rate for one or several of the plateaus. DIN i is the neuron from Fig. 2, A–C. Both DINs i and ii spiked when the SP joint was in ventral positions, though with slightly different spike frequencies (Fig. 2D, i and ii). DINs iii and iv did the opposite of DINs i and ii, in that they spiked at the highest frequencies when the SP joint angle was in the dorsal range. DIN iii also responded when the antenna was held in its resting position, near 0°. This DIN showed directional selectivity in the form of hysteresis: it spiked at the resting position after levation from ventral joint angles but not after depression from dorsal joint angles. DIN iv was descending contralaterally, whereas DIN iii was descending ipsilaterally. Still other simple position-sensitive DINs spiked at ventro-lateral and dorso-medial joint angles but not in the resting range (Fig. 2D, v). Of the 14 simple position-sensitive DINs recorded, none was preferably responding in the resting range. This indicated that the input to simple position-sensitive DINs might be driven by patches of sensory hairs at the SP joint, which are only deflected if the SP joint angle is dorsal or ventral from the resting position.

Fig. 2. Simple position-sensitive DINs. A: typical spike pattern of a ventral simple position-sensitive DIN during SP joint movement. Dashes indicate single spikes of the DIN during 4 consecutive sweeps (horizontal lines) of the same stimulus (bottom trace). Periods of SP joint movement are shaded in gray. The stimulus velocity was 13°/s. B: bars indicate the mean spike frequency of the DIN shown in A during levation (left) and depression (right) at different joint angles and different stimulus velocities. The bin size was 5°. Four or five consecutive sweeps of the same stimulus were averaged. Ramps as well as plateaus (−52°, 0°, +52°) were taken into account. y-Axes for stimulus velocity of 127°/s are scaled differently from other velocities. C: mean spike frequency during the different ramps for SP joint stimulation at different velocities. D: range fractionation of 4 ipsilateral (i–iii, v) and 1 contralateral (iv) simple position-sensitive DINs. Bars show mean spike frequency on each of the 5 plateaus (compare to A). Firing rates of DINs were maximal in the ventral, the dorsal, or both extreme positions, but never in the resting range (2nd and 4th plateaus). Stimulus conditions: n = 4 staircase repetitions per DIN, ramps with 30–40°/s velocity.
Taken together, the group of simple position-sensitive DINs mediate information on the current SP joint angle. These DINs showed an increased spike rate at ventral and dorsal SP joint angles, irrespective of whether the antenna was moving or not. Simple position-sensitive DINs descended ipsi- or contralaterally. They are only weakly sensitive to movement direction and velocity.

**Dynamic position-sensitive DINs.** Dynamic position-sensitive DINs signal antennal movement within specific SP joint angle ranges (Fig. 3). In contrast to simple position-sensitive DINs, dynamic position-sensitive DINs stopped spiking, or reduced their spike frequency strongly, once antennal movement ceased. This was irrespective of the current SP joint angle. Dynamic position-sensitive DINs were therefore strongly sensitive to antennal movement. Some dynamic position-sensitive DINs were additionally selective for the direction of movement, and increased their spike rate during movement toward either extreme position.

Figure 3A shows the response of a representative dynamic position-sensitive DIN during imposed SP joint movement. Spikes were elicited at all movement velocities used, ranging from 1 to 800°/s. The DIN did not spike, except when the antenna moved toward the dorso-medial and the ventro-lateral extreme angles within a narrow joint angle range. This type of response was consistent during repeated stimulation (Fig. 3A), and DINs with similar properties were recorded in two further animals. The DIN was strongly sensitive to three parameters of SP joint movement: 1) the DIN was position sensitive, as it only fired within a narrow range around the two extreme positions; 2) the DIN was strongly movement sensitive, as it only spiked during SP joint movement and stopped spiking as soon as the plateaus were reached; and 3) the DIN was strongly direction sensitive, as spikes only occurred during movements toward the extreme angles but never during movements away from these. To underline the strong movement sensitivity of this DIN, Fig. 3B shows a sequence in which staircases with 32 ramps were used for SP joint stimulation. In this case, the DIN still fired only when the joint angle approached the extreme positions. The response was limited to the last two ramps of the staircase. No response was recorded during the plateaus between the last two ramps, even though they clearly lay within the position-sensitive range of this DIN (Fig. 3, A and B). In summary, this DIN appeared to signal that the SP joint approached its extreme angle during antennal elevation and depression, respectively.

Like the simple position-sensitive DINs, the group of dynamic position-sensitive DINs showed range fractionation (Fig. 3C). DIN i is the neuron from Fig. 3, A and B. DIN ii spiked preferentially at dorso-medial joint angles beyond 5°. DIN iii covered the opposite part of the joint angular range, spiking preferentially at ventro-lateral joint angles beyond −25°. Both histograms (Fig. 3C, ii and iii) show a sharper peak during movements away from the preferred position than during movements toward it, indicating directional selectivity.
The mean spike frequency on the plateaus was below 1.5 Hz for all DINs shown in Fig. 3C, highlighting the movement sensitivity of dynamic position-sensitive DINs.

As a population, the three types of DINs shown in Fig. 3C can convey considerable positional information: strong, simultaneous firing of DINs i and ii would have occurred only during movement toward the dorso-medial extreme position, whereas strong, simultaneous firing of DINs i and iii would have occurred only during movement toward the ventro-lateral extreme position. Firing of DIN ii alone would have occurred only during movement away from the dorso-medial extreme position. Strong firing of DIN iii alone would have occurred only during movements away from the ventro-lateral extreme position. We also recorded from dynamic position-sensitive DINs with sensitivity ranges and tuning curves different from those shown in Fig. 3C.

Together, dynamic and simple position-sensitive DINs appeared to signal antennal posture with considerable fidelity. Dynamic position-sensitive DINs add a look-ahead value to positional information, in that their firing indicates which positions are to be reached next.

An example for an exceptional type of dynamic position-sensitive DINs is illustrated in Fig. 4. Such DINs responded to SP joint movement exclusively at high joint angle velocities (100–800°/s) and fired at most one or two spikes per ramp. The DIN in Fig. 4 responded only to movements in the ventral joint angle range (ramps 1 and 4), making it position sensitive. Spike reliability increased with velocity, as documented by an average spike rate of 0.6 spikes per ramp at 127°/s, and 1.2 spikes per ramp at 374°/s (considering only ramps 1 and 4). Stimulation at still higher velocities led neither to a stronger response nor to increased reliability. Other DINs of this type responded preferentially to dorsal ramps or to all ramps of fast staircases. It appears that these DINs responded optimally to stimuli that were faster than those we could deliver, i.e., faster than 800°/s. Another possibility is that these DINs responded to costimulation of mechanoreceptors other than those of the SP joint, e.g., campaniform sensilla on the pedicel.

Unspecific movement-sensitive DINs. Unspecific movement-sensitive DINs are sensitive to SP joint movement but insensitive to other parameters. They are unspecified in that their spike rate is independent of the direction and velocity of antennal movement, and also independent of the joint angle range in which the movement occurs. In that sense, they do the opposite of simple position-sensitive DINs, which are relatively insensitive to SP joint movement. Unspecific movement-sensitive DINs were recorded ipsi- and contralaterally.

A typical example of an unspecific movement-sensitive DIN is shown in Fig. 5. These DINs fired irregular bursts of spikes and irregular single spikes independent of SP joint stimulation (Fig. 5, A and B). When exposed to a staircase with 32 ramps and relatively fast ramp velocity (38°/s), both irregular bursting and low-frequency background activity persisted (Fig. 5A). Additionally, characteristic double spikes were elicited during the ramps (Fig. 5, A and C). The double spikes occurred reliably on all 32 ramps, independent of joint angle and movement direction. When the stimulus was a staircase with very slow ramp velocity (1°/s; Fig. 5B), the DIN only showed the irregular background activity. Interspike intervals were significantly shorter during stimulation with fast, small-amplitude deflections (as in Fig. 5A) than during stimulation with slow, large-amplitude deflections (as in Fig. 5B). This is illustrated by the corresponding cumulative probability plots of the interspike intervals (Fig. 5D, black solid vs. gray dotted lines), which differed significantly ($P = 0.006$, 2-sided Kolgomorov-Smirnov test, $n_1 = 831, n_2 = 280$). The discrepancy between the two distributions in Fig. 5D was due to the double spikes, which typically had interspike intervals between 80 and 120 ms. During faster deflection, there were no interspike intervals longer than 1 s (Fig. 5D, black line reaches 1.0 at ~1 s). This corresponded to the 950-ms-long interval between ramps. Therefore, every ramp elicited at least one additional spike in the DIN. During slower stimulation, interspike intervals of >1 s occurred (Fig. 5D, gray dotted line). We conclude that the bursting pattern seen in Fig. 5, A and B, was not driven by antennal stimulation, whereas additional spikes were elicited by transient deflection of the joint, provided it was fast enough.

When the SP joint was moved over the whole working range using the standard staircase with four ramps, the DIN still responded with only one to three additional spikes per ramp. As a result, the DIN showed an apparent velocity sensitivity (Fig. 5E) that was due to the increasing significance of the additional spikes with decreasing ramp duration (see also Fig. 6). Accordingly, the number of spikes per ramp depended on ramp velocity much as predicted by a hyperbolic function for constant spike rate and decreasing ramp duration (Fig. 5F). The additional one to three spikes during faster ramps caused a deviation from that hyperbolic prediction, which is evident only for faster, and therefore shorter, ramps (Fig. 5F, inset). Finally, unspecific movement-sensitive DINs also showed an increase in spiking activity in response to touch stimuli to the abdomen (data not shown). As these stimuli did not elicit active antennal movements, we conclude that the DIN received additional input from other mechanoreceptors not located on the antenna.

Figure 6 explains the apparent velocity sensitivity exhibited by unspecific movement-sensitive DINs and simple position-sensitive DINs with simple, modeled data. A neuron that does not respond to antennal stimuli exhibits the same mean spike frequency at all stimulus velocities (Fig. 6, gray line and inset, trace 1 from top). If this neuron responds to antennal stimu-
lation with two additional spikes, which occur independent of the stimulus velocity, this results in an increased spike rate during faster ramps (Fig. 6, circles). The two additional spikes fall into an increasingly shorter interval during faster ramps (Fig. 6, inset, traces 2–5). The two additional spikes thus have a larger impact on the mean spike rate during shorter ramps. Apart from the mean spike rate, this affects the interspike interval distribution and the instantaneous spike rate of the DIN. The slope of the apparent velocity sensitivity depends on the number of additional spikes elicited (Fig. 6, compare triangles, circles, and crosses). The apparent velocity sensitivity of simple position-sensitive and unspecific movement-sensitive DINs could be explained by such a small number of additional spikes. The tuning curves of ON- and OFF-type velocity-sensitive DINs clearly deviated from this model.

ON-type velocity-sensitive DINs. We found two types of genuinely velocity-sensitive DINs. The first were ON-type...
velocity-sensitive DINs, which are excited by movement of the SP joint in such a way that their spike frequency is linearly correlated with SP joint angle velocity. Generally, these DINs have a low resting spike frequency and show no increase in spike frequency in the absence of SP joint movement.

A representative example of an ON-type velocity-sensitive DIN is shown in Fig. 7. As soon as the antenna was moved (Fig. 7A, gray shaded areas), the DIN started spiking vigorously. This activity lasted only as long as the antenna kept moving. The mean latency of the first spike was $12.9 \pm 1.9$ ms (mean $\pm$ SD, $n = 6$ ipsilateral ON-type velocity-sensitive DINs, where mean values for each DIN were derived from 4 subsequent ramps at velocities faster than $300^\circ$/s). This activity pattern was observable during all recordings from this DIN type and during all ramps. This spike pattern was reliably elicited during repetitive trials (Fig. 7A). All ON-type velocity-sensitive DINs showed a strong sensitivity to antennal movement velocity. Faster movements elicited spikes at higher frequencies, up to mean frequencies of $>200$ Hz (Fig. 7B). This was due to a relatively constant, large number of spikes fired during ramps of all velocities (Fig. 7C). This contrasts strongly with the apparent velocity sensitivity as exemplified in Fig. 6. Apart from the velocity, ON-type velocity-sensitive DINs were weakly selective for the direction of antennal movement and the SP joint angle. For example, the second and third ramps (dorsal joint angle range) elicited weaker responses than the first and third ramps in several DINs of this group (Fig. 7). Regardless of such weak position sensitivity, the spike frequency was proportional to movement velocity in all parts of the joint angle range (Fig. 7B, compare open and filled triangles). Note that the x-axis in Fig. 7B is log-scaled and that the fitting function was linear (solid black line, Fig. 7B). ON-type velocity-sensitive DINs were regularly recorded in the ipsi- and the contralateral connective.

OFF-type velocity-sensitive DINs. This group of DINs mirrors the response properties of ON-type velocity-sensitive DINs. OFF-type velocity-sensitive DINs exhibit a high baseline spike frequency, ranging between 15 and 30 Hz in the
absence of antennal movement. This baseline activity differed considerably between different DINs (compare Fig. 8, A and D). Upon onset of SP joint movement, the spike rate of OFF-type velocity-sensitive DINs decreased (Fig. 8, A–D, gray shaded areas), as if the neurons were inhibited by movement-sensitive input elements. This putative inhibition persisted until the movement ceased, in some cases even longer (Fig. 8D, second and fourth ramps). The decrease in spike frequency depended linearly on movement velocity: the mean spike rate decreased as the velocity increased (Fig. 8, E and F, black linear fits).

This suggested that the strength of the putative inhibition increased with increasing joint angle velocity. OFF-type velocity-sensitive DINs were not sensitive to any other parameter of antennal movement, except velocity. The reduction of activity did not depend on movement direction or joint angle.

In summary, we found that SP joint angle velocity was mediated from the brain to the thorax via two computational channels, potentially acting in a push-pull manner. ON-type velocity-sensitive DINs started spiking during SP joint movement and increased their spike rate with increasing joint angle.

Fig. 8. OFF-type velocity-sensitive DINs. A: original recording of a representative contralateral OFF-type velocity-sensitive DIN (top trace) and the stimulus (bottom trace). Periods of SP joint movement are shaded in gray. The DIN’s spike frequency was reduced during antennal movement. B: spike raster plot for 5 sweeps of the same stimulus; the DIN activity was reduced during all ramps of all 5 trials. Arrowhead marks the trial shown in A. C: peristimulus time histogram of the 5 sweeps shown in B. Spike frequency dropped markedly during all ramps. D: spike pattern (top traces) during 4 consecutive sweeps of the same stimulus (bottom trace) of an ipsilateral OFF-type velocity-sensitive DIN. This DIN had a higher baseline firing rate than the DIN in A. E: mean spike rates during ramps of different velocities for the DIN in A–C. F: same plot details as in E for the DIN in D. Black lines are linear fits with slopes of \(-13.91 (R = -0.988; E)\) and \(-27.00 (R = -0.965; F)\).
velocity, and OFF-type velocity-sensitive DINs reduced their spike rate during SP joint movement in a velocity-dependent way.

**DISCUSSION**

The primary focus of this study was to analyze which information about antennal posture and movement is mediated from antennal mechanoreceptors to the thoracic ganglia, which control front leg movements. As a consequence, we recorded from antennal mechanosensory DINs and systematically varied antennal posture, movement velocity, and movement direction. We found DINs that were strongly sensitive to one of these parameters and weakly sensitive to the others. Five groups of DINs mediate detailed information about antennal posture and movement to the thorax. Two groups mediate postural information, two groups mediate the joint angle velocity, and one group responds to SP joint movement in general. We did not find any DINs that were sensitive to only one stimulus parameter, so that even simple position-sensitive DINs were weakly sensitive to antennal movement. As all neurons were recorded anteriorly to the entry point of the neck connective to the prothoracic ganglion, and classified as descending, we assume that all DINs connect to thoracic neural networks.

To this end, individual DIN morphologies remain elusive, and we cannot distinguish whether individual neurons are descending from the brain or from the SOG. In both cases, DINs may receive direct input from antennal mechanosensory afferents (e.g., Bräunig et al. 1983). However, this work will provide the basis for a computational analysis of the population of antennal mechanosensory DINs in insects. It highlights distinct computational features of DINs that can be contrasted against, for example, sensory neurons and local brain neurons. The data will also guide and simplify the individual identification of DINs with particularly interesting response properties.

**Directional information.** We did not find any predominantly direction-selective DINs that responded only to levitation or depression of the antenna. Similarly, Ritzmann et al. (2008) did not report such units from the cockroach central complex. Still, directional information is mediated to the thorax, for example, by the combined encoding of position and movement in the population of DINs. Moreover, many neurons were weakly direction selective, including simple position-sensitive, dynamic position-sensitive, and ON-type velocity-sensitive DINs. This is evident in the differences between their responses during levation and depression (Figs. 2, 3, and 7). Finally, directional information might be derived from the activation pattern of the antennal muscles. This would be unambiguous, because the SP joint is a simple hinge joint with only two antagonistic muscles (Dürr et al. 2001).

**Postural information.** Our results show that postural information, putatively derived from the activity of hair field afferents (Okada and Toh 2001), is mediated to thoracic neural networks via simple and dynamic position-sensitive DINs. These DINs are of particular relevance to the leg-retargeting behavior that stick insects show upon antennal contact during locomotion, as they potentially contribute to encoding the contact height of the antenna (Schütz and Dürr 2012). A DIN with properties comparable to the dynamic position-sensitive DINs shown in Fig. 3C, ii and iii, was also described in the cricket (Gebhardt and Honegger 2001). Given the importance of positional information for the spatial coordination of limbs, dynamic position-sensitive DINs could be a common feature of descending antennal mechanosensory pathways.

Simple position-sensitive DINs exhibited relatively low spike rates in the range of 10 Hz, even at their preferred joint angles. Therefore, assuming a rate code, any single DIN is unlikely to convey sufficient information about the current joint angle. Stick insects move their antennae with cycle frequencies of up to 2 Hz. Assuming symmetrical movements about the resting angle, the SP joint angle would be dorsal or ventral from the resting position for only 250 ms during each cycle. As a consequence, simple position-sensitive DINs could only fire two or three spikes while the joint stayed within its sensitivity range. Position-sensitive DINs could, however, signal the antennal joint angle as labeled lines. A prerequisite for this is range fractionation, i.e., different sensitivity ranges between neurons. Indeed, both simple and dynamic position-sensitive DINs show a simple form of range fractionation. In both groups, different DINs fire preferentially at ventral or dorsal joint angles. Range fractionation was, among other systems, shown in sensory afferents of the femoral chordotonal organs (fCO) in locust and stick insect legs (Burns 1974; Büschges 1994; Hofmann et al. 1985; Kittmann and Schmitz 1992; Matheson 1990, 1992; Zill 1985). Like position-sensitive DINs, some fCO afferents exhibit relatively low spike frequencies. Other than the DINs described here, however, some fCO afferents exhibit peak spike frequencies at intermediate joint angles (Matheson 1992). We did not find such response types within the population of antennal mechanosensory DINs. This may indicate that position-sensitive DINs receive input from sensory neurons of antennal hair fields, which are strongly activated only if the antennal joint angle is outside the resting range (Okada and Toh 2001).

The simultaneous firing of simple and dynamic position-sensitive DINs, and the contrast between the spike rates of position-sensitive DINs with different sensitivity ranges, could further increase the precision of positional information mediated by the DIN population. A position tuning curve of particular interest is that of the dynamic position-sensitive DIN shown in Fig. 3A. This DIN was only spiking during movements toward the ventral and dorsal extreme positions. Spikes in this DIN therefore signaled that the SP joint was approaching its turning point. This kind of signal appears well suited for the coordination of movements in different joints, and for the control of alternating motor patterns. Indeed, stick insects with intact antennal hair fields do not use the entire antennal joint angle work ranges during natural movement. Instead, they reverse movement direction before the joint limit is reached (Krause et al. 2013). Our DIN recordings show that the information necessary to trigger such return movements is extracted from sensory signals. Still, it is not immediately clear why that information is forwarded to thoracic neural networks. One explanation could be the temporal coordination of antennal and front leg movements, as observed by Dürr et al. (2001).

**Joint angle velocity.** Movement of the SP joint leads to a velocity-dependent excitation of ON-type velocity-sensitive DINs, to a velocity-dependent reduction of spike frequency in OFF-type velocity-sensitive DINs, and to additional spikes in unspecific movement-sensitive DINs. The two groups of velocity-sensitive DINs could serve the same function. In prin-
ciple, they could act upon the same thoracic interneurons in a push-pull type of mechanism. Ritzmann et al. (2008) also found both excited and inhibited antennal mechanosensory units in the cockroach central complex. As in the DINs reported here, the spike rate of these units depended on antennal movement velocity. Although the delay of their response was not estimated precisely, the central complex units responded with much longer delays than the DINs presented here (~40–60 ms later). This suggests that antennal movement velocity could generally be mediated by an excited and an inhibited pathway in different insects. It also further underlines that there could be two pathways for the transfer of antennal mechanosensory information: one fast, direct pathway connecting the deutocerebrum and SOG to the thorax and one slow, indirect pathway involving the central complex. An alternative model is that the central complex receives a copy of the descending antennal mechanosensory signal, which is then integrated with other sensory information to select an appropriate behavioral pattern based on the overall state of the animal (Strausfeld and Hirth 2013). This model does not suggest a slow pathway connecting the brain to thoracic motor centers via the central complex, but a more indirect contribution of the central complex via an influence on DINs (Ritzmann et al. 2012).

An ON-type velocity-sensitive DIN that responded to SP joint movement was also described in the cricket (DBN-i-1-2; Gebhardt and Honegger 2001). This DIN responded to deflections of the ipsilateral antenna with phasic depolarizations and spikes, much like the ON-type velocity-sensitive DINs described here. One important difference, though, is that the spike rate of DBN-i-1-2 increased with movement velocities up to 300°/s and then declined slightly when the joint angle velocity was increased further. The tuning curve was therefore bell shaped, not linear up to very high velocities, as was the case in the ON-type velocity-sensitive DINs we recorded in the stick insect.

It is somewhat enigmatic why the nervous system should mediate antennal joint angle velocity to the thorax. One reason could be that the movement velocity is needed as a more global variable. For example, the tuning curves of position-sensitive DINs depended somewhat on the joint angle velocity. In the cockroach, stronger wind stimuli resulted in stronger activation of DINs responding to flagellar bending (Burdoohan and Comer 1990). Assuming these DINs also responded to flagellar bending during an active contact, their spike rate would depend upon the preceding antennal movement velocity. Higher velocities would lead to stronger deflections when the same obstacle was met. Velocity-sensitive DINs might therefore be relevant for interpreting the responses of other neurons, as was also suggested by Ritzmann et al. (2008). Related to this, velocity information was shown to be relevant for tactually mediated turning. In a model of antennal wall-following behavior in cockroaches, both proportional and derivative (PD) feedback about contact distance were required for inducing appropriate turns of the body axis (Cowan et al. 2006). Analogously, one might argue for a similar PD controller underlying tactually mediated climbing. In climbing, the appropriate timing of leg movements in response to obstacle contact will depend on distance and velocity of approach. In such a control scheme, the proportional component could be delivered by simple and dynamic position-sensitive DINs, while velocity-sensitive DINs appear ideal to signal derivative feedback.

Finally, in addition to their proprioceptive role, velocity-sensitive DINs will respond strongly to antennal deflections caused by external forces such as approaching predators. Relevance to active tactile exploration. We measured the sensitivity of DINs to imposed SP joint movement while the animal was fixed and quiescent, after ablation of all legs. This situation was different from active tactile sensing, where 1) movements are driven by muscular contractions, 2) both antennal joints are moving at the same time, and 3) DINs might receive additional input from other sensory modalities. However, the experimental situation might resemble a state of passive sensing in which antennal deflections are introduced by external events, such as contact with approaching conspecifics or predators (Comer et al. 2003; Okada and Akamine 2012).

How similar would the DIN response patterns be during active antennal movement? Staudacher and Schildberger (1998) analyzed state-dependent gating effects in DINs that responded to mechanosensory, visual, and auditory stimuli in walking and standing crickets. Visual responses and the auditory response of some DINs to courtship signals were gated by walking activity, but not the response to mechanical stimulation of the cercal system or ultrasound. The latter two potentially signal predators. If these results can be generalized, one could expect that antennal mechanosensory information should not be gated, since it is known to be relevant for escape behaviors (see Comer and Baba 2011; Staudacher et al. 2005). Recently, numerous studies have reported state-dependent differences in sensory processing (e.g., Chiappe et al. 2010; Jung et al. 2011; Longden and Krapp 2009; Maimon et al. 2010; Rien et al. 2012; Zorovic and Hedwig 2011). Essentially, all of these studies point in a similar direction: tuning curves of single neurons may change with the behavioral state of insects (and other animals, see Maimon 2011 for a review). These changes can be relatively large and affect the gain as well as the sensitivity range of individual neurons. They do not, however, affect the stimulus modality the neurons respond to. Indeed, Gebhardt and Honegger (2001) suggested that an identified DIN may have different tuning curves in different animals. In two experiments they reported, the same identified DIN was sensitive to the antennal joint angle during imposed movement, but the response differed between two animals. Therefore, while the tuning curves of individual DINs might be different depending on the state of the animal and across individuals, the same five groups of DINs mediating antennal posture and movement velocity to thoracic ganglia are likely to be involved. One possible source of the DIN modulation could be the central complex.

Tactually elicited retargeting of front legs occurs within 40 ms in walking stick insects (Schütz and Dür 2011). ON-type velocity-sensitive DINs conducted spikes to the prothoracic ganglion within 12.9 ms after antennal stimulation. Hence, ON-type velocity-sensitive DINs could contribute to the retargeting, as their response latencies are sufficiently short. The latency between motoneuron stimulation near the metathoracic ganglion and subsequent extensor tibiae muscle force production is 8.5 ms in the stick insect (Guschlbauer et al. 2007). Assuming similar latencies in the prothoracic ganglion, a direct pathway from ON-type velocity-sensitive DINs to motoneurons could lead to changes in leg movement trajectories within <25 ms after antennal contact. The delay of 40 ms measured in behavioral experiments was 15 ms longer, which could be
needed for an additional processing step within a layer of thoracic interneurons. In any case, the delays observed were short enough to explain the fast retargeting of leg movements observed in behavioral experiments, even taking into account that passive forces may have to be overcome before a change in the movement trajectory can occur during ongoing movement (Ache and Matheson 2012; Hooper et al. 2009). Touch-sensitive DINs in the cricket and cockroach were even faster and responded within 4 and 7 ms, respectively (Burdoohan and Comer 1990, 1996; Schöneich et al. 2011). With correction for the recording sites, which differed in the experiments on crickets and cockroaches, the delay would have been even shorter in crickets and slightly longer in cockroaches. The different latencies may indicate either interspecific differences or that contact information generally reaches thoracic networks faster than proprioceptive information. This could be tackled by recording from touch-sensitive DINs in the stick insect while delivering precisely timed touch stimuli.

In summary, the population of DINs mediates sufficient information about antennal posture and movement from the head to the thorax to drive the intersegmental spatial coordination of antennae and front legs. Antennal mechanosensory DINs thus contribute to obstacle negotiation and adaptive locomotion in stick insects.

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AUTHOR CONTRIBUTIONS

Author contributions: J.M.A. performed experiments; J.M.A. analyzed data; J.M.A. and V.D. interpreted results of experiments; J.M.A. prepared figures; J.M.A. drafted manuscript; J.M.A. and V.D. edited and revised manuscript; J.M.A. and V.D. approved final version of manuscript; V.D. conception and design of research.

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