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

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**Abstract**

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. Based on the 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 dependency 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 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.

**Keywords**: tactile sensing, interneurons, coordination, proprioception, ensemble coding
Introduction

Both mammals and arthropods use actively movable tactile sensors, such as whiskers or antennae, to sample the adjacent space for near-range orientation (see Staudacher et al. 2005; Diamond et al. 2008 and Prescott et al. 2011 for reviews). 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; Okada and Toh, 2000; Cowan et al. 2006), 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). In order to exploit antennal tactile 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 to the central complex of the protocerebrum with relatively long delays (Ritzmann et al. 2008; Harley and Ritzmann, 2010), where they could contribute to the selection of appropriate locomotor patterns. Second, there is a rapid, direct neural pathway involving descending interneurons which may convey spikes to the thoracic ganglia within less than 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; Staudacher, 1998; Gebhardt and Honegger, 2001; Schöneich et al. 2011), which control leg movements (Burrows, 1996; Büschges and Gruhn, 2007; Büschges et al. 2008). Here, we used the stick insect *Carausius morosus* (de Sinèty 1901) for characterizing a large population of antennal mechanosensory descending interneurons (DINs) which mediate antennal posture and movement to thoracic ganglia. These neurons constitute an important component of the fast, direct pathway involved in descending motor control.
Insects use antennal mechanosensory cues for adaptive locomotion

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 which 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 *Carausius 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 re-targeting of the ipsilateral front leg towards 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. Re-targeting 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. Descending interneurons should therefore transmit short-latency information about antennal contact location to thoracic motor networks in stick insects.

Antennal posture is essential for the acquisition of near-range spatial information.

In *Carausius 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 need 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 joint (HS) and the next distal scape-pedicel joint (SP). 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 towards 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. Antennal 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 focusing on descending interneurons which 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.

**Antennal mechanosensory descending interneurons (DINs)**

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 bottleneck 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 was 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 to which degree 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 sub-groups, mediating precise information about antennal
posture and movement to thoracic neural networks.
Material and Methods

Animals and Preparation

Adult, female stick insects (*Carausius morosus*) with intact antennae from a colony bred at Bielefeld University were firmly fixed dorsal side up using dental glue (Protemp, 3M ESPE AG, Neuss, Germany). All legs and the left antenna were removed. The head-scape joint (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 annulus, where the flagellum is stiff (Dirks and Dürr, 2011; Figure 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 (Weidler and Diecke, 1969; Bässler, 1977). 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 tracheae 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 lateral to the ganglion in five positions, using 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 to 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 minutien pin was inserted into the proximal part of the right flagellum (Figure 1A). With the HS joint fixed, the antenna could be moved about the SP joint using a contact-free, magnetic stimulator (Gebhardt and Honegger, 2001; Figure 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, the 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 (Figure 1A, ventral antennal posture; Figure 1B). After having been moved to the resting position (Figure 1A, medial posture, black; Figure 1B), it was held there for 950 ms, and then moved to the dorso-medial extreme position (Figure 1A, dorsal antennal posture; Figure 1B). Following another hold period of 950 ms, the reverse movement was imposed, bringing the antenna back to the ventro-lateral extreme position (Figure 1B). Ramp velocities and holding times were kept the same for subsequent staircases, with staircases being separated by a 2.9 s inter-stimulus interval.

Throughout this paper, stimulus periods of imposed antennal movement are referred to as ramps, whereas hold periods with no antennal movement are referred to as plateaus. Ramp velocities were varied pseudorandomly between 1 and 800 °/s, covering the velocity range of sinusoidal joint angle time courses with 100° peak-to-peak amplitude and cycle frequencies up to 2.5 Hz. Staircases of the same velocity were typically repeated four times (ranging from 2 to 10 times) before a different velocity was used. Staircases with ramp velocities slower than 12°/s were repeated at least twice. A complete staircase always covered a joint angle range of 104°, which slightly exceeded the natural angular working-range of the joint.
(approximately 100°, Krause et al. 2013). This working-range was typically covered by 2 ramps in each direction (ranging from 2 to 16). With the HS joint fixed and all but the proximal annuli of the flagellum cut off, stimulation was designed to predominantly stimulate two hair fields and two hair rows of the SP joint (Krause et al. 2013).

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 macro objective (TechSpec VZM 200, Edmund Optics, Barrington, USA) 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 hairfields, we cannot exclude co-stimulation of other pedicellar or flagellar mechanoreceptors, e.g. campaniform sensilla, in form of cuticular strain. We mention and discuss this wherever relevant.

All experiments were either performed in the dark, or with blindfolded animals (eyes covered with black paint). This suppressed potential visual stimulation of multimodal DINs. The terms ipsi- 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 Figure 1B. The first two of these ramps will be referred to as levation, 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.
Whole-nerve recordings were obtained *en passant* from one or both neck connectives using unipolar tungsten hook electrodes connected to pre-amplifiers (MA-101, Electronics work shop, University of Cologne, Germany; total amplification: 2000x).

Thin-walled borosilicate glass microelectrodes (GB100TF-10, Science Products, Germany) filled with either 1 Mol/l LiCl (shaft) and 5% Lucifer Yellow (Sigma-Aldrich, Taufkirchen, Germany; tip) or 1 Mol/l KCl (shaft) and 4% neurobiotin tracer (Vector labs, Burlingame, USA; 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 descending interneuron. 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 (Figure 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 6500 and 12000 Hz using a MICRO 1401 A/D converter and Spike 2 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 to 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 (Figure 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 did respond 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 complimentary function to the posture- and movement-sensitive DINs which this study focuses on.

After each experiment, intracellular spikes were used to calculate spike-triggered averages of the neck-connective recording(s). For all DINs presented in this paper, 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 Spike 2, data were re-sampled at 5000 Hz and exported to Matlab (Version 7.9, Mathworks, Natick, USA), where they were analyzed and plotted using 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 four). 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 (Figure 1A, 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.
The main objective of this study was to characterize the response properties of antennal mechanosensory descending interneurons (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, so that all recorded neurons projected at least into the prothoracic ganglion. Furthermore, all successful stainings obtained revealed neurites in the prothoracic ganglion. All DIN groups described in this study therefore potentially provide information about antennal posture and movement to neural networks of the prothoracic ganglion.

Based on our analysis of 110 recordings from DINs, a large variety of mechanoreceptive response properties was sampled. In the following, we provide a representative overview of five groups of antennal mechanosensory DINs. All of these mediate information about the scape-pedicel (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 the 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 towards DINs with larger axons. In the following, each of the five DIN groups will be characterized with regard to their functional relevance, using representative examples.

1) Simple position-sensitive DINs effectively signal the SP joint angle, as their spike rate was increased within a certain joint angular range (Figure 2).
The representative simple position-sensitive DIN shown in Figure 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 (Figure 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 (Figure 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 (Figure 2B). The spike distributions depended on the movement direction in that they were narrower and showed a stronger increase towards ventral joint angles during depression than during levation (Figure 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 towards more extreme joint angles remained (Figure 2B, compare rows). Low numbers of additional spikes fired during antennal movement also resulted in an apparent velocity-sensitivity (Figure 2C, see also Figure 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 (Figure 2C). In contrast, the spike frequency of ON-type velocity-sensitive DINs increased by a factor of about 50 and the variance remained constant over the same velocity range (for comparison, see Figure 7).

The simple position-sensitive DIN in Figure 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 (Figure 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 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 Figure 2 A-C. Both DINs i and ii spiked when the SP joint was in ventral positions, though with slightly different spike frequencies (Figure 2Di, 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 following levation from ventral joint angles, but not following 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 (Figure 2Dv). 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.

Taken together, the group of simple position-sensitive DINs mediates 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 descend ipsi- or contralaterally. They are only weakly sensitive to movement direction and velocity.

2) Dynamic position-sensitive DINs signal antennal movement within specific SP joint angle ranges (Figure 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 towards 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 towards the dorso-medial and the ventro-lateral extreme angles within a narrow joint angle range. This type of response was consistent during repeated stimulation (Figure 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 towards the extreme angles, but never during movements away from these. To underline the strong movement-sensitivity of this DIN, Figure 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 (Figure 3A, B). In summary, this DIN appeared to signal that the SP joint approached its extreme angle during antennal levation and depression, respectively.

Like the simple position-sensitive DINs, the group of dynamic position-sensitive DINs showed range fractionation (Figure 3C). DIN i is that from Figure 3A 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 (Figure 3C ii and iii) show a sharper peak during movements away from the
preferred position than during movements towards it, indicating directional selectivity. The mean spike frequency on the plateaus was below 1.5 Hz for all DINs shown in Figure 3C, highlighting the movement sensitivity of dynamic position-sensitive DINs.

As a population, the three types of DINs shown in Figure 3C can convey considerable positional information: strong, simultaneous firing of DINs i and ii would have occurred only during movement towards the dorso-medial extreme position, whereas strong, simultaneous firing of DINs i and iii would have occurred only during movement towards 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 different sensitivity ranges and tuning curves than those shown in Figure 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 Figure 4. Such DINs responded to SP joint movement exclusively at high joint angle velocities (100–800°/s) and fired at most one to two spikes per ramp. The DIN in Figure 4 responded only to movements in the ventral joint angle range (ramps one and four), 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 did neither lead 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 as if these DINs responded optimally to stimuli which were faster than those we could deliver, i.e., faster than 800°/s. Another
possibility is that these DINs responded to co-stimulation of mechanoreceptors other than those of the SP joint, e.g. campaniform sensilla on the pedicel.

3) *Unspecific movement-sensitive DINs* are sensitive to SP joint movement but insensitive to other parameters. They are unspecific 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 Figure 5. These DINs fired irregular bursts of spikes and irregular single spikes independent of SP joint stimulation (Figure 5A, 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 (Figure 5A). Additionally, characteristic double-spikes were elicited during the ramps (Figure 5A, 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, Figure 5B), the DIN only showed the irregular background activity. Interspike-intervals were significantly shorter during stimulation with fast, small-amplitude deflections (as in Figure 5A) than during stimulation with slow, large-amplitude deflections (as in Figure 5B). This is illustrated by the corresponding cumulative probability plots of the inter-spike intervals (Figure 5D black solid vs. gray dotted graph), which significantly differed (p=0.006, two-sided Kolgomorov-Smirnov test, n1=831, n2=280). The discrepancy between the two distributions in Figure 5D was due to the double-spikes, which typically had inter-spike intervals between 80 and 120 ms. During faster deflection, there were no interspike-intervals longer than 1 s (Figure 5D, black graph reaches 1.0 at about 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 over 1 s occurred (Figure 5D, grey dotted graph). We conclude that the bursting pattern seen in Figure 5A 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 1-3 additional spikes per ramp. As a result, the DIN showed an apparent velocity sensitivity (Figure 5E) which was due to the increasing significance of the additional spikes with decreasing ramp duration (see also Figure 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 (Figure 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 (Figure 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 using simple, modeled data. A neuron which does not respond to antennal stimuli exhibits the same mean spike frequency at all stimulus velocities (Figure 6, grey graph and first trace in inset). If this neuron responds to antennal stimulation with two additional spikes, that occur independent of the stimulus velocity, this results in an increased spike rate during faster ramps (Figure 6, filled circles). This occurs because the two additional spikes fall into an increasingly shorter interval during faster ramps (Figure 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 (Figure 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.

4) **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 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 Figure 7. As soon as the antenna was moved (Figure 7A, grey 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 ± 1.9 ms (mean ± SD, N = 6 ipsilateral ON-type velocity-sensitive DINs, where mean values for each DIN were derived from four subsequent ramps at velocities faster than 300 °/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 (Figure 7 A). 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 over 200 Hz (Figure 7B). This was due to a relatively constant, large number of spikes fired during ramps of all velocities (Figure 7C). This contrasts strongly to the apparent velocity-sensitivity as exemplified in Figure 6. Apart from the velocity, ON-type velocity-sensitive DINs were weakly selective to 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 (Figure 7). Regardless of such weak position-sensitivity, the spike frequency was proportional to movement velocity in all parts of the joint angle range (Figure 7B, compare open and filled triangles). Note that the x-axis in Figure 7B is log-scaled, and that the fitting function was linear (solid black line, Figure 7 B). ON-type velocity-sensitive DINs were regularly recorded in the ipsi- and the contralateral connective.

5) 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 Figure 8A and D). Upon onset of SP joint movement, the spike rate of OFF-type velocity-sensitive DINs decreased (Figure 8A-D, grey 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 (Figure 8D, second and fourth ramp). The decrease in spike frequency depended linearly on movement velocity: the mean spike rate decreased as the velocity increased (Figure 8E 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 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 descending interneurons 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 levation 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 (Figures 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 which 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 Figure 3Cii 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 symmetric 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, static position-sensitive DINs could only
fire two to 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 the sensory afferents of the femoral chordotonal organs (fCO) in locust and stick insect legs (Burns, 1974; Zill, 1985; Matheson, 1990; Matheson, 1992, Hofmann et al. 1985; Kittmann and Schmitz, 1992; Büschges, 1994). 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 Figure 3A. This DIN was only spiking during movements towards 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 has been 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 principle, 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 (ca.
40 to 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 (Burdohan 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 was 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 which 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 Staudacher et al. 2005; Comer and Baba, 2011).

Recently, numerous studies reported state-dependent differences in sensory processing (e.g., Longden and Krapp, 2009; Maimon et al. 2010; Chiappe et al. 2010; Jung et al. 2011; 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, however, not 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 re-targeting of front legs occurs within 40 ms in walking stick insects (Schütz and Dürr, 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 re-targeting, 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 less than 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 re-targeting of leg movements observed in behavioral experiments, even when taking into account that passive forces may have to be overcome before a change in the movement trajectory can occur during ongoing movement (Hooper et al. 2009; Ache and Matheson, 2012). Touch-sensitive DINs in the cricket and cockroach were even faster, and responded within 4 and, respectively, 7 ms (Schöneich et al. 2011; Burdohan and Comer,
Correcting 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 either indicate inter-specific 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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References


**Figure captions**

**Figure 1:** Stimulation and recording sites. A) Schematic of the head (gray), the basal antennal segments (black), and the stimulus situation. The head-scape (HS) joint was fixed, and the SP joint was left free to move. A metal minutin pin (filled gray) was inserted into the base of the flagellum and moved contact-free via a magnet (filled gray, black arrows). Additionally, the antenna is shown with the SP joint in both extreme positions (gray). Note that the HS and SP joint axes are slanted in the real animal. B) The SP joint angle during the standard stimulation protocol (staircase of ramps and plateaus). For most analyses presented in this paper, the joint angle velocities were varied between 1 °/s and 800 °/s (ramps shown in gray). All other parameters, i.e., ramp amplitude, number of ramps and plateau duration (plateaus shown in black) were kept constant at 52°, 4 ramps, and 0.95 s, respectively. The ramps of this standard staircase were numbered 1 to 4, according to their order of occurrence. C) Schematic of the intra- and extracellular recording sites in the prothoracic ganglion and the neck connectives. The platform on which the ganglion and the connectives were fixed is shown in black. sog = suboesophageal ganglion, meso = mesothoracic ganglion. The original DIN recording (lower trace) represents a group of neurons that strongly responded to touching the flagellum (arrowhead), but not to later stimulation of SP joint proprioceptors. Scale bars indicate 20 mV and 1 s, respectively.
Figure 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 (lower 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 (rows). The bin size was 5°. Four to five consecutive sweeps of the same stimulus were averaged. Ramps as well as plateaus (-52°, 0°, +52°) were taken into account. The y-axes in the fourth row panels are scaled differently. C) Mean spike frequency during the different ramps (symbols) for SP-joint stimulation at different velocities. D) Range fractionation of four ipsilateral (i-iii, v) and one contralateral (iv) simple position-sensitive DIN(s). Bars show the mean spike frequency on each of the five plateaus (compare to A). The firing rates of DINs were maximal in the ventral, the dorsal, or both extreme positions, but never in the resting range (second and fourth plateau). Stimulus conditions: n = 4 staircase repetitions per DIN, ramps with 30 to 40 °/s velocity.
**Figure 3:** Dynamic position-sensitive DINs. A) Spike pattern of a representative dynamic position-sensitive DIN. Dashes indicate single spikes of a representative dynamic position-sensitive DIN during 4 consecutive sweeps (horizontal lines) of the same stimulus (lower trace). This DIN spiked exclusively during movement towards the dorso-medial and ventro-lateral extremes of the joint angle working-range. B) The same DIN fired only during the last two ramps in the dorsal and ventral extreme range upon stimulation with 32 small-amplitude ramps of the same velocity. Upper trace: intracellular recording; lower trace: stimulus. The three most ventral ramps of the preceding staircase are shown on the left. C) Dynamic position-sensitive DINs show range fractionation. The normalized spike frequencies during stimulation of the SP joint are shown for three different dynamic position-sensitive DINs. i) Same DIN as in A and B. ii) An ipsilateral dynamic position-sensitive DIN that spiked preferentially during movement at dorso-medial joint angles. iii) An ipsilateral dynamic position-sensitive DIN that spiked preferentially during movement at dorso-medial joint angles. All DINs show differences in their response range depending on movement direction. The mean spike frequency on the plateaus, where the antenna was not moving, was lower than 1.5 Hz for all DINs. Stimuli were repeated four times, the joint angle velocity was 40 °/s in all cases. Dotted vertical lines indicate the resting position.

**Figure 4:** DINs firing single spikes during fast ramps. A) Representative example of a dynamic position-sensitive DIN firing only single spikes during ramps with high joint angle velocities. This DIN descended contralaterally. Upper trace: intracellular recording, lower trace: SP joint angle. The ramp velocity was 127 °/s. B) Four sweeps of the same stimulus (lines) and the spikes elicited (dashes). This DIN fired only during movements faster than 40 °/s, and only during ramps one and four. Such DINs exhibit an extreme form of dynamic position-sensitivity.
**Figure 5:** Unspecific movement-sensitive DINs. A) Original recording of a representative unspecific movement-sensitive DIN (upper trace) during stimulation with 32 low-amplitude ramps (lower trace). The DIN responded reliably to movements of small amplitude at 38 °/s (dotted lines), but also received a stimulus-independent drive (irregular bursts). B) During slow antennal movement, (1 °/s, lower trace), the burst activity and low-frequency background spike activity persisted (upper trace). C) Typical response of the DIN to the ramps shown in A, the first of the two ramps in the inset is indicated by the arrowhead in A. D) Cumulative probability distributions of inter-spike intervals for stimulation with two staircases as shown in A (black solid line) and two staircases as shown in B (four ramps at 1 °/s; gray dotted line). E) Mean spike frequency and F) mean number of spikes during ramps of the standard staircase at different velocities. The gray lines indicate the expected dependency of spike number on ramp velocity in case of constant spike frequency (see also Figure 6). The number of spikes elicited followed the expected spike numbers closely, except at the highest velocities (inset).
**Figure 6:** Apparent velocity-sensitivity can arise due to a constant number of additional spikes induced by transient stimuli. The figure shows modelled data. Neurons responding with a constant number of additional spikes elicited by each ramp, independent of the ramp velocity, show an increase in spike rate with increasing ramp velocity. Filled triangles: one additional spike per ramp, filled circles: two additional spikes per ramp, crosses: three additional spikes per ramp. Grey line: spike rate of a neuron with the same properties that does not respond to the stimulus. Inset: five identical spike trains (gray dashes) with different stimulus durations (black boxes) and two additional spikes per stimulus (black dashes). The resulting mean spike frequency per stimulus is given for each modelled spike train. The longest stimulus interval (uppermost black box) was defined as 2 s. The response pattern shown in the inset corresponds to the graph with filled circles in the main figure.

**Figure 7:** ON-type velocity-sensitive DINs. A) Dashes show spikes of a representative ON-type velocity-sensitive DIN during 4 consecutive sweeps of the standard staircase at 40 °/s velocity (lower trace). B) The mean spike frequency during movement depended linearly on movement velocity. Mean spike rates of up to 212 Hz were reached (ramp 1, 700 °/s). The linear fit (black line) has a slope of 0.279 (R = 0.995). C) The number of spikes recorded during ramps of different velocities varied, but not in a manner expected for constant spike rate. The measured numbers of spikes cross the reference lines for constant frequency multiple times, across the whole range of velocities (compare to Figure 5F). ON-type velocity-sensitive DINs are therefore genuinely velocity-sensitive.
Figure 8: OFF-type velocity-sensitive DINs. A) Original recording of a representative contralateral OFF-type velocity-sensitive DIN (upper trace) and the stimulus (lower 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 five sweeps of the same stimulus, the DIN activity was reduced during all ramps of all five trials. C) Peri-stimulus time histogram of the five sweeps shown in B. The spike frequency dropped markedly during all ramps. D) Spike pattern (upper traces) during four consecutive sweeps of the same stimulus (lower trace) of an ipsilateral OFF-type velocity-sensitive DIN. This DIN had a higher baseline firing rate than the DIN in A). E) The mean spike rates during ramps of different velocities for the same DIN as in A-C. F) Same plot details as in E) for the DIN in D. The black lines are linear fits with slopes of -13.91 (R = -0.988; E) and -27.00 (R = -0.965; F), respectively.
**Tables**

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**Table 1:** The number of recordings obtained from each DIN group. The number of recordings from ipsilateral/contralateral DINs per group are given, along with the total number of recordings (Σ).