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Proprioceptive encoding of head position in the black soldier fly, *Hermetia illucens* (L.) (Stratiomyidae)

Angelique Paulk* and Cole Gilbert†

Department of Entomology, Cornell University, Ithaca, NY 14853, USA

*Present address: ARL – Division of Neurobiology, University of Arizona, Tucson, AZ 85721, USA

†Author for correspondence (e-mail: cg23@cornell.edu)

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Summary

Because the eyes of insects cannot be moved independently of the head, information about head posture is essential for stabilizing the visual world or providing information about the direction of gaze. We examined the external anatomy and physiological capabilities of a head posture proprioceptor, the prosternal organ (PO), located at the base of the neck in the black soldier fly, *Hermetia illucens* (L.) (Family: Stratiomyidae). The PO is sexually isomorphic and is composed of two fused plates of about 130 mechanosensory hairs set in asymmetrical sockets whose orientation varies across the organ. A multi-joint mechanical coupling between the head, neck membrane, and contact sclerites deflects the hairs more or less to increase or decrease their level of excitation. The PO sensory afferents project to the central nervous system (CNS) *via* a pair of bilateral prosternal nerves (PN) to the fused thoracic ganglia. Simultaneous recording of spiking activity in the PN and videotaping of wind-induced and voluntary head movements around all three axes of head rotation reveal that a few PN afferents are active at rest, but activity increases tonically in response to head deflections. Activity is significantly modulated by change in head angles around the pitch (±40°), yaw (±30°) and roll (more than ±90°) axes, although the dynamic range of spiking activity differs for each axis of rotation. Prosternal nerve afferents are bilaterally excited (inhibited) by pitch down (up); excited (inhibited) by head yaw toward the ipsilateral (contralateral) side; excited by roll down toward the ipsilateral side, but little inhibited by roll toward the opposite side. Although bilateral comparison of activity in PN afferents reliably encodes head posture around a given rotational axis, from the point of view of the CNS, the problem of encoding head posture is ill-posed with three axes of rotation and only two streams of afferent information. Furthermore, when the head is rotated around more than one axis simultaneously, mechanical interactions in the neck modify the responses to postural changes around the three rotational axes, which adds further ambiguity to reliable encoding of head posture. The properties of the PO in this relatively basal fly species are compared to those of higher flies and possible mechanisms of disambiguation are discussed.

Key words: mechanoreception, prosternal organ, insect, neck, posture, ill-posed problem.

Introduction

Most creatures capable of moving their head relative to their trunk have evolved cervical proprioceptors to monitor the posture of the head (Mittelstaedt and Mittelstaedt, 1992). In insects, head posture may be encoded by a variety of proprioceptors that appear to have evolved independently a number of times among different orders (Peters, 1962), with the most widespread condition being small fields of mechanoreceptive hairs located on the anterior end of the second lateral cervical sclerite [e.g. Plecoptera (Wittig, 1955), Orthoptera (Goodman, 1959), Mantodea (Pringle, 1938) and Hymenoptera (Lindauer and Nedel, 1959)]. In some other groups [e.g. Odonata (Mittelstaedt, 1950; Fuldner, 1954-1955), Dermaptera (Popham, 1960), Coleoptera (Korschelt, 1924) (C.G., unpublished)] hair fields are located in different areas of the cervical region simply as clusters of one to several dozen hairs grouped together in the arthrodial membrane. Another condition exists in Diptera, the true flies, in which the hair plates were first described (Lowne, 1870) from the blow fly, *Calliphora* (=*Musca*) *vomitoria*, as a pair of bilaterally symmetrical, separated groups of hairs (~100 hairs each) located on cephalad extensions of the presternum, the anteriormost sclerite in the ventral cervical region. These hair plates have been termed the prosternal organ (PO) and the morphology described by Lowne is similar for other muscoid flies (Peters, 1962; Preuss and Hengstenberg, 1992; Gilbert and Bauer, 1998). In more primitive flies, the hair plates are anteriorly fused along the sagittal midline (Peters, 1962) and in

the most primitive flies in which the PO has been found (family: Anisopodidae) it is present as a single midline cluster of hairs (Gilbert and Edgecomb, 1996).

Although the location of such proprioceptive hair fields varies among insects, they are all assumed to similarly monitor head position to provide feedback for control of head posture (Mittelstaedt, 1950; Lindauer and Nedel, 1959) and to provide information about the direction of gaze important for visually guided behaviors (Mittelstaedt, 1957; Poteser et al., 1998). In flies, sensory feedback about position of the head is required for proper alignment of visual fields of motion-sensitive neurons involved in optomotor control of flight (Kern et al., 2006). Such feedback is important for maintaining stability of the head, as well as interpreting visual translation during head saccades. The mechanistic function of proprioceptive feedback is best studied in the PO of higher Diptera, principally *Calliphora erythrocephala* (Calliphoridae) and *Neobellieria* (=*Sarcophaga*) *bullata* (Sarcophagidae) (Liske, 1977; Liske, 1978; Horn and Lang, 1978; Preuss and Hengstenberg, 1992; Gilbert et al., 1995; Gilbert and Bauer, 1998). After unilateral shaving of the PO hairs (Preuss and Hengstenberg, 1992) or section of the prosternal organ nerve (Gilbert and Bauer, 1998), flies compensate by rolling the head down to the operated side, as if to equalize excitation of the hair plates on each side and the compensatory roll disappears if both sides are operated. Unilaterally waxing down the hairs, which presumably excites them, has the opposite effect, causing the flies to roll their heads away from the waxed side, again balancing the excitation on both sides (Preuss and Hengstenberg, 1992). Additionally, when a single hair plate is shaved, male *N. bullata* flies cannot aerially capture female flies to mate, as do sham operated males, indicating the male tracking behavior, and even sexual fitness, can be limited by alteration of PO input (C.G. and M. Kim, unpublished).

Each PO hair is singly innervated by a mechanosensory neuron (Lowne, 1890-1892; Richter, 1964) proposed to be excited by deflection of the hair by the anteriorly placed contact sclerites. In muscoid flies, when the head is at rest, approximately a dozen hairs on each PO hair plate are deflected by the contact sclerites (Preuss and Hengstenberg, 1992). Both Peters (Peters, 1962) and Preuss and Hengstenberg (Preuss and Hengstenberg, 1992) proposed that differential deflection of hairs on both hair plates encodes head position around the pitch and roll axes. For example, pitch down provides bilaterally symmetrical stimulation, whereas roll provides asymmetrical stimulation, i.e. increasing the number and angle of deflected PO hairs on one side while reducing deflection of hairs on the other side. Electrophysiological recordings of the PN during head pitch and roll, as well as asymmetrical electrical stimulation of the PN, provide physiological support for these functional hypotheses (Gilbert et al., 1995).

To better understand the functional evolution of the cervical system throughout the Diptera, we have examined the quantitative relationships between head movements about the pitch, yaw and roll axes and the physiological activity in the PN of the black soldier fly *Hermetia illucens* (L.) (family: Stratiomyidae), a more primitive fly with anteriorly fused hair plates. This fly has a world-wide distribution, chiefly in warmer climes (Iide and Mileti, 1976; Sheppard et al., 2002) and a lekking mating system that involves visual tracking and complex flight behaviors, such as hovering and mid-air grappling (Tomberlin and Sheppard, 2001). From morphological examinations, we determined that the orientation of the PO hairs varies across the PO suggesting various directional sensitivities across the hair plates. Electrical activity in the PN is primarily tonic in response to changes in head position and the organ responds to rotation around all three axes. Pitch down (up) results in increased (decreased) firing of the PN. Yaw toward (away from) the recorded side increases (decreases) activity in the PN. Roll down to the recorded side results in increased PN excitation, but roll up does not change PN activity relative to the resting rate. Furthermore, encoding of head position around the pitch or roll, but not yaw, axis is affected by the position of the head around the other two axes. These results are discussed in light of the evolution of the PO from a single midline structure to a pair of separate hair plates.

Materials and methods

Animals

Eggs and larvae of the black soldier fly *Hermetia illucens* (L.) (Family: Stratiomyidae), were obtained from the Coastal Plain Experiment Station (CPES) of the University of Georgia, Tifton, GA, USA and reared on larval housefly medium (Shono et al., 2002). Adult flies were fed sugar and water *ad libitum*. In some cases, adults laid eggs on the larval medium, which allowed a second generation of larger flies to be reared in the laboratory. A full rearing protocol has been described (Sheppard et al., 2002). Flies varied in size from 40–110 mg (wet mass) and 0.5–2.5 cm (body length), but only flies $\geqslant$1.5 cm body length were used in these experiments (Fig. 1A).

Morphology of the prosternal organ

To determine the number of mechanosensory hairs of the PO, the anteriorly fused PO hair plates and surrounding cuticle from 20 freshly killed flies (ten male, ten female) were mounted on a slide under a drop of glycerin, a cover slip was placed on top, and the hairs counted with the aid of a compound microscope at 100× magnification. Only hairs in sockets with an asymmetrically thickened wall (Gaffal et al., 1975) were counted as mechanoreceptors. To examine possible effects of body size on number of hairs, the length of a hind femur of each of the 20 flies was measured under a stereomicroscope fitted with an ocular micrometer. To obtain an overview of the PO, flies were prepared for scanning electron microscopy by freeze killing, desiccated for 3 days, mounted on stubs, then sputtered with gold–palladium to a thickness of 45 nm, viewed using a Zeiss DSM 960 scanning electron microscope at 3 kV, and photographed digitally.

Head movements

Five male and three female flies ranging in wet mass from

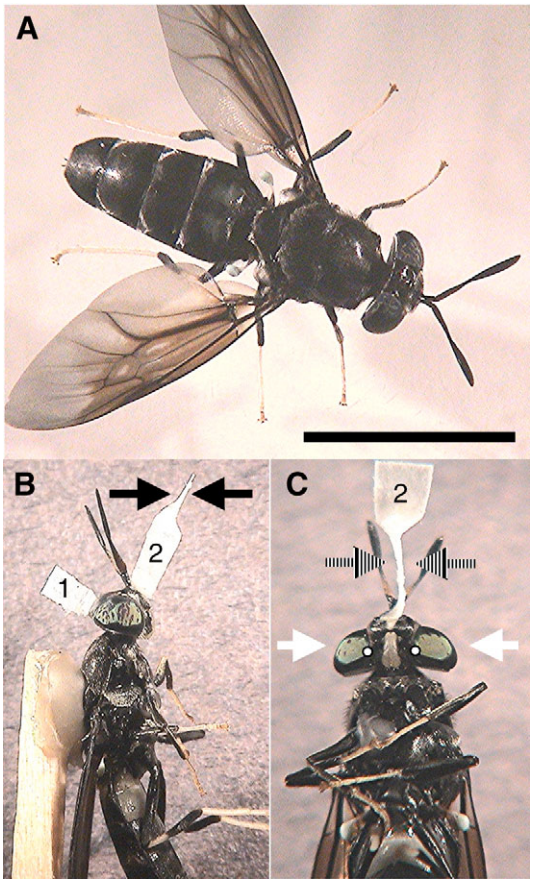

Fig. 1. *Hermetia illucens,* head angle directions, and the experimental setup. (A) Dorsal view of a standing fly. Scale bar, 1 cm and applies to all panels. (B) Lateral and (C) ventral views of a mounted fly with both flags fixed to the head. Air puffs to the two flags move the head around the pitch (black arrows to distal flag 2), yaw (hatched arrows to proximal flag 2), and roll (white arrows to flag 1) axes. White circles flanking the mouth in C indicate the digitized points.

60–100 mg were prepared as follows. Flies were chilled on ice, then waxed on the dorsum of the thorax to a toothpick using dental wax melted with a low-temperature optical cautery (Acuderm, Fort Lauderdale, FL, USA), and finally positioned ventral side up under a stereomicroscope. Two flags of paper cardstock were waxed perpendicular to the surface of the fly's head in the midsagittal plane (Fig. 1B,C): One short flag (1) (mean mass of three flags=0.55 mg) was attached dorsally and a second flag (2) (mean mass of three flags=1.05 mg) was attached anteriorly in the longitudinal body axis. Flag 2 was twisted medially so as to have two perpendicular surfaces for creating forced movements around the pitch and yaw axes. The average wet mass of a fly's head is 5.00±0.34 mg (N=3). Flies carrying flags appeared to move their heads normally in hovering flight and in tethered situations. Thus, we assume that the flags did not significantly load the neck. Moreover, none of the measurements reported here depend upon the dynamics of head movement, which may be most affected by the added mass and drag of the flags.

To rotate the fly's head by various amounts around the pitch, yaw and roll axes, puffs of air controlled by a Picospritzer II (General Valve, Fairfield, NJ, USA), using a MacAdios II SE A/D converter (GW Instruments, Cambridge, MA, USA) with Igor Pro 3.1 software on a Power Macintosh 7100/80av computer, were delivered through pulled glass capillary tubes (o.d. 1.0 mm; tip diameter approximately 5 μm) directed at the flags attached to the fly's head. Flies were positioned under a stereomicroscope so that both the head and an LED that was illuminated for the duration of the air puff could be viewed by a CCD color camera (NC-15D, NEC) through the microscope at 10× magnification and recorded on videotape using a modified Sony VCR (A. R. Vetter, Rebersberg, PA, USA). Images were digitized offline using MGI Video Wave 4.0 (Roxio, Santa Clara, CA, USA) and a video to digital converter (Real Networks, Seattle, WA, USA). Each frame of video (at least 4 min per fly) was extracted using Platypus Animator 5.4 (C Point Pty. Ltd., Para Hills, Australia).

A custom program written in Matlab 6.1 (Mathworks, Natick, MA, USA) was used to calculate angular head positions frame by frame from two digitized points on the posteroventral head capsule just to the left and right of the mouth in ventral view (Fig. 1C). Head yaw position could be measured directly as the angular difference between line segments joining the two digitized points in subsequent frames. Head pitch and roll were viewed indirectly and had to be computed by assuming that the head pivoted around the neck on a lever arm that was 0.65 the width of the mouth, as determined empirically from several flies. Change in pitch or roll resulted in translation or foreshortening, respectively, of the line segment between the digitized points. To calibrate the accuracy of the computed pitch and roll head positions, freshly killed flies (N=2) were positioned using the methods described above under the microscope together with a front-surface mirror, such that a direct view of the pitch or roll position, as well as the normal indirect view, was captured. Computed and directly measured positions were then determined for head pitch angles (±32°) and roll angles (±90°). The relationship between computed *versus* directly measured pitch angles was y=1.27x+0.8 (r^2=0.87, N=261), while that between computed and directly measured roll angles was y=1.10x+4.6 (r^2=0.97, N=272). Thus, the computed angles were slightly overestimated and all pitch and roll angles reported here have been decremented by these slopes to correct for the overestimate. The mirrors were not used during experiments to allow for greater magnification of the head, and thus increased precision. Digitizing error determined by repeated measurements of the same frame was less than 2.0°.

Physiological recordings of prosternal afferent activity

To gain access to the prosternal nerves (PN), after the air puff apparatus was set up around the fly, a small patch of membranous cuticle immediately posterior to the presternum was peeled away. Tracheae and fat body were gently removed to reveal the paired PNs. Suction electrodes were fashioned from pulled glass microfilament capillary tubes (o.d. 1.0 mm;

tip diameter approximately 5 μm) placed in a custom microelectrode holder with an Ag/AgCl pellet and Luer port (E. M. Wright, Guilford, CT, USA) advanced by a micromanipulator. A ground electrode was formed by painting a line of Pelco colloidal silver (Ted Pella, Redding, CA, USA) along the side of the capillary tube to nearly 0.5 mm from the tip and wrapping a few turns of silver wire (75 μm diameter) around the upper shaft of the capillary tube in contact with the silver paint. Electrodes were filled with insect saline (Strausfeld et al., 1983) and a single PN was sucked up into the capillary tube by a 10 ml syringe attached through tubing to the Luer port of the holder. Electrical activity was recorded in the left PN in four flies (three males, one female) and in the right PN in the other four flies (two males, two females). Only recordings with extracellular spikes of at least 100 μV were used. The signal was passed through a differential AC amplifier (A-M Systems, Everett, WA, USA) and recorded *via* a MacAdios II SE A/D converter (GW Instruments, Cambridge, MA, USA) on a Power Macintosh 7100/80av computer running Igor Pro 3.1 at a sampling rate of 50 kHz. Raw extracellular recordings were converted offline into text format and analyzed in Matlab.

Analysis of physiological activity

Since video recordings of head position had a temporal resolution of 30 fps, the voltage of the extracellular recordings, which typically consists of compounded action potentials, was also integrated over 33 ms bins (Fig. 2). To allow comparison across flies, values of integrated spiking activity were normalized for each fly by dividing by the largest integrated activity value for that fly. A 'resting' activity level for each fly averaged across all frames in which the fly's head was at rest was then subtracted from all the normalized integrated activity levels. Thus, data are presented as relative increases or decreases of PN activity. The PO is assumed to be bilaterally symmetrical and data are presented as though all activity was recorded on the left side. Head angles are calculated such that both yaw towards and roll down to the side of the recorded PN were considered positive and pitch up is positive.

Positional analysis: pure head pitch, yaw, or roll

To investigate encoding of head position by the PO, an air puff was delivered to rotate the head around the pitch, yaw, or roll axis. The fly, however, was also volitionally free to move its head in any direction during the puffs, thus the head was often not at rest around one or two other axes. To determine the relationship between PN activity and rotation around a single axis, sequences during which the position of the head around one axis varied were analyzed separately, while the position around the other two axes was constant and within ±2.5° of resting positions. Further analyses investigated sequences in which the position around some axes are held constant, but not at the resting position. Finally, to determine interactions among all three axes, analyses are presented of head positions perturbed from rest around all three axes.

Temporal analysis

To examine the temporal properties of PN activity, sequences were selected in which head position around all three rotational axes was different from rest, but held at a constant (plateau) position for at least eight data points (264 ms). Concurrent PN activity was plotted over time during the plateau and examined for phasic properties by linear regression. Head positions preceding the plateau may have affected any temporal properties of the PN activity, as hair plates in some insects exhibit phasic firing only when maximally deflected and excited (Pringle, 1938; Liske, 1989), thus the plateaus were separately analyzed according to the 2^3 different combinations of increasing or decreasing pitch, yaw and roll angles that could precede any period of constant head position.

Statistical analyses

All statistical analyses were performed in Matlab 6.1. To test whether data from all eight flies could be pooled, the basic relationships between pure pitch, yaw and roll head angles with spiking activity in the PN were

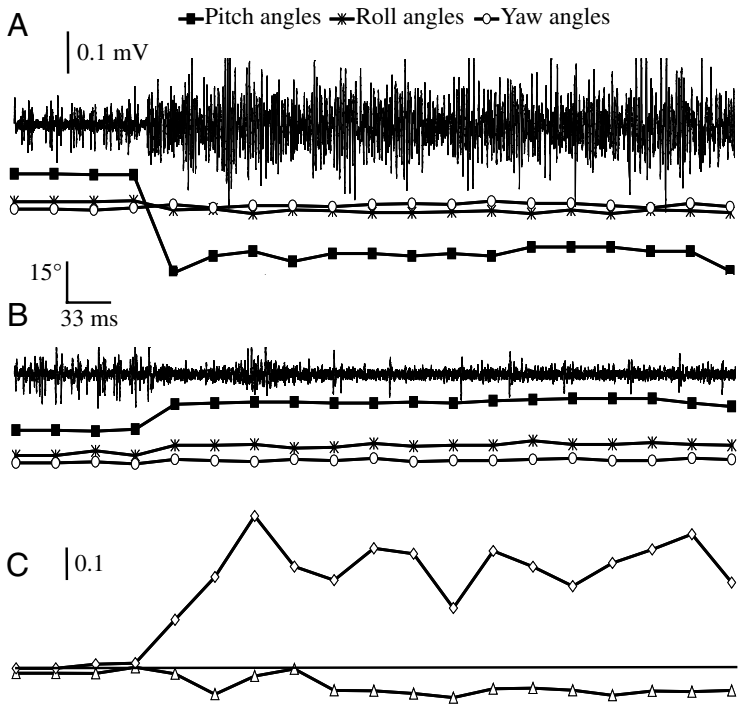

Fig. 2. Activity in the prosternal organ nerve (PN) during head movements. (A) Raw recording shows sustained PN excitation associated with sustained pitch down and resting yaw and roll head positions (lower three traces). (B) Sustained PN inhibition associated with pitch up and resting yaw and roll. (C) Integrated spiking activity levels for the raw traces in A (diamonds) and B (triangles). Scale bars apply to A and B.

compared using one-way ANCOVA models. After the relationships were determined to be relatively consistent across flies, the data were pooled, including both males and females, and the entire range of head angles and PN activities of the eight flies were analyzed as a whole.

Results

Morphology of the prosternal organ

In the ventromedial cervical region, the presternum and cervical sclerites form a relatively stable sclerotized cuticular frame surrounding the PO and associated cervical membrane (Fig. 3A,B). At lower magnification, the bilateral contact sclerites (CS) located anterolateral to the PO appear to be lightly sclerotized glabrous folds of arthrodial membrane. Closer examination, however, reveals an uneven surface covered with oriented microtrichiae (Fig. 3C). The surrounding cuticle is smooth compared to the contact sclerites, suggesting that the morphology of the contact sclerite cuticle is specialized in the neck. The PO is located on the anterior portion of the presternum with the hairs on the left and right side of the midline organized into a single fused sub-triangular hair plate (Fig. 3D) weakly divided by a narrow band of glabrous cuticle extending from the posterior. Hair socket placements and sizes vary across the PO (Fig. 3D–F), with very little row or column organization. The surface of the PO is slightly convex and positioned so that much of the PO faces anteriorly, not ventrally, when the fly is at rest. The hair sockets have large, asymmetrically sculptured collars that probably limit the direction of movement of the hairs (Fig. 3D–F). The collars of lateral hairs are raised nearest the lateral edge of the PO, extending anteriorly and posteriorly, with a lower rim of cuticle nearest the midline of the PO, suggesting that the hairs are limited to lateral movements toward the PO midline (Fig. 3F). Hairs near the midline also have a lateral, sculptured collar around the socket nearest the edge of the PO, but have reduced cuticle posterior to the hair socket, possibly extending their ranges of deflection to posterior and lateral movements (Fig. 3E).

The PO has an average of 133.89±11 total hairs, with no significant difference in the number of PO hairs between the males (129.5±9.01, N=10) and females (138.2±11.83, N=10) (ANOVA; P<0.081). There is a slight positive trend between number of PO hairs and body size (data not shown), but neither the slope of the overall relationship, nor that for males or females analyzed separately, is significantly different from zero (F-test; overall: P<0.15; males, P<0.66; females, P<0.72). In addition to the normal mechanosensory hairs, many animals had one or two pairs of much taller hairs (Fig. 3B). The more anterior hair of each pair was usually taller and occasionally the shorter hair was absent.

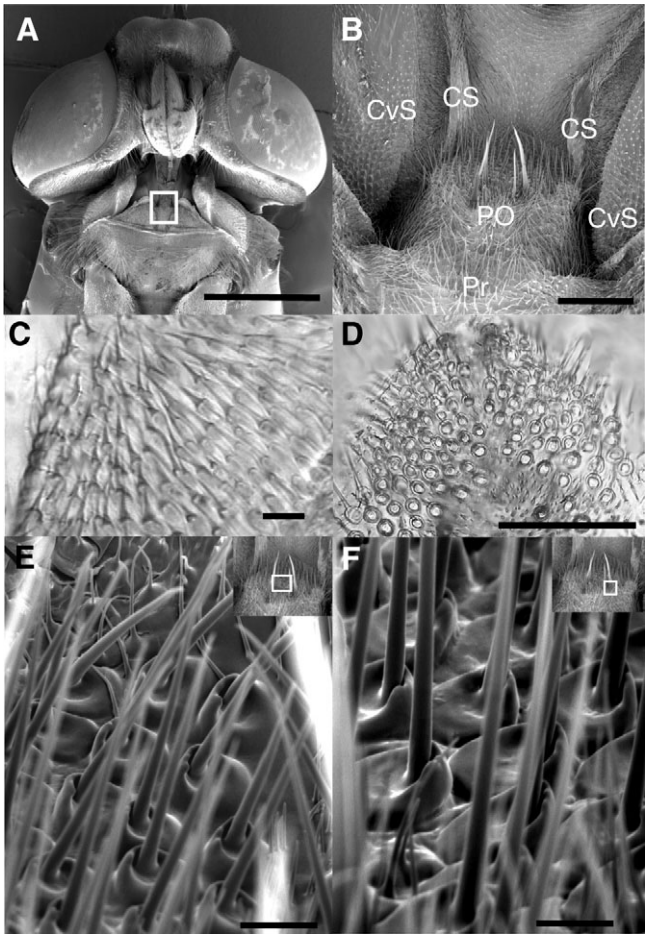

Fig. 3. The prosternal organ in *Hermetia illucens*. (A) Scanning electron micrograph of the ventral cervical region of an *H. illucens* female. The white box indicates the region that is magnified in B. Scale bar, 5 mm. (B) The cervical sclerites (CvS) are lateral to contact sclerites (CS) that are little more than folds of cervical membrane anterior to the prosternal organ (PO), which is an anterior extension of the presternum (Pr). Scale bar, 50 μm. (C) Higher magnification light micrograph reveals that the surface of the CS is covered with oriented microtrichia. Scale bar, 1 μm. (D) The PO is composed of two hair plates with differently oriented sockets separated by a strip of glabrous cuticle. Scale bar, 50 μm. (E,F) Scanning electron micrographs reveal variation in asymmetry of the socket orientation with region on the PO, as indicated by the square in the insets. Scale bars, 3 μm.

Encoding head position around each rotational axis

Extracellular recordings of activity in the PN in *H. illucens* typically consist of spikes of various amplitude that compound on one another when the PO is stroked or when the head is moved (Fig. 2A), which is similar to afferent activity of cervical hairs in flesh flies (Gilbert et al., 1995) and mantids (Liske, 1989). To verify that data from all eight flies could be pooled, relationships between pure head pitch, yaw and roll angles and PN activity were compared across individuals (Fig. 4). The relationships are significantly different among flies (ANCOVA; P<0.001), however, multiple comparison tests of the slopes indicate that the majority of the slopes among flies are not significantly different from one another. Considering this variation, the relationships exhibited in the pooled data are consistent with data from individuals. There are

no significant differences between the sexes in any portion of this analysis.

Around each axis, rotation in one direction increases PN activity from the resting level, whereas rotation in the opposite direction tends to decrease activity (but see roll below). However, the relationships and the angular range of head rotation vary for each axis (Fig. 4). Around the pitch axis the angular range of rotation is ±40° with pitch down (up) leading to increased (reduced) excitation of prosternal afferents (slope: –0.0072 act °, r^2=0.48, N=2055, P<0.05). Thus, encoding of

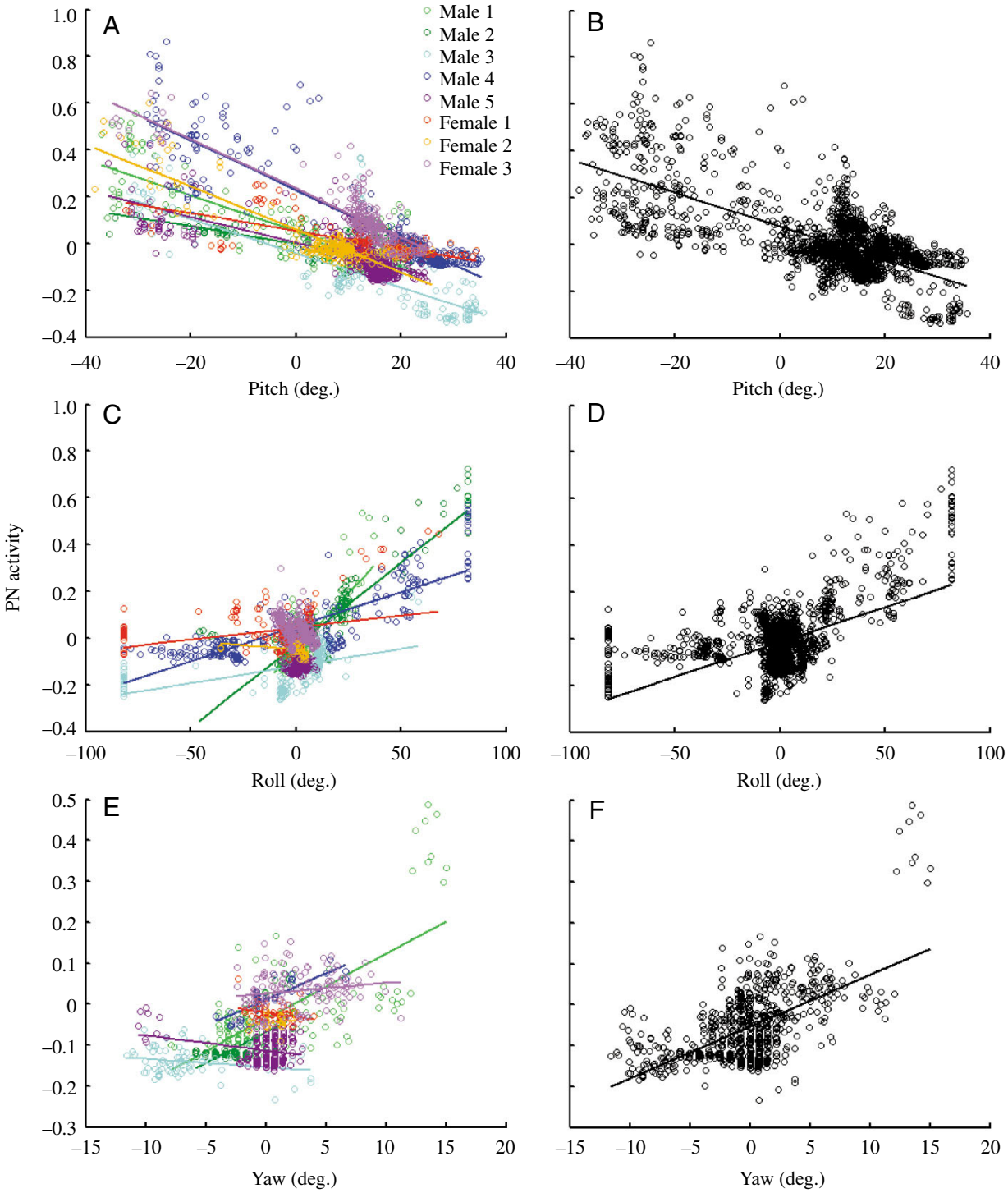

Fig. 4. Relationships of prosternal organ nerve (PN) activity with pure pitch, roll and yaw head movements. (A,C,E) Data from all eight individual flies identified by different symbols (inset in A) and regression lines determined by ANCOVA. (B,D,F) Pooled data for each axis of rotation and grand regressions. Note different axis scales.

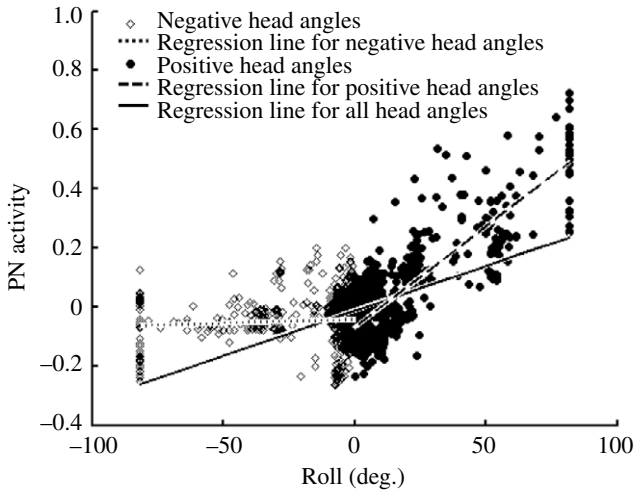

Fig. 5. Relationship of prosternal organ nerve (PN) activity with roll head position divided into positive angles (roll down, filled symbols; broken regression line) and negative angles (roll up, open symbols, dotted regression line). The solid line indicates the regression line for the whole data set.

pitch is bidirectional and laterally symmetrical. Around the yaw axis the fly has limited mobility with an angular range of ±30°, although only a limited range of angles, ±15°, is evident in this figure, which shows variation in pure yaw, i.e. when pitch and roll are held close to rest. Yaw angles were occasionally larger in other sequences in which pitch and roll positions of the head also varied. Yaw rotations of the head toward (away from) the recorded side lead to increases (decreases) in excitation (slope: 0.0127 act °, r^2=0.34, N=747, P<0.001). Thus, encoding of yaw is also bidirectional, but is laterally asymmetric.

Around the roll axis, the angular range of rotation is greatest, with experimentally puffed angles ranging from ±90° and one fly voluntarily rolled its head 180°. Roll down to the recorded side leads to increased PN activity, but roll to the contralateral side elicits little change in PN activity. Closer examination of the data set for head roll (Fig. 5) highlights this relationship. Linear regression achieves the highest correlation between positive roll angles and PN activity (r^2=0.66, N=938, F-test, P<0.001) with a slope of 0.0068 act °, which is much larger than the measured slope across all the roll values (0.0030 act °; r^2=0.31, N=1758) and is similar to the slope of pitch *versus* PN activity. The PN activity at negative roll angles, although lower than that at rest, does not have a slope that differs significantly from zero (slope: 0.0003 act °, P<0.0575, r^2=0.0044), indicating that negative roll angles only minimally affect PN activity. Thus, PO encoding of roll is more unidirectional than is the encoding of pitch and yaw. The response is not strictly unidirectional, however. The reduced excitation relative to rest associated with negative angles could indicate directionality, *per se*, just not in a graded manner over the entire range of negative angles. The linear relationship fit to the positive angles could extend to the negative angles for the range of about 0° to –20°, after which it becomes flat. Similar analysis for directional non-linearities in pitch and yaw revealed similar slopes to those derived from analysis the entire data sets and marked decreases in the explanatory power of the regressions.

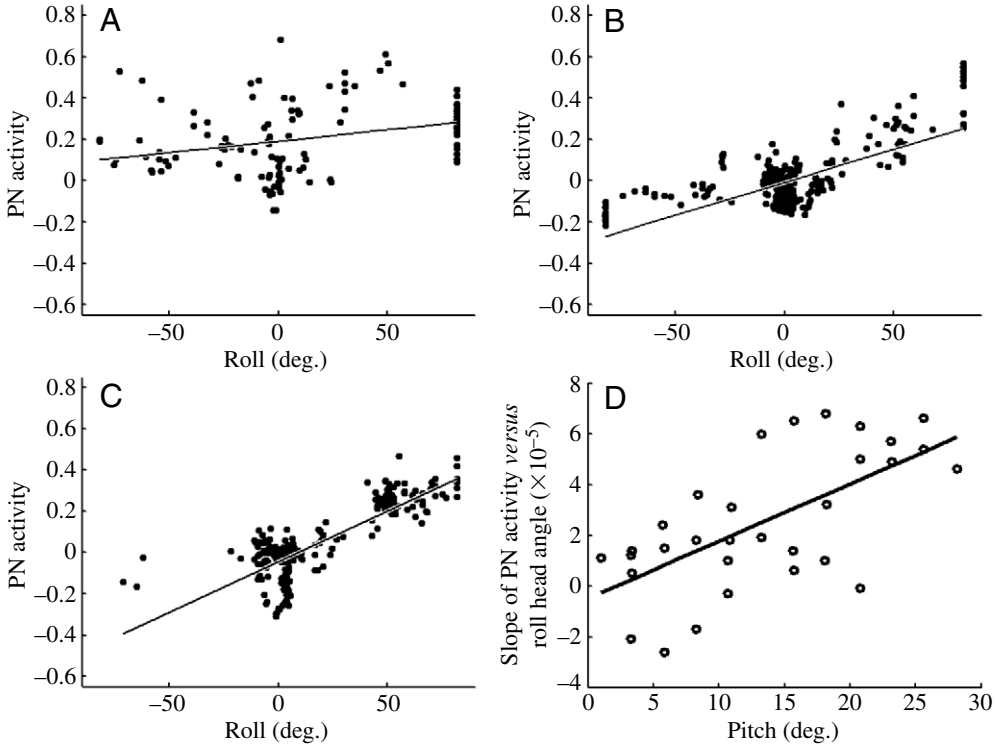

Fig. 6. Effect of different values of head pitch on the encoding of roll. (A–C) Plots of prosternal organ nerve (PN) activity *versus* roll with different constant pitch increments in each panel. The average pitch values within ±2.5° for each plot are as follows: (A) 0.99°, (B) 18.2° (rest) and (C) 23.2°. (D) The slopes of the linear regressions (solid lines) from A–C, plus others (data not shown), are plotted against their associated constant pitch angle. The fitted line is significantly different from zero, indicating that varying the pitch posture of the head affects the relationship of PN activity *versus* roll.

Table 1. *Interactions among three axes of head rotation*

Variable head axis plotted against PN activity	Range of constant increments for variable head angles (deg.)	*N*	Mean constant head angle (deg.)	Slope (act/°/°)	*P* value	r^2 value
Roll	Pitch: 1.0 to 28.2	31	Yaw: 0.7	0.0002	0.0001	0.42
	Yaw: –9.2 to 3.0	6	Pitch: –3.0	–0.0001	0.0212	0.77
Pitch	Roll: –10.5 to 14.3	45	Yaw: 1.5	–0.0002	0.0022	0.20
	Yaw: –4.0 to 3.1	14	Roll: 3.0	0.0008	0.0012	0.80

Rows indicate that the relationship of roll or pitch *versus* prosternal nerve (PN) activity is affected by the head position around a second axis while the head is held constant around the third axis. The relationship of the first row is partially illustrated in Fig. 6 to make the method of analysis clear, but see the text for further details.

Interactions among pitch, yaw and roll

Since natural head posture may be a combination of rotations around several axes, we investigated whether rotation around one axis could influence the PN response to movements around another axis. For example, to examine the influence of pitch on roll, we analyzed sequences in which yaw angles were constant, i.e. within 2.5° of rest, and pitch angles were held constant at several increments, e.g. 0.99°, 18.2° (rest), and 23.2°, while a range of roll angles ($N \geqslant 100$) was tested at each pitch increment (Fig. 6A–C). Then, the slope of the relationship between PN activity and roll angle for each constant pitch increment was plotted against that pitch value and a linear regression calculated (Fig. 6D). If pitch angle has no effect on the relationship of PN activity to roll, the slope of the regression in Fig. 6D should be flat, but this is not the case (slope=0.0002; r^2=0.42, P=0.0001), indicating that head pitch angle strongly influences how the PO encodes head roll. The greater the pitch up of the head, the steeper the slope of PN activity *versus* head roll angle, i.e. the PO becomes more sensitive to roll when the head is pitched up. Similar analyses were performed for many combinations of pitch, yaw and roll to examine interactions among the axes. Several such linear regressions were statistically significant (P<0.05) (Table 1). Specifically, the encoding of roll is affected by varying pitch (as described above) or yaw angles, although the effect of increasing yaw is to decrease the sensitivity of the PO to roll. Moreover, the relationship of PN activity *versus* roll, which was shown above to be unidirectional when head position around the other two axes is at rest, can be seen to be bidirectional when pitch is not at rest.

Encoding of pitch is also affected by non-resting posture around the two other axes, roll and yaw. The effect of increasing roll is to make the slope of PN activity *versus* pitch more negative, i.e. the PO becomes more sensitive to pitch when the head is rolled down. The r^2 value, however, is small, 0.20, and has less explanatory power than the other interactions described here. Yaw has a similar effect on encoding of pitch as it has on encoding of roll, i.e. the PO becomes less sensitive to pitch as the head is yawed. When head yaw was the variable axis and either roll or pitch was held at constant increments no significant interaction effects were recorded (data not shown).

The foregoing analyses investigated relationships among PN activity and head position around each of the axes while position around a given axis was held constant. Flies, however, may

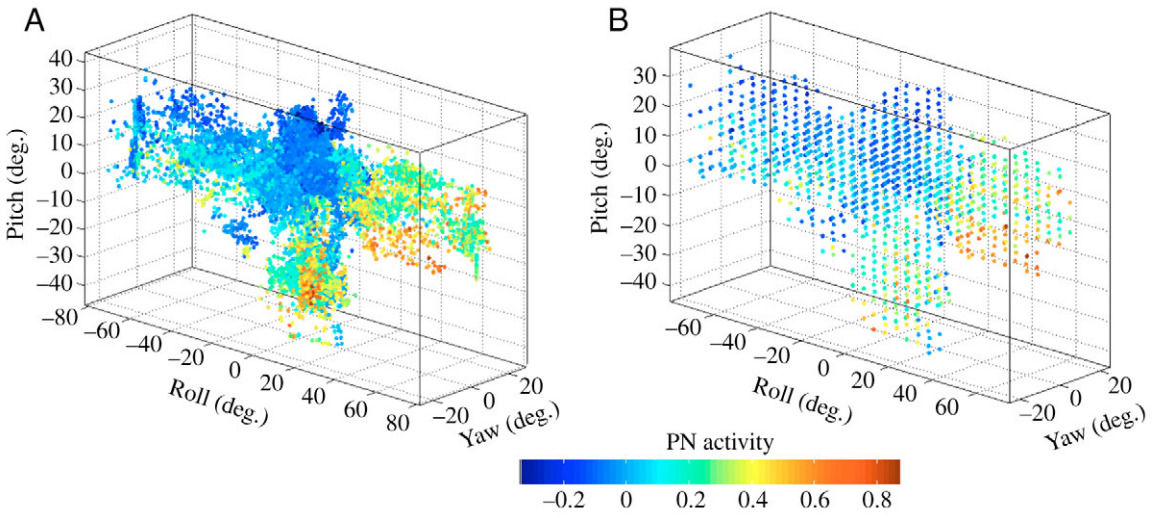

Fig. 7. Four-dimensional plots of prosternal organ nerve (PN) activity *versus* head angle. (A) Complete raw data set (B) angular data binned into 5° cubes for clarity. In both panels the level of PN activity is expressed by the false color scale.

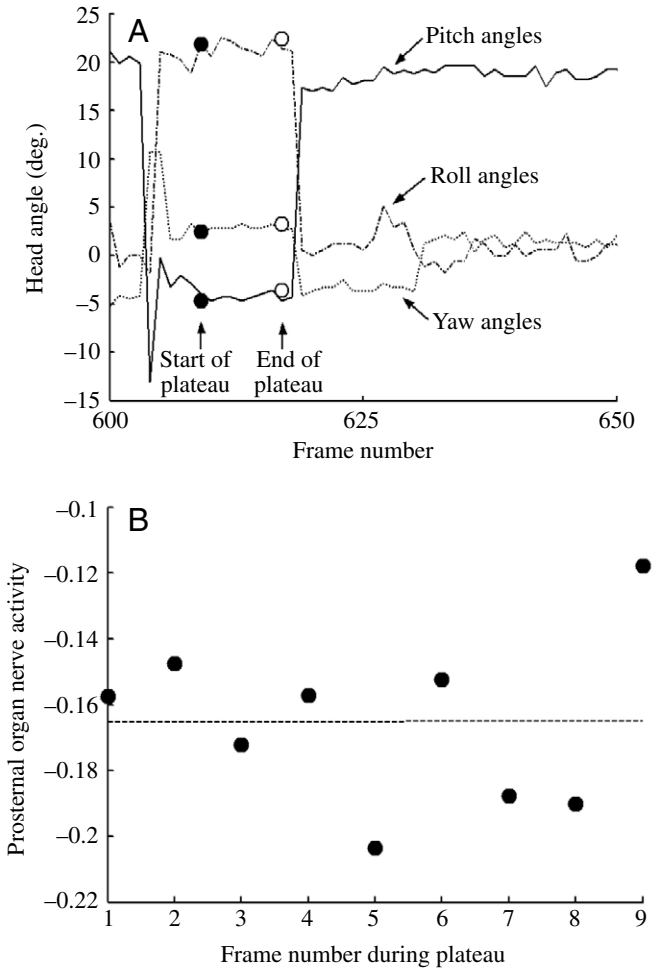

Fig. 8. Prosternal organ nerve (PN) activity is tonic while the head is held at arbitrary constant positions around all three axes. (A) A constant head position, or plateau, signified by the filled (open) circle at the beginning (end), is illustrated. The slope of each angular plateau is between ±0.1° frame^{-1}. (B) PN activity regressed on time during the plateau. The slope of this line is not significantly different from zero indicating that the PN afferents responded tonically during this period.

move their heads simultaneously in all three directions with each having an effect on PN activity, and four-dimensional plots of the data reveal the complexity of these relationships (Fig. 7). The same general trends are evident in the three-dimensional data as are found in the one and two-dimensional analyses. Namely, PN activity varies negatively with pitch, i.e. pitch down leads to increased activity. PN activity varies positively with roll and yaw, but while the roll trend was apparent, the positive yaw trend was not clear in this three dimensional view and may be obscured by the other two directions.

A further observation that becomes apparent in the three-dimensional plots is that the entire volume is not filled, i.e. there are some combinations of head rotations that never occurred. Whether such gaps indicate biomechanical limitations of the cervical linkage or simply that we did not test a particular combination is not clear.

Temporal properties of prosternal organ afferents

To investigate whether the PN afferents respond tonically or phasically to head rotation, PN activity was regressed against time for sequences in which head position was held constant for intervals at least 264 ms and up to 1.4 s (Fig. 8). For tonic activity, the regression slopes should not differ from zero and for phasic activity slopes should be significantly negative. Each of the 2^3 possible conditions of head rotation preceding the plateau position, e.g. pitch excitatory, roll inhibitory, yaw excitatory, etc was tested (N=6–25). The average slopes within each condition were not significantly different from zero (t-tests, P>0.05), with the exception of the condition in which the change in pitch and yaw were inhibitory and the change in roll was excitatory (N=25). Thus, generally PN activity is tonic during periods of stable head position, but one condition revealed a slightly phasic response. A closer look at the raw data suggests that individual, large spiking units may fire phasically, while the remaining units fire tonically (Fig. 2A), but we have not yet separately analyzed individual units. The presence of phasic units among tonic activity is very similar to sensory recordings of cockroach hair plates (Pringle, 1938) or mantid cervical hair plates (Liske, 1989).

Discussion

Morphology of the prosternal organ

Within the order Diptera, the PO is a relatively novel structure at the phylogenetic level of the Stratiomyidae. It is absent from all taxa in the primitive paraphyletic Lower Diptera (formerly Nematocera) except the families Anisopodidae (Gilbert and Edgecomb, 1996) and Axymyiidae (C.G., personal observation), in which the PO is a single midline cluster of hairs. Anisopodidae has been considered to be the sister taxon to the higher monophyletic suborder Brachycera (Oosterbrook and Courtney, 1995). Among taxa in the lower Brachycera, where Stratiomyidae is placed (Yeates, 2002; Yeates and Wiegman, 1999), the structure of the PO is quite variable compared to that among the advanced taxa in the higher Brachycera, in which the PO comprises two widely separated groups of hairs (Peters, 1962). In the lower Brachycera, the morphology is transitional between a single cluster of hairs and two separated clusters. In some families, such as Bombyliidae, both anteromedially fused and completely separated hair plates are present (Yeates, 1994). This transitional morphology may be a response to selection for increased precision or sensitivity in encoding head positions around some axes, particularly roll, which could be accomplished by increased separation of the left and right hair fields.

Directional selectivity of the PO may also be increased by two other morphological properties: (1) the hair sockets and (2) the contact sclerite. The asymmetrically raised cuticular collar or lip around each hair socket in *H. illucens* appears to provide a physical barrier that limits the directional deflection of each hair and thus imparts directional sensitivity to individual PO sensory neurons. Such has been demonstrated for similar

appearing sockets of cervical hairs in honey bees (Thurm, 1964), cercal hairs in cockroaches (Camhi and Tom, 1978), and the bristles of the head in muscoid flies (Theiß, 1979), although Gaffal et al. (Gaffal et al., 1975) caution that asymmetries of internal structures, such as the socket septum, although related to external socket asymmetries, may be sufficient to provide the directional sensitivity. The orientation of the sockets varies across the PO, as in higher flies (C.G. and R. M. Burger, unpublished). In *H. illucens* more lateral hairs have anterolateral collars associated with restricting bending in the lateral direction and permitting bending obliquely toward the midline of the PO. More medial hairs have the collar more directly anterior and hairs would bend more readily toward the posterior. Thus, there may be an incipient directional map laid out by the differential orientation of the sockets across the PO in this fly, as has been demonstrated for many macular type proprioceptive organs that sense the orientation to gravity in invertebrates and vertebrates (Schöne, 1975).

Another morphological mechanism to increase the directional precision of the PO to head posture is a specialization of the posterior surface of the contact sclerite (CS), which is covered by microtrichia (Fig. 3C) that appear similar to the 'armored' membrane located around the leg, head–thorax, and head–proboscis joints in many other brachycerous flies (Gorb, 1997; Gorb, 2001). Such membrane is composed of spine- or scale-like protuberances, which can be used to fold regions of membrane in specific spatial relation to one another based on how the microtrichia link together (Gorb, 2001). The microtrichia on the *H. illucens* CS are spaced quasi-regularly at a slightly narrower width than the PO hairs. Thus, the CS microtrichia could act as a comb that temporarily engages the hairs of the PO during a given head rotation to further constrain the deflection axis of the hair during a particular rotation of the head. As the head returns to its resting posture, the microtrichia would disengage the PO hairs. A similar mechanism may exist in higher flies, such as Calliphoridae (Preuss and Hangstenberg, 1992) and Sarcophagidae (C.G. and R. M. Burger, unpublished), that also have a hirsute posterior margin of the contact sclerite.

Encoding of head position by the prosternal organ

Sensory afferent neurons of the PO of *H. illucens* respond to rotation of the head about all three rotational axes: pitch, roll and yaw. Their responses to pitch, and to some extent roll, validate the biomechanical model of PO function developed by Peters (Peters, 1962) and expanded by Preuss and Hengstenberg (Preuss and Hengstenberg, 1992). As the head pitches down, both left and right contact sclerites would depress PO hairs further than their resting position thereby increasing excitation, whereas pitch up would release some hairs from their deflected resting position thereby decreasing excitation and producing a bidirectional code for pitch angle. The present electrophysiological results from *H. illucens* quantitatively support this model prediction over a range of ±40° of pitch for a relatively primitive fly with a single, anteriorly fused hair plate. The response, however, is not symmetrical; i.e. the dynamic range for pitch down from 0° to –40° is larger than that for pitch up from 0° to +40°. This bidirectional asymmetry probably reflects the fact that at rest only a few hairs of either hair plate are depressed. Similar qualitative electrophysiological results (Gilbert et al., 1995) have been obtained from an advanced muscoid fly in which the PO comprises widely separated hair plates and the CS area is a large proportion of the hair plate area. In many flies of the lower Brachycera, however, including *H. illucens*, the contact sclerites are little more than small, lightly sclerotized patches or folds in the gular arthrodial membrane (Fig. 3) (C.G., A.P. and R. S. Edgecomb, unpublished). Nevertheless, some hairs are deflected and excited at rest, as Preuss and Hengstenberg (Preuss and Hengstenberg, 1992) proposed, and thus the encoding of pitch is bidirectional in *H. illucens*. Many anteromedial hairs, however, are probably never touched by the CS in any head posture, but during pitch down are probably deflected by less differentiated midsagittal membrane.

The model for encoding roll proposes that some hairs of the PO are stimulated at rest and as the head is rolled down to one side the hairs would be unilaterally deflected by the CS, thereby increasing excitation. Conversely, with roll up, hairs would be released from excitation, again producing a bidirectional code for roll, as with pitch. Behavioral (Preuss and Hengstenberg, 1992; Gilbert and Bauer, 1998) and electrophysiological (Gilbert et al., 1995) results provide qualitative support for this model prediction in muscoid flies with separated hair plates. The present experiments on a more primitive fly with fused hair plates reveal more quantitative detail. Some PO afferents are active at rest and their excitation increases linearly in response to roll down by as much as 90° at roughly the same rate as for pitch. Roll up, however, leads only to a slight decrease in excitation from the resting level that is relatively uniform across the range of head angles. This non-linearity in bidirectional coding appears almost unidirectional. Clearly some hairs that are deflected at rest are released from that deflection with roll up, but not to the extent that they are with pitch up, which produces linear bidirectional coding over the full dynamic range of head movement around that axis. With roll up in the range from about –20° to –90°, the signal in the afferents of one hair plate is ambiguous. Such ambiguity could have been the substrate for natural selection that drove the separation of the hair plates in more advanced flies of the higher Brachycera. We have preliminary data, however, from a muscoid fly, *Neobellieria bullata* (Sarcophagidae) that the encoding is also non-linear with a strong increase of excitation in response to roll down for angles as large as 90° and only a small range, 0° to –15°, over which excitation is reduced for roll up (Paulk et al., 2001).

Finally, the current functional model does not address how the PO could encode yaw. Muscoid flies can move their heads around the yaw axis more than ±20° (Land, 1975; van Hateren and Schilstra, 1999) and many other flies, including *H. illucens*, can as well. It is not clear, however, how such movement would be transmitted to the sensory hairs of the

PO. Peters (Peters, 1962) did not discuss such a possibility, but Preuss and Hengstenberg (Preuss and Hengstenberg, 1992) discuss the lack of yaw-specific kinematic effects of the CS on the hairs of the PO. The only evidence until now that the PO may be involved in encoding yaw comes from an experiment by Liske (Liske, 1977). In tethered flying muscoid flies he perturbed the head around yaw axis and subsequently measured syndirectional flight torque. A control experiment described in his unpublished thesis (Liske, 1978) documents the abolition of the syndirectional torque response when the PO is ablated. Unfortunately, the prosternum also contains a proprioceptive chordotonal organ, the tendon of which inserts on the presternum very near the PO, and Liske is not explicit that it remained intact after the excision of the PO (Liske, 1978). Thus, in muscoid flies head yaw may or may not be encoded by the PO. In *H. illucens* however, the PO sensory afferents respond bidirectionally to head yaw. They are more excited during movements of the head toward the recorded afferents and less excited by movements in the opposite direction.

The foregoing discussion of how the PO can bidirectionally (for the most part) encode pure pitch, roll, and yaw begs the question of how head position is unambiguously encoded when a given head position may be composed of rotations around more than one axis. Some head postures, such as pitch up, improve encoding of position around other axes, such as roll. The increased sensitivity of roll in that case, may be due to the reduction of confounding PN activity associated with pitch. Such interactions would allow greater precision for roll, which has the most angular degrees of freedom and is crucial to visual stabilization during saccadic turning (van Hateren and Schilstra, 1999). Such functional speculations on the adaptive significance of the interaction of multi-axis rotations, however, overlook the precise biomechanics of the neck membrane and sclerites during head movements. We tried to observe these, but the ventral cervical area in soldier flies is not clearly visible with the head in normal posture. Another fly, such as an asilid, with a relatively long neck would be more suitable for studying cervical biomechanical interactions as the head rotates around several axes to understand better which hairs are being bent when the head is in different postures. Such a study may be necessary to understand the apparent ambiguity in excitation caused by multi-axis head rotation.

A simple neural scheme could allow the CNS to disambiguate pitch and roll by taking the common afferent activity in the left and right hair plates to determine pitch and the remaining difference in bilateral afferent activity to determine roll. Adding rotation around a third axis, i.e., yaw however, creates an ill-posed problem for the CNS – the head has three degrees of rotational freedom, but only two bilateral sources of information in the PO. One solution would be for PO hairs to have regional specialization, rather than having all hairs equivalent and simply encoding more or less deflection on their side. There are several morphological characteristics of the PO consistent with regional specialization. First, the hairs closest to the midsagittal plane are located such that they are rarely, if ever, contacted by a CS. Perhaps these hairs are only deflected by the gular membrane and only encode pitch. This would allow the two lateral regions of the hair plates to encode rotation around only two axes. But the two axes are roll and yaw, which are the most ambiguous due to their lateral asymmetries. Second, the distribution of hair socket orientations also suggests that there may be regional specialization across the PO that could contribute to disambiguation of head position. More lateral hairs, with sockets oriented toward the midline, could be deflected during head yaw. Whereas more medial hairs, with sockets oriented more anteroposteriorly, could be deflected during pitch or roll. Such differentiation of socket orientation could be the substrate for regional specialization of sensory hairs to direct directionally appropriate compensatory responses, which have not yet been demonstrated in this fly. If regional groups of hairs encode different directions of head movement, one might expect that the sensory fibers project to different areas of the thoracic neuropil where they could contact the dendritic fields of motorneurons that innervate different neck muscles. We have made backfills from the PO of *H. illucens* (C.G., A.P. and R. S. Edgecomb, unpublished), but have not noticed any gross differences in the projection patterns of afferent axons. Thus, the means by which this primitive fly, as well as the higher flies, disambiguate the signals of the sensory afferents of the prosternal organ to produce directionally appropriate responses in visually guided behaviors remains an open question.

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