

## RESISTANCE REFLEX THAT MAINTAINS UPRIGHT HEAD POSTURE IN THE FLESH FLY *NEOBELLIERIA BULLATA* (SARCOPHAGIDAE)

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### Summary

In flesh flies *Neobellieria bullata*, we investigated a resistance reflex that maintains upright head posture around the roll axis relative to the thorax. The gain of the reflex depends upon the fly's behavioral state: moving flies immediately correct 90% of the amplitude of experimentally imposed roll perturbations, returning the head almost to the fully upright position; motionless flies allow perturbations to persist for minutes before correcting only 70% of perturbation amplitude.

To investigate the role of various neural pathways, we examined the control of head posture after sectioning relevant proprioceptive or motor nerves. Excision of the prosternal chordotonal organ causes no decrements in the control of head posture. Unilateral deafferentation of a cervical proprioceptive organ, the prosternal organ, induces roll towards the cut side. Unilateral section of the frontal nerve, a mixed motor nerve that supplies the neck

depressors and levators, leads to unilateral deficits in correcting perturbations towards the contralateral side. After bilateral proprioceptive or frontal motor nerve section, approximately 40% of perturbation amplitude is still corrected. To determine the contributions of the passive elastic properties of the neck skeleto-muscular system, flies were tested under reversible nitrogen anesthesia. They immediately corrected 40% of perturbation amplitude. Taken together, the results demonstrate that passive elasticity plus active prosternal nerve afference to contralateral depressors innervated by the frontal nerve in combination constitute a sufficient and necessary reflex loop to control head roll posture.

Key words: flesh fly, *Neobellieria bullata*, resistance reflex, posture, proprioception, gating, neck, mechanoreceptive hair.

### Introduction

One of the principal questions in systems neuroscience focuses on the interaction between reflexes that function to regulate posture and voluntary movements that require new and dynamic equilibrium postures. Three-dimensional control of head posture in flies (for a review, see Hengstenberg, 1993) is influenced by many sensory modalities: by vision through the compound eyes (Land, 1973, 1975; Geiger and Poggio, 1977; Hengstenberg, 1988) and ocelli (Hengstenberg, 1993), by acceleration of the halteres relative to the body (Sandeman and Markl, 1980; Nalbach and Hengstenberg, 1994), by gravity perceived through the legs and antennae (Horn, 1982; Horn and Lang, 1978) and by proprioceptive information from the prosternal organ (Gilbert *et al.* 1995; Liske, 1977; Preuss and Hengstenberg, 1992). There is also a pair of prosternal chordotonal organs whose tendons insert on the presternum (Hertweck, 1931) and whose function is unknown, but which may be involved in the control of head posture. A complete review of the sensori-motor innervation of the 22 pairs of neck muscles through four motor roots (frontal, cervical, ventral cervical and anterior dorsal nerves) that control the posture of

the head around three rotational axes is beyond the scope of the present study, but the interested reader is referred to Strausfeld *et al.* (1987), Milde *et al.* (1987) and Gilbert *et al.* (1995). The present work is designed to investigate quantitatively the necessary and sufficient sensori-motor pathway(s) of a resistance reflex that maintains upright head posture about the roll axis with respect to the thorax.

A proprioceptive pathway for control about the roll axis has been demonstrated from morphological (Peters, 1962), behavioral (Preuss and Hengstenberg, 1992) and physiological (Gilbert *et al.* 1995) evidence. Peters (1962) proposed a functional model of proprioceptive coding of head posture from his description of the anatomy of the prosternal organ, which in muscoid flies comprises two bilaterally symmetrical fields of approximately 100 hairs positioned on weakly sclerotized cephalad extensions of the presternum, a ventral midline sclerite in the neck membrane (Fig. 1). When the head is centered in the midsagittal plane, approximately 12 of the anteriormost hairs of each field are deflected down by a so-called contact sclerite, a medial extension of the lateral (second) cervical

sclerite, which is the principal sclerite articulating the head with the thorax. As the fly's head rolls to one side, more hairs on that side are deflected through the mechanical linkage of the two sclerites, while some of the anteriormost hairs on the opposite side are released from deflection. Thus, by comparing the activity of hair afferents in both prosternal nerves, a fly could accurately sense perturbations of its head posture. In an elegant test of Peters' (1962) functional model, Preuss and Hengstenberg (1992) waxed or shaved the hairs of the prosternal organ and subsequently recorded head posture about the roll axis of tethered standing and flying blowflies *Calliphora erythrocephala*. After unilaterally increasing the stimulation of one of the organs by waxing the hairs in deflected positions, the head maintained an angular bias of approximately 12° down to the contralateral side. In a separate treatment, when stimulation was unilaterally decreased by shaving most of the hairs from one of the organs, the head rolled down to the shaved side, initially by an amplitude of 50° that decayed to a bias of 20° after 30 s of flight. In both experiments, no angular bias was recorded when the treatments were subsequently applied bilaterally, thus validating Peters' (1962) model. These behavioral results were corroborated and extended in electrophysiological experiments (Gilbert *et al.* 1995), in which stimulation of a prosternal nerve induced head roll downwards to the contralateral side with the magnitude of the roll being 30° or less and depending upon the frequency of stimulation. There are other potential sources of proprioceptive information about head posture relative to the thorax, e.g. mechanosensory hairs on the vertex of the head (Thiess, 1979) and the prosternal chordotonal organs (Hertweck, 1931). The current state of knowledge about the sensory input to a proprioceptive reflex is that the prosternal organ is necessary for complete maintenance of upright head posture about the roll axis. Examination of the quantitative sufficiency of prosternal organ afference and the possible necessity of other sensory systems for complete reflexive control of head roll posture is one focus of the present study.

Motor pathways that control head posture about the roll axis have been inferred from anatomical (Strausfeld *et al.* 1987; Strausfeld and Seyan, 1985) and physiological (Milde *et al.* 1987; Gilbert *et al.* 1995) experiments. Anatomical studies suggest that roll of the head is mediated indirectly, i.e. by muscles that originate on the thorax and insert on the lateral cervical sclerite rather than on the head. Changes in posture around the roll axis would be mediated by changing the position of the cervical sclerite relative to the thorax (Strausfeld *et al.* 1987). These indirect neck muscles are innervated by motoneurons carried either in the frontal nerve (FN), a mixed sensory and motor nerve leaving the prothoracic neuromere, or in the cervical nerve, a pure motor nerve arising from the subesophageal ganglion. Four of the frontal nerve motoneurons, FN MN 1–4, innervate the muscles predicted to roll the head (Strausfeld *et al.* 1987), a levator, an adductor and two depressors. Physiological evidence that contraction of muscles innervated by FN MNs is sufficient to roll the head is twofold: electrical stimulation of the frontal nerve rolls the head from an

upright position downwards by an amount, 32° or less, related to the stimulus frequency; and cutting the frontal nerve abolishes downward roll of the head elicited by stimulation of the contralateral prosternal nerve (Gilbert *et al.* 1995). Furthermore, at least one unidentified FN MN is excited by downward (upward) movement of a visual pattern in the ipsilateral (contralateral) visual field (Milde *et al.* 1987; Gilbert *et al.* 1995; Strausfeld *et al.* 1995), movement that also induces downward roll of the head in behavioral experiments (Hengstenberg *et al.* 1986). Thus, the current state of knowledge about the motor pathways controlling the position of the fly's head about the roll axis is that activity in the frontal nerve is qualitatively sufficient to roll the head, that the frontal nerve is necessary for proprioceptive reflexive control, at least through the prosternal organ, and that visual, haltere and proprioceptive pathways converge on the pool of frontal nerve motoneurons. Whether another motor pathway, perhaps innervated through the cervical nerve, is necessary or sufficient for complete quantitative control of head roll posture is another focus of the present experiments.

In this study, we examine a resistance reflex that maintains head posture about the roll axis upright relative to the thorax to determine the quantitative contribution of putative sensory and motor elements. In developing a behavioral assay, we demonstrate that the gain of the reflex depends upon the fly's behavioral state. Afference from the prosternal chordotonal organ is not necessary for accurate control of head posture about the roll axis in stationary flies. However, prosternal organ afference and contralateral depressors innervated by motoneurons of the frontal nerve together are necessary and sufficient for reflexive correction of approximately 60% of the amplitude of roll perturbations. Experiments with anesthetized flies further demonstrate that the passive elasticity of skeleto-muscular elements of the neck accounts for approximately 40% of the correction of roll perturbations.

## Materials and methods

### *Experimental animals*

Experiments were performed on flesh flies *Neobellieria* (= *Sarcophaga*) *bullata* (Diptera: Sarcophagidae), maintained on sucrose and water supplied *ad libitum* in our laboratory colony at 27 °C, 65% relative humidity and with a 16 h:8 h L:D photoperiod. Flies were chilled, and a toothpick was then waxed to the dorsal surface of the thorax, parallel to the anterior/posterior body axis of the fly, using dental wax. A small paper flag (1 mm × 3 mm) to be used as a 'handle' was then waxed to the dorsal surface of the head at an angle within 10° of vertical. The mass of the flag (mean mass of 10 flags, 0.66 mg) did not load the head significantly (mean wet mass, 10.43 ± 1.8 mg; mean ± s.d., *N* = 20 flies reared in our colony which has provided stable conditions of nutrition and larval density for many generations). Flies bearing flags maintained and moved their head posture in a similar manner to normal flies in which one of the fly's stout orbital setae was used as a 'handle'. All experiments were conducted in normal room light in the laboratory.

For experiments on intact moving flies, the dorsal toothpick was clamped in a holder with the fly in its normal upright posture holding a small Styropor sphere with its legs. Flies that rotated the sphere are referred to as 'walking' (several flies that were actually walking on the laboratory tabletop were tested with qualitatively similar results) and flies that had released the sphere and were flapping their wings are referred to as 'flying'. Most experiments were performed on flies suspended from the holder in their normal upright posture that were not holding a sphere with their legs or flapping their wings; such flies are referred to as 'motionless'. Except for experiments comparing intact motionless flies with intact walking or flying flies, all flies were dissected by opening a ventral window into the prothoracic cavity (Fig. 1). The arthroial membrane was removed between the coxae of the first pair of legs, the probasisternum and the presternum. An insect saline solution (O'Shea and Adams, 1981) was subsequently applied to the dissected area. The probasisternum and its connected coxal muscles, the first and second sternal anterior rotators (terminology of Miller, 1965), were removed. The tendon of the chordotonal organ that inserts on the lateral margin of the presternum was also removed as a result of the other operations. The prosternal tracheal sacs were then teased aside or partially removed to expose the first neuromere of the fused thoracic ganglia and both the left and right prosternal nerves and frontal nerves, which extend from the anterior surface of the neuromere. As experiments warranted, one or several nerves were cut, the saline was topped up, the flies were returned to their normal upright posture and at least 5 min was allowed to pass before any experiments were performed. Details of individual experimental manipulations are described

below with the results from each experiment. Individual flies were video-taped over the course of several hours and often through several experimental manipulations.

The final experiments with reversibly anesthetized flies were performed using a similar protocol to that described above with slight modification. After chilling, flies were waxed ventrally to a toothpick between the second pair of legs. The legs and wings were removed, and the stumps were waxed. The flies were then placed in a small chamber made anoxic by the introduction of 95% pure nitrogen for at least 10 min. The flies were then quickly transferred to a holder and aligned as described below. In flies (Krishnan *et al.* 1997), as in other insects (Wegener, 1993), motionlessness due to nitrogen anoxia occurs within a few minutes and is paralleled by a loss of central nervous system function and a lack of electrical excitability of evoked muscle potentials (Krishnan *et al.* 1997). Recovery from anoxia is complete, but depends upon the duration of anoxia and begins only after tens of minutes for flies kept anoxic for 10 min. During our experiments, which typically lasted less than 5 min, we periodically tested for recovery from anoxia by touching the genitalia and bristles on other parts of the body of the fly. Only data from those flies that remained totally unresponsive were analyzed.

#### Data recording and analysis

Individual flies were positioned dorsal side up facing a front surface mirror angled at 45° to vertical and viewed through a Wild stereo microscope at 64× focused on the fly's reflection. A NEC CCD camera attached to the microscope through a phototube provided a standard 33 frames s<sup>-1</sup> video signal to a NEC television monitor and a modified Sony video-tape

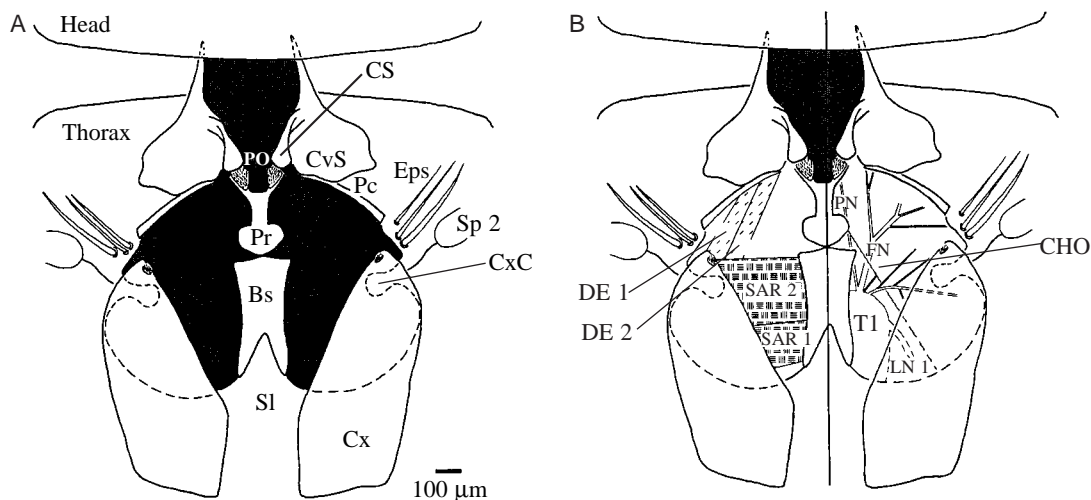
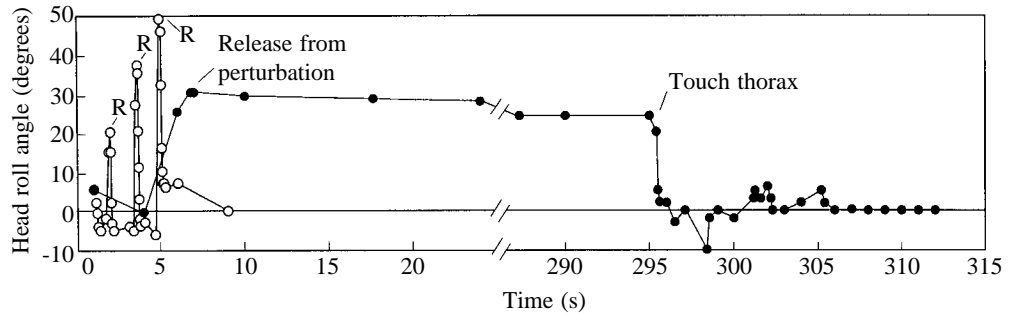


Fig. 1. Diagram of the anatomy of the head and prothoracic region of *Neobellieria bullata* in ventral view detailing the structures relevant to this study. Anterior is up. (A) Superficial elements of the intact fly. The arthroial membrane is shaded. Bs, basisternum; CS, contact sclerite; CvS, lateral cervical sclerite; Cx, coxa of the first leg; CxC, coxal condyle; Eps, episternum; Pc, postcervicale; PO, prosternal organ; Pr, presternum; SI, sternellum; Sp 2, spiracle of the mesothorax. (B) Split view of the same region. On the left side, the arthroial membrane has been removed to reveal the underlying muscles. DE 1, DE 2, depressor muscles of the lateral cervical sclerite; SAR 1, SAR 2, sternal anterior rotator muscles of the coxa. On the right side, the muscles have been removed and the tracheal air sacs teased away to reveal the underlying nervous system. CHO, chordotonal organ; FN, frontal nerve; LN 1, first leg nerve; PN, prosternal nerve; T1, first neuromere of the fused thoracic ganglia.

Fig. 2. Head posture about the roll axis *versus* time for a tethered flying flesh fly *Neobellieria bullata* (open symbols) and a suspended, motionless fly (filled symbols). Upon release from three experimental perturbations (R), the tethered flying fly immediately returns its head to an upright posture, whereas at the cessation of perturbation, the head of the motionless fly remains approximately in the same perturbed posture until the thorax is touched, at which time the head also returns to an almost fully upright posture.



recorder (Vetter). Experiments were conducted in which the position of the fly's head was manually perturbed using forceps to roll it to the left or right in random order using the paper flag. The head either returned immediately to a more vertical position within 300 ms (experiments with moving and anesthetized flies) or remained approximately in the perturbed position and only returned to a more vertical position following a gentle touch to the body (experiments with motionless flies). In the latter case, once the return of the head to upright was elicited, it occurred with similar speed given the temporal resolution of our camera. The flies were typically touched on a leg or an abdominal bristle, but contact with any region, including the thorax, wings and antennae, was sufficient to elicit a correcting reflex. The angle of the head about the roll axis was measured directly from the television screen either in real time or subsequently from video tapes. The anatomical frame of reference was defined by bilaterally symmetrical structures at the bases of the wings, the tegulae, which determined the transverse axis with the thoracic vertical axis ( $0^\circ$ ) taken as the perpendicular bisector of the line joining the two tegulae. A transparent circular protractor was aligned with the thoracic coordinates. The transverse and dorsoventral axes of the head were determined by the symmetry of the antennal bases and rows of frontal setae, respectively. The dorsoventral angle of the head relative to thoracic vertical was measured, with perturbation angle defined as the angle to which the head was rolled in experiments using moving (see Fig. 3) or anesthetized (see Fig. 8) flies. In experiments with motionless flies (see Figs 4–7), because the head slips back a few degrees towards vertical after release of the paper flag, perturbation angle is defined as the angle at which the head remained stationary during the first 10 s after perturbation. Then the correcting reflex was elicited (see Fig. 2 for clarification of this distinction). The final angle is defined as the angular deviation of the head from upright after a reflexive or, in the case of anesthetized flies, a passive return of the head towards upright by the fly. The correction angle is the difference between the perturbation angle and the final angle. Repeated measurements from the same video image were within  $3^\circ$ . By convention, perturbations and corrections towards the fly's right (left) are positive (negative) angles. Means are reported as  $\pm 1$  standard deviation (S.D.). The slopes of regression lines were compared

using Minitab statistical software with a general linear least-squares model two-factor analysis of variance (ANOVA GLM) with correction angle as the response and the factors being perturbation angle and treatment, e.g. intact moving flies, opened dissection window, both prosternal nerves cut, etc.

## Results

### *State-dependence of the head roll resistance reflex*

The resistance reflex that maintains the posture of the head around the roll axis upright relative to the thorax depends upon the behavioral state of the fly (Fig. 2). If the head of a tethered fly is rolled while the fly is walking or flying, the perturbation in the position of the head is immediately compensated, and the head is realigned with the thorax. The realignment to normal upright posture, i.e.  $0^\circ$ , from a perturbation angle of  $35^\circ$  is initiated immediately upon release of the head and is completed in less than 300 ms. Alternatively, if the fly is suspended and motionless, perturbations in the roll angle of the head are not immediately corrected. Indeed, head posture may remain stable for longer than 5 min. Flies eventually spontaneously correct the perturbation after some elapsed time, e.g. just before taking their first step at the initiation of walking, or the correction can be experimentally elicited by tactile stimulation, such as brushing an antenna or touching hairs on the thorax. We found no special trigger hairs or regions of the body; anywhere we touched elicited reflexive postural correction. Regardless of how the correction was initiated, the rotational speed of the head during the return to upright posture was  $100\text{--}150^\circ\text{s}^{-1}$ .

### *Gain of the head roll resistance reflex*

The accuracy of correction of head roll also depends upon the behavioral state of the fly. Tethered flying ( $N=7$ ) or walking ( $N=3$ ) flies correct more than 90% of the amplitude of experimental head perturbation (Fig. 3), such that the final head position is within  $20^\circ$  of vertical for perturbations  $\pm 75^\circ$ . The actual 'zero' of the system, the upright head position, may be inferred from the y-intercept of the regression line ( $-2.6^\circ$ ) or, alternatively, from the mean value of the final head position ( $2.7 \pm 7.8^\circ$ ). After eliciting the reflex with tactile stimulation, suspended motionless flies (Fig. 4,  $N=11$ ) are almost as precise

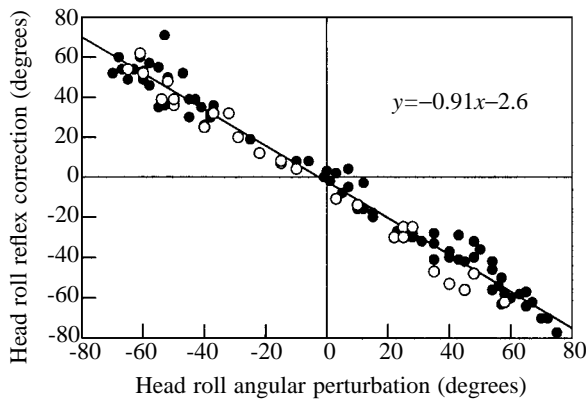


Fig. 3. Correction angle after perturbation of the head around the roll axis for intact moving flies, either tethered flying (filled symbols) or walking (open symbols), following release from perturbation. Positive (negative) angles indicate head roll postures or movements downwards to the fly's right (left). Regression statistics:  $r^2=0.98$ ,  $P<0.001$ .

in their corrections as are flying or walking flies ( $r^2=0.90$  versus 0.98, respectively), but are less accurate (gain of 0.71 versus 0.91, respectively). Motionless flies only correct approximately 70% of the amplitude of perturbation around the roll axis. The upright head position is still close to zero (y-intercept  $-2.6^\circ$ , mean of final head positions  $3.5\pm 8.1^\circ$ ) and within a measurement error of  $0^\circ$ . The slopes of the regression lines for moving and motionless flies are significantly different for the entire data set ( $F_{1,191}=10.75$ ,  $P=0.001$ ) as well as for a reduced data set that examined only perturbation angles  $\pm 45^\circ$  ( $F_{1,145}=22.72$ ,  $P<0.001$ ), which suggests a fundamental difference between these two behavioral states, not only in terms of gating sensory afference but also in terms of the gain of the reflex, i.e. similar angular perturbations lead to larger angular corrections in moving flies compared with motionless flies. Flying and walking flies, or more generally flies in motion, achieve more immediate reflex correction of head position with greater accuracy than do suspended motionless flies. Nevertheless, motionless flies still correct approximately 70% of the perturbation around the roll axis. Subsequently described experiments focus on the correction of experimental perturbation in suspended motionless flies to determine the sources of sensory information about the error in head position and what, if any, motor systems are involved in repositioning the head.

#### Propriosensory input to the head roll resistance reflex

In opening the dissection window, the tendons of the prosternal chordotonal organ are cut bilaterally so that comparison of dissected flies with their intact counterparts provides information on the contribution of the chordotonal organ to the control of head posture about the roll axis. Removal of both chordotonal organs reveals no significant effect on the reflexive positioning of the head in dissected suspended motionless flies relative to their intact counterparts

(Fig. 4). Perturbation experiments with the dissection window opened yield a regression equation of  $y=-0.73x-3.1$  ( $r^2=0.92$ ,  $N=8$ ), which is not significantly different from that of intact flies ( $F_{1,223}=0.01$ ,  $P=0.958$ ). The y-intercept ( $-3.1^\circ$ ) is slightly more negative than in intact suspended flies, but it is within observational error. Thus, in motionless flies, chordotonal input is not a necessary source of sensory information for reflexive correction of head posture around the roll axis. Whether such input plays a role in moving flies, e.g. in the increased gain of the reflex, remains an open question. Flight could not be induced in flies with an open dissection window.

Although there are a variety of mechanosensory thoracic bristles that may contact the head and provide information about its position relative to the thorax, we have some evidence to suggest their lack of involvement in providing sensory information for the roll reflex (see below). The principal source of proprioceptive information about head roll appears to be the prosternal organ. To test quantitatively the functional model developed by Peters (1962) and Preuss and Hengstenberg (1992) that comparison of left and right prosternal afference maintains the upright position of the head, we unilaterally or bilaterally sectioned the prosternal nerves, perturbed the head around the roll axis and elicited a correcting reflex.

When the left prosternal nerve is cut, the corrected head posture is biased to the ipsilateral side (mean final head angle  $-9.6\pm 12.4^\circ$ ,  $N=6$  flies). Likewise, cutting the right prosternal nerve induces a shift to the right (mean final head angle  $8.2\pm 13.0^\circ$ ,  $N=2$  flies). When these data sets are combined and displayed as final head angle versus perturbation angle standardized as measurements ipsi- or contralateral to a sectioned left prosternal nerve, the data fall along two lines (Fig. 5). Perturbations towards the intact right side are well corrected with a slight, but non-significant, bias to the cut side (mean final head angle  $-2.2\pm 6.4^\circ$ ) and a regression slope of 0.09. The correction is not significantly different from that

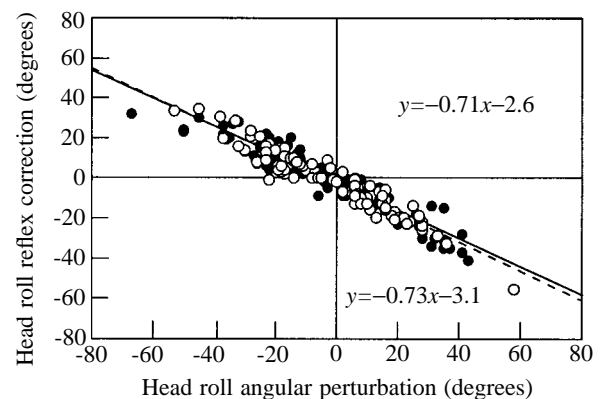


Fig. 4. Correction angle after perturbation of the head around the roll axis for suspended motionless flies, intact (filled symbols, solid regression line;  $r^2=0.90$ ,  $P<0.001$ ) or after dissection that removed the chordotonal organs (open symbols, broken regression line;  $r^2=0.88$ ,  $P<0.001$ ), following perturbation and release elicited by a subsequent touch to the thorax. Positive (negative) angles indicate head roll postures or movements downwards to the fly's right (left).

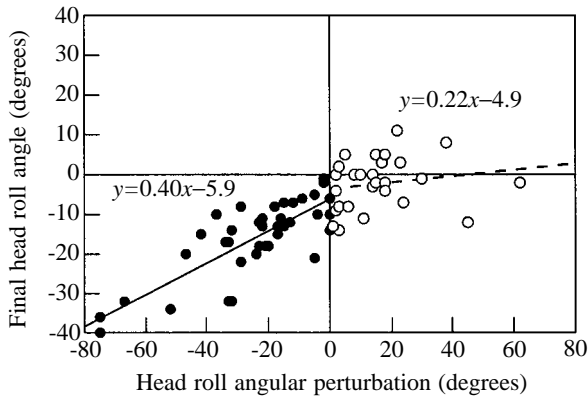


Fig. 5. Final corrected head angle following perturbation and release elicited by a subsequent touch to the thorax for suspended motionless flies after unilateral section of the prosternal nerve. Data are plotted as sections of the left nerve, with open symbols indicating perturbations to the intact (right) side (broken line;  $r^2=0.19$ ,  $P<0.017$ ) and filled symbols indicating perturbations to the sectioned (left) side (solid line;  $r^2=0.62$ ,  $P<0.001$ ). Perturbations to the intact side are well corrected, whereas those towards the sectioned prosternal nerve are not.

made by control flies with only the dissection window open ( $F_{1,123}=1.42$ ,  $P=0.236$ ). However, perturbations towards the severed left side are not very well corrected, with larger uncorrected final head angles for larger perturbations. The regression equation is  $y=0.40x-5.9$ , indicating that the fly corrects a little more than half the amplitude of the imposed perturbation. This is a significantly poorer correction than that achieved by control flies with only the dissection window open ( $F_{1,129}=6.31$ ,  $P=0.013$ ).

If both prosternal nerves are cut, left and right afferences are zero and the functional model predicts that their comparison should not result in any error signal being sent to the motor output. Thus, any perturbation in the head position should not be actively corrected. This is not, in fact, the result observed (Fig. 6, open symbols). Perturbation experiments with bilateral prosternal nerve section demonstrate that the fly still corrects approximately 45% of the amplitude of the perturbation around the roll axis ( $y=-0.45x-1.5$ ,  $r^2=0.64$ ,  $N=6$  flies). The slope of the regression line indicates significantly poorer correction than that achieved by control flies with only a dissection window open ( $F_{1,194}=27.33$ ,  $P<0.001$ ). There is also more variability in the final head position compared with that in control flies, as is evident in the lower  $r^2$  value (0.64 *versus* 0.88). Furthermore, the  $y$ -intercept of  $-1.5^\circ$  indicates that whatever system is responsible for the 45% correction is either inherently centered at zero or has another source of sensory information, albeit somewhat imprecise, but which symmetrically encodes head position relative to the thorax.

#### Motor pathways of the head roll resistance reflex

Motoneurons in the frontal nerve (FN) innervate, among other tissues, a pair of ipsilateral depressor muscles, DE 1 and

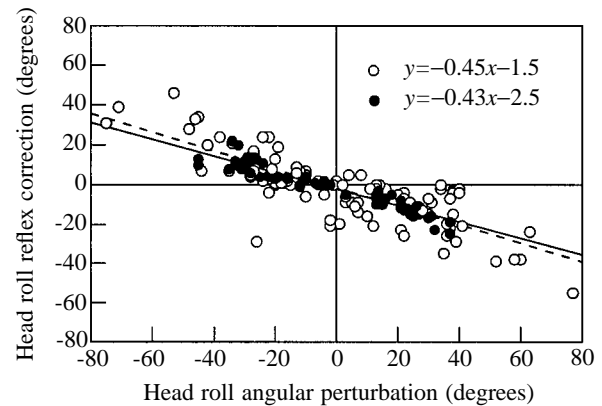


Fig. 6. Head correction following perturbation and release elicited by a subsequent touch to the thorax for suspended motionless flies after bilateral section of the prosternal nerves (open symbols, broken regression line;  $r^2=0.64$ ,  $P<0.001$ ) or frontal nerves (filled symbols, solid regression line;  $r^2=0.77$ ,  $P<0.001$ ).

DE 2, a levator and an adductor muscle. The putative function of DE 1 and DE 2 is to depress the ipsilateral side of the head. The levator and adductor could elevate the ipsilateral side of the head. Motor units in the frontal nerve are usually silent when the head is in its upright position (Gilbert *et al.* 1995). Thus, increased firing in the depressor motoneurons of the left FN should cause the head to roll down towards the left and *vice versa*. Increased firing in the levator and adductor motoneurons of the right FN should also roll the head down to the left. To determine the relative contributions of depressor and levator muscles, as well as other neck muscles innervated from the thorax, we unilaterally or bilaterally sectioned the frontal nerve, perturbed the head around the roll axis and elicited a correcting reflex.

Perturbation experiments with a unilaterally sectioned right or left frontal nerve reveal differential patterns of reflexive correction. When these data are combined and displayed as final head angle *versus* perturbation angle standardized as measurements ipsi- or contralateral to a sectioned left frontal nerve, the data fall along two lines (Fig. 7). Perturbations towards the left, ipsilateral to the cut FN, are well corrected with little offset in mean final head angle ( $-4.0\pm 6.8$ ,  $y=-0.005x-4.2$ ,  $r^2=0.0002$ ,  $N=4$  flies). However, perturbations towards the right, contralateral to the cut FN, are poorly corrected with a larger uncorrected final head angle for larger perturbations. The regression equation is  $y=0.37x+2.5$  ( $r^2=0.25$ ,  $N=4$  flies), indicating that the fly corrects only approximately 40% of the amplitude of the imposed perturbation. The two slopes are significantly different ( $F_{1,60}=7.62$ ,  $P=0.008$ ). Thus, when the thoracic innervation to levators and adductors ipsilateral to the direction of perturbation is cut, but the innervation to the contralateral FN depressors remains intact, the fly is able to correct the error. Conversely, when the innervation of FN depressors contralateral to the direction of perturbation is cut, but the innervation to the ipsilateral FN levators and adductors remains



intact, the fly is unable to correct more than 50% of the amplitude of the perturbation. Thus, the ipsilateral levator and adductor are neither necessary for complete correction nor are they sufficient. Flies in which both frontal nerves are cut (Fig. 6, filled symbols) still correct approximately 43% of the amplitude of the perturbation ( $y = -0.43x - 2.5$ ,  $r^2 = 0.77$ ,  $N = 4$  flies), which is significantly less than the correction achieved by control flies with only a dissection window open ( $F_{1,154} = 65.02$ ,  $P < 0.001$ ). Taken together, these results suggest that the motoneurons of the FN contralateral to the direction of perturbation are necessary for complete correction of perturbations of the head around the roll axis.

However, there must be at least one other effector system, since even without frontal nerve innervation of neck muscles the fly corrects its head posture to some extent. Similar to the argument advanced above for sensory input, the  $y$ -intercept of  $-2.5^\circ$  for the regression of the bilateral FN section results (Fig. 6, filled symbols) indicates that the effector system responsible for the 43% correction is either inherently centered at zero or receives low-gain, but symmetrical, sensory information through prosternal (or some other) afferents that is sufficient for partial correction of posture of the head about the roll axis. As mentioned in the Introduction, contraction of some of the neck muscles innervated by the cervical nerve could possibly exert torque around the roll axis. However, testing their necessity by cutting the cervical nerve is difficult as it requires a much more invasive thoracic dissection.

#### Passive elastic contribution of the neck skeleto-muscular system

The approximately 45% correction of perturbation amplitude by flies with both prosternal nerves or both frontal nerves cut suggests that another parallel system contributes to the resistance

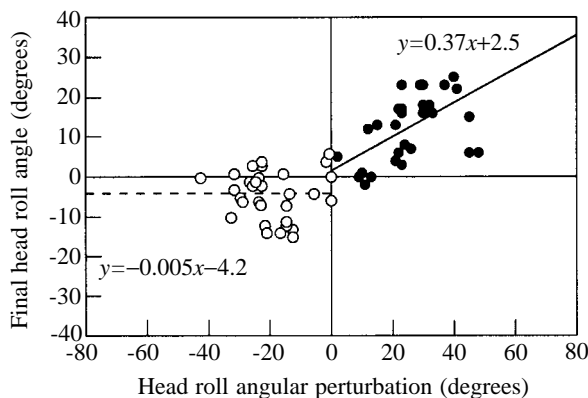


Fig. 7. Final corrected head angle following perturbation and release elicited by a subsequent touch to the thorax for suspended motionless flies after unilateral section of the frontal nerve. Data are plotted as sections of the left nerve, with open symbols indicating perturbations to the sectioned (left) side (broken line;  $r^2 = 0.0002$ ,  $P = 0.96$ ) and filled symbols indicating perturbations to the intact (right) side (solid line;  $y = 0.37x + 2.5$ ,  $r^2 = 0.25$ ,  $P = 0.003$ ). Perturbations ipsilateral to the sectioned side are well corrected, whereas those contralateral to the sectioned frontal nerve are not.

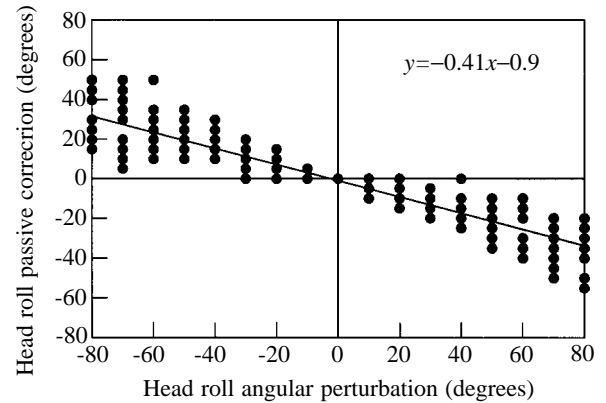


Fig. 8. Head correction immediately following release from perturbation for suspended motionless flies under nitrogen narcosis. Regression statistics:  $r^2 = 0.81$ ,  $P < 0.001$ .

reflex. Furthermore, an experiment in which both prosternal nerves and both frontal nerves were cut was performed on one fly (data not shown), which still corrected approximately 35% of the amplitude of the perturbation around the roll axis between  $\pm 30^\circ$ , yielding a regression of  $y = -0.35x - 1.2$  ( $r^2 = 0.98$ ,  $N = 16$  angles). To test whether this correction is due to a parallel active sensori-motor system, or is perhaps sufficiently explained by the contributions of the passive elastic properties of the neck skeleto-muscular system, we performed perturbation experiments on flies reversibly anesthetized with nitrogen (Fig. 8). Head position was randomly perturbed in  $10^\circ$  increments and immediately upon release returned to a position that corrects 40% of the amplitude of the perturbation and that is centered about zero. The best-fitted regression line is  $y = -0.41x - 0.9$  ( $r^2 = 0.81$ ,  $N = 20$  flies), which is not significantly different from that describing results from flies with both prosternal nerves cut (Fig. 6;  $F_{1,477} = 1.38$ ,  $P = 0.240$ ) or both frontal nerves cut (Fig. 6;  $F_{1,436} = 0.05$ ,  $P = 0.829$ ). The viscoelastic properties of the neck skeleto-muscular system appear to be relatively resilient. The release of stored elastic strain due to head perturbation appeared to have similar dynamics and amplitude whether the torque resulting in head perturbations had been applied briefly (for less than 1 s) or for longer (for several seconds). However, we did not systematically investigate the viscoelastic properties. The results from anesthetized flies demonstrate that the passive spring torques derived from skeleto-muscular components of the neck, including the arthroal membrane, are sufficient to provide some corrective head turning and to explain quantitatively the correction recorded in the experiments after bilateral section of sensory (prosternal) or motor (frontal) nerves.

## Discussion

### Functional model of the head roll resistance reflex

As initially postulated by Peters (1962) and first tested by Preuss and Hengstenberg (1992), the mechanosensory hairs of

the prosternal organ sense head posture around the roll axis. Our present results confirm and quantitatively extend the previous findings. By perturbing head posture, we determined how accurately the resting head posture is actively maintained and through which effector pathways. When a single prosternal nerve is cut, head position is shifted to the side ipsilateral to the cut nerve by approximately  $8^\circ$  in motionless flies. After bilateral section of the prosternal nerves, 45% of the amplitude of perturbations in head roll is still corrected, although precision is reduced (Fig. 6). Preuss and Hengstenberg (1992) also found that the standard error of the mean head position was more than twice as large after bilateral shaving, but in their experimental design the fly's head was unperturbed and maintained an upright posture. They speculated that another active proprioceptive organ or passive spring torques contributed by the bilaterally symmetrical neck skeleto-muscular system represented alternative pathways for maintaining head posture around the roll axis. The present results from experiments in which both prosternal nerves or both frontal nerves were sectioned (Fig. 6), or from the single experiment in which all four nerves were cut, also indicate that some other system centered about  $0^\circ$  is sufficient to correct approximately 40% of the amplitude of imposed angular perturbation. Our results with anesthetized flies demonstrate that the passive elastic properties of the neck skeleto-muscular system (including the arthroal membrane) are sufficient to account quantitatively for the 40% correction (Fig. 8). No other proprioceptive system is necessary, including the prosternal chordotonal organ. The function of this organ remains unknown, but it has been suggested to have a role in the control of head posture. In motionless flies, this is not the case with respect to posture around the roll axis. The results from experiments on intact flies are not statistically different (Fig. 4) from those on flies from which both chordotonal organs had been removed. Whether the chordotonal organ is involved in the control of head posture in moving flies remains an open question.

Motor pathways necessary for the resistance reflex are innervated through the frontal nerve, as demonstrated by the unilateral residual bias in final head position after unilateral section of the frontal nerve. The significant residual bias is associated with perturbations contralateral, but not ipsilateral, to the sectioned frontal nerve. Thus, some muscle(s) innervated by the frontal nerve is required for complete roll of the head from a contralateral angular offset back to an upright position. The neck is a complex multi-joint linkage, and the requisite muscle(s) could either be providing the torque to move the head or the rigidity to the linkage such that torque produced by another, intact muscle is transferred to the head. Motoneurons of the frontal nerve innervate several different neck muscles as well as some prothoracic body wall muscles (Strausfeld and Seyan, 1985; Strausfeld *et al.* 1987). The neck muscles innervated by the frontal nerve are the two depressors, DE 1 and DE 2, a levator, an adductor and several 'internal muscles' of the lateral cervical sclerite that have been proposed to anchor the sclerite to the occipital condyle. The laterality of

the residual bias indicates that neither the levator nor the adductor is sufficient to mediate head roll posture after denervation of muscles innervated by the contralateral frontal nerve. Nor are muscles innervated through the cervical nerve sufficient to roll the head when the frontal nerve is cut. However, the muscles innervated by the contralateral frontal nerve are necessary to roll the head reflexively back into an upright posture after perturbation. This conclusion is consistent with the results of an experiment (Gilbert *et al.* 1995) in which electrical stimulation of a prosternal nerve induced downward roll of the head to the contralateral side, but only when the contralateral FN was intact.

There must also be an active system that acts like a mechanical clutch to keep the head firmly appressed to the thorax. In motionless flies, the perturbed head posture slips a few degrees towards upright after release from perturbation, but then remains approximately at the perturbed angle until the fly is aroused. In contrast, the perturbed head posture in anesthetized flies is not stable and is corrected, albeit incompletely, immediately upon release from perturbation. A functionally similar cervical clutch mechanism has been demonstrated in dragonflies (Gorb, 1991, 1995), in which the head is actively appressed to the thorax when the animal is stationary and is free to roll when the animal is flying. The similarity ends there, however, since the maintenance of upright (relative to gravity) head posture in dragonflies is primarily under inertial rather than muscular control (Mittelstaedt, 1950). The neuromuscular identity of such a cervical clutch in *N. bullata* is unknown, but it must be innervated through a motor root other than the frontal nerve, because the perturbed head posture remains stable in flies with bilateral section of the frontal nerves (Fig. 6). The four so-called 'internal' neck muscles, SC-CO DV 1–4, which have been postulated to pull the lateral cervical sclerite against the condyle, are each innervated only by a frontal nerve motoneuron (Strausfeld *et al.* 1987). Alternative candidate muscles innervated by other nerves not sectioned in these experiments were discussed in the Introduction. The sclerite retractor, SC-RE, an indirect muscle that is innervated by the cervical nerve, has been proposed to appress the head to the thorax (Strausfeld *et al.* 1987). Other muscles innervated by the cervical nerve, VL and OH 1–2, and the ventral cervical nerve, OH 3–5, remained intact in these experiments and could also serve as the clutch. Of these, VL and OH 3–5 receive directionally selective visual input and thus are less likely to play a role in holding the head to the thorax.

#### *Modulation of the resistance reflex*

Prosternal organ afference is necessary and sufficient (with the passive elastic properties of the neck) to specify the amount of muscular contraction required to resist perturbation of head roll posture. However, the afferent activity does not always have access to the neck muscles to perform the resistance reflex. The reflex is gated off or on by the fly's behavioral state and by activity in other sensory modalities. When the fly is stationary, the reflex is gated off, and head posture can retain



large angular offsets long after the source of the perturbation is removed (Fig. 2). Prosternal organ afferents adapt very little to such sustained angular offsets and continue to generate action potentials (C. Gilbert and M. Kim, unpublished observations), but such activity does not have access to the motoneurons, directly or through interneurons, and the reflex is gated off. Gating on may result from external stimulation, e.g. the tactile stimulation of our experimental paradigm, or internal stimulation, perhaps proprioceptive, associated with moving. Intact moving flies resist any perturbation of their head around the roll axis, immediately returning their head close to an upright posture relative to their thorax (Figs 2, 3). Activity in the prosternal organ afferents has access to neck motoneurons mediating roll posture. The mechanism that gates the access of prosternal afference to neck motoneurons could be provided by haltere afference. When a fly is moving, either walking or flying, its halteres swing (Sandeman and Markl, 1980), and the campaniform sensilla stimulated thereby (Fayyazuddin and Dickinson, 1996) could provide either an excitatory signal that allows the prosternal afference access to neck motoneurons or an inhibitory signal, through an interneuron, that prevents the muscles from clamping the head to the thorax in motionless flies.

Even such behaviorally gated prosternal proprioceptive access to the neck motor system appears to be modulated by stimulation through exteroceptive sensory modalities during flight and during walking. In tethered flight, perturbations of the halteres (Nalbach and Hengstenberg, 1994) induce compensatory angular offsets of the head around the roll axis as large as 50° that can last several seconds. In stable tethered flight, flies visually track slow oscillations of panoramic surrounds with equally slow changes in the roll posture of their heads. Again, such postural changes can result in angular offsets as large as 60° lasting several hundred milliseconds (Hengstenberg, 1988). Finally, tethered walking flies holding a ball equal to or greater than their own mass counter-roll their heads by an amount that increases with the angle from vertical at which the ball is held (Horn and Lang, 1978). The effect is reduced when sensory hairs on the antenna are excised. These compensatory head movements must be active since they counteract the passive elastic properties of the neck skeleto-musculature, and the resistance reflex mediated by the prosternal organ must be modulated during such movements. Whether the reflex is totally suppressed, or is operative but adopts a new set point, is an open question. In experiments on flies walking with their transverse axis inclined vertically, the head counter-rolls approximately 30° towards vertical whether the prosternal organ is intact or excised (Horn and Lang, 1978), indicating that transmission from the prosternal organ afferents to the neck muscles is completely suppressed when activity in the gravity receptors increases. Similar types of experiments with tethered flying flies to investigate the interaction of the effects of moving visual stimuli on access of prosternal organ afference to the neck muscles have not yet been performed. Movements of the head around the same axis induced either by exterosensory or by proprioceptive stimuli are presumably

mediated by the same motoneurons and muscles (Strausfeld and Seyan, 1985). The neural site of reflex modulation is most probably central, rather than peripheral at the muscles, because almost all the neck muscles are innervated by single, and therefore probably excitatory, motoneurons (Milde *et al.* 1987; Strausfeld *et al.* 1987). Such reflex modulation in other better-studied arthropod systems also occurs centrally, often on the terminals of the sensory afferents presynaptic to motoneurons or interneurons (Clarac *et al.* 1992; Watson, 1992; Watson *et al.* 1993). The neurobiology of the thoracic ganglia of flies is still poorly understood, but not intractable, and further study of this system will provide valuable insight into the complexity and plasticity of the neural networks controlling posture.

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