Central gating of fly optomotor response

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We study the integration of multisensory and central input at the level of an identified fly motoneuron, the ventral cervical nerve motoneuron (VCNM) cell, which controls head movements of the animal. We show that this neuron receives input from a central neuron signaling flight activity, from two identified wide-field motion-sensitive neurons, from the wind-sensitive Johnston organ on the antennae, and from the campaniform sensillae of the halteres. We find that visual motion alone leads to only subthreshold responses. Only when it is combined with flight activity or wind stimuli does the VCNM respond to visual motion by modulating its spike activity in a directionally selective way. This nonlinear enhancement of visual responsiveness in the VCNM by central activity is reflected at the behavioral level, when compensatory head movements are measured in response to visual motion. While head movements of flies have only a small amplitude when flies are at rest, the response amplitude is increased by a factor of 30–40 during flight.

behavioral state | electrophysiology | motion vision | proprioceptor

The perceptual response to a sensory stimulus depends on the behavioral state of the animal. This is especially evident when responses between awake and sleeping or anesthetized animals are compared (1–5). In addition, attention can strongly modulate the perceptual response (6–11). Furthermore, recent studies have shown that the response properties of sensory neurons depend on the locomotor state of the animal (12–16). However, it is not clear at what level of the nervous system the integration of sensory input and internal state occurs and according to which rules. We address this question in an identified ventral cervical nerve motoneuron (VCNM) that is part of the neck motor system of the blowfly *Calliphora vicina*.

Blowflies are well known for their aerobatic maneuvers. This flight behavior has important consequences for vision, because fast turns can lead to motion blur and thus impair vision of spatial details. These adverse effects on vision can, in principle, be partly compensated for by eye movements. However, in all insects, the eyes are fixed to the head capsule and only small eye movements are possible (17). Therefore, effective compensatory eye movements can only be made by turning the head. Blowflies can turn their head relative to the thorax around all three body axes but with different amplitudes (yaw, ±20°; pitch, ±20°; and roll, ±90°). Accordingly, movements of the thorax during flight are compensated for by countermovements of the head relative to the thorax (18–21). These head movements are mediated by the neck motor system, which consists of 21 pairs of muscles, with each of them innervated by a single motoneuron (22, 23). The three oblique horizontal muscles, OH3–5, are supplied by the ventral cervical nerve, comprising the axons of three identical motor neurons, VCNM1–3 (22). Unilateral contraction of these muscles leads to a sidewise deflection of the head (yaw response), whereas bilateral contraction pulls the head back toward the body. From the anatomical overlap of the terminal region of two wide-field motion-sensitive neurons, the horizontal system north (HSN) and horizontal system equator (HSE), and the dendrites of VCNMs, a possible connection between these neurons was suggested (22). In addition, again based on anatomical findings, VCNMs were supposed to receive input from the halteres (24). Halteres are small club-shaped appendages oscillating during flight at the same frequency as but in antiphase to the wings. Furthermore, the halteres oscillate during walking in the absence of wing vibration (25).

So far, only extracellular recordings from VCNMs exist (26–28). These studies showed that VCNMs respond to horizontal front-to-back motion. Using intracellular recordings, we demonstrate that the VCNM receives input from wide-field motion-sensitive neurons of the lobula plate; from the Johnston organ of the antennae; from the campaniform sensillae of the halteres; and, most prominently, from a central input reflecting the behavioral state of the fly. These integrative properties of the motoneuron can also be observed in the optomotor response of the fly.

Results

In a first set of experiments, we measured the responses of the VCNM to visual motion along the horizontal axis simultaneously with the responses of its presumed input elements, the horizontal system (HS) cells (29–31). Like HS cells (Fig. 1*A*, red trace), the VCNM responded to horizontal motion stimuli with a graded shift in membrane potential (Fig. 1*A*, black trace). Ipsilateral front-to-back motion [preferred direction (PD)] of the pattern led to a depolarization of the cell, and ipsilateral back-to-front motion [null direction (ND)] led to a hyperpolarization of the cell. Contralateral motion elicited only weak responses. However, the visual stimuli used in this study did not elicit action potentials in the VCNM. This is consistent with the much lower resting potential of the VCNM (around −60 mV) as opposed to HS cells (around −50 mV). To investigate possible contact sites between HS cells and the VCNM, we performed triple staining (Fig. 1*B*) of an HSN cell (red), an HSE cell (green), and a VCNM (yellow). The axon terminal of an HSE cell (in green) and the dendrite of the VCNM (in yellow) from another preparation are shown in greater detail in Fig. 1*B Inset*. Clearly, the terminal processes of the HSN cell, the HSE cell, and the VCNM dendrite are found in close proximity. Together with similar preferred axes of motion, this suggests that the VCNM receives visual input from HS cells. To investigate the connectivity between HS cells and the VCNM directly, we performed dual intracellular recordings from different horizontal-sensitive cells of the lobula plate (HS and the centrifugal horizontal CH cells) and VCNM by injecting current into lobula plate cells and recording the response of the VCNM (Fig. 1*C*), and vice versa (Fig. 1*D*). Current of both polarities injected into the lobula plate cells led to a change of the same sign in the membrane potential of the VCNM. The same was true when current was injected into the VCNM and the membrane potential was recorded in lobula plate cells. Such a bidirectional bipolar coupling of two cells indicates the presence of electrical synapses (32, 33) between the VCNM and HS and CH cells. However, the coupling strength between CH cells and the VCNM was weaker than between HS cells and the VCNM. Because CH cells are electrically coupled to HS cells (34, 35), the coupling of CH cells and the VCNM might be indirect via HS cells.

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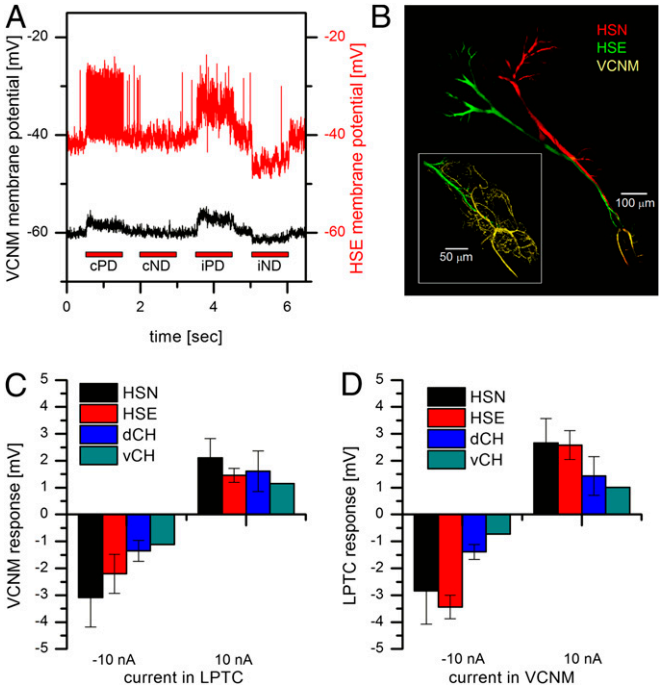

Fig. 1. Visual response properties and electrical coupling of the VCNM. (*A*) Example response of simultaneous recordings of an HSE cell (red) and the VCNM (black) to horizontal motion in front of the ipsilateral and contralateral eye. Both cells respond to ipsilateral front-to-back motion (iPD) with a depolarization and to ipsilateral back-to-front motion [ipsilateral null direction (iND)] with a hyperpolarization. In addition, the HSE cell responds to contralateral back-to-front motion (cPD) with an increase in excitatory postsynaptic potential (EPSP) frequency and the VCNM responds with a small depolarization. The same experiment has been done on eight paired recordings of HS cells and the VCNM. cND, contralateral null direction. (*B*) Two-photon images of the VCNM and HS cells. The HSN cell (red) was filled with the red fluorescent dye Alexa 568 (Molecular Probes), and the HSE cell (green) was filled with the green fluorescent dye Alexa 488 (Molecular Probes). The VCNM (yellow) was filled with both dyes. Shown is an x-y maximum intensity projection. The inset shows a 3D reconstruction of the dendrite of the VCNM and the axon terminal of an HSE cell. (*C*) Current injection of −10 nA and +10 nA in various cells of the lobula plate LPTC sensitive to horizontal motion elicited different levels of hyperpolarization and depolarization in the VCNM. (*D*) Same as in *C*, but the current was injected into the VCNM and the response was measured in lobula plate cells. Data represent the mean ± SEM of the HSN cells (n = 5), HSE cells (n = 4), dCH (dorsal centrifugal horizontal, n = 3), and vCH (ventral centrifugal horizontal, n = 1).

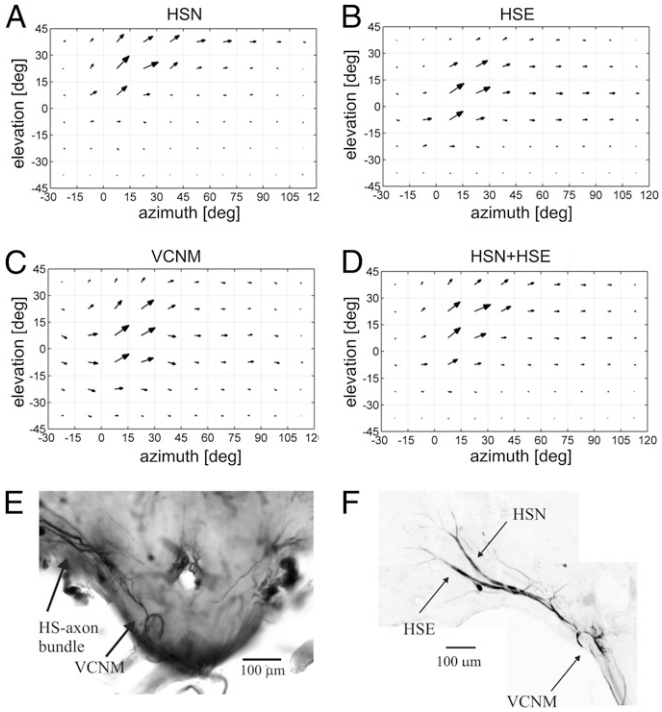

Fig. 2. Receptive fields and neurobiotin coupling. Receptive fields of the HSE cell (*A*, n = 4), HSN cell (*B*, n = 4), and VCNM (*C*, n = 3). The orientation of the arrow indicates the local PD, and the length of the arrow indicates the local motion sensitivity at this azimuth and elevation. (*D*) Linear summation of the receptive fields of the HSE and HSN cells. (*E*) Injection of neurobiotin into the HSE cell led to costaining of the VCNM (data from 33). (*F*) Injection of neurobiotin into the VCNM led to costaining of the HSE and HSN cells. This confirms the results about the electrical coupling between the VCNM and the HS cells (Fig. 1).

From these experiments, we conclude that the VCNM receives its visual motion input from HSN and HSE cells. To test whether these two types of HS cells are sufficient to explain the visual response properties of the VCNM completely, we measured the receptive fields of the HSN cell, the HSE cell, and the VCNM (Fig. 2 *A*–*C*). The receptive field of the VCNM (Fig. 2*C*) is similar to the one measured extracellularly from the OH muscle (27, 28). Like HS cells, the VCNM responds mainly to frontal horizontal motion stimuli. The linear superposition of the receptive fields of HSN and HSE cells (Fig. 2*D*) matches well the measured flow field of the VCNM (Fig. 2*C*). This suggests that HSN and HSE cells form the sole direct visual input to the VCNM. Additional evidence for the proposed connectivity between HS cells and the VCNM is obtained from dye coupling. As was shown previously, neurobiotin is a small enough molecule to pass through gap junctions in invertebrates, and thus can be used to visualize gap junction-coupled neurons via dye coupling (33, 36). Because fluorescein does not pass through gap junctions, we used the fluorescein dye to identify the injected cell. We injected the fluorescein-neurobiotin dyes either into an HS cell (Fig. 2*E*, data from ref. 33) or into the VCNM (Fig. 2*F*). The fluorescein-labeled cell was identified under the fluorescence microscope, and a picture was taken. If the dyes were injected into an HSN or HSE cell, the VCNM always showed neurobiotin immunolabeling (n = 3). Dye injection into the VCNM always led to costaining of HSN and HSE cells (n = 3). These results further support an electrical coupling of HSN and HSE with the VCNM.

In addition to motion stimuli, wind stimuli delivered to the head of the fly elicited depolarizing responses in the VCNM, sometimes eliciting action potentials (Fig. 3*A*). However, the spiking response was rather transient and depended on the resting potential of the neuron. If the resting potential of the VCNM was more negative, head wind stimuli elicited only a graded potential change. However, combining wind and motion stimuli always led to a nonlinear superposition of the individual response components with respect to spiking behavior of the VCNM (Fig. 3*B*); whereas wind stimuli and motion stimuli presented separately usually did not lead to action potentials, the simultaneous stimulation always resulted in the generation of action potentials in the VCNM. We further investigated this nonlinear enhancement of the motion response by head wind stimulation at the level of the target muscle of the VCNM. Extracellular recording from the OH muscle during simultaneous wind and motion stimulation led to a strong and directionally selective motion response of the muscle (Fig. 3*C*). Dipteran flies are known to sense wind by means of the Johnston organ (37–39), which is located in the second segment of the antennae. The Johnston organ detects displacement between the second and the third segments of the antennae. To test whether the wind response of the VCNM is mediated by the Johnston organ, we immobilized the antennae with wax (Fig. S1*A*) or removed the arista, a feather-like appendage of

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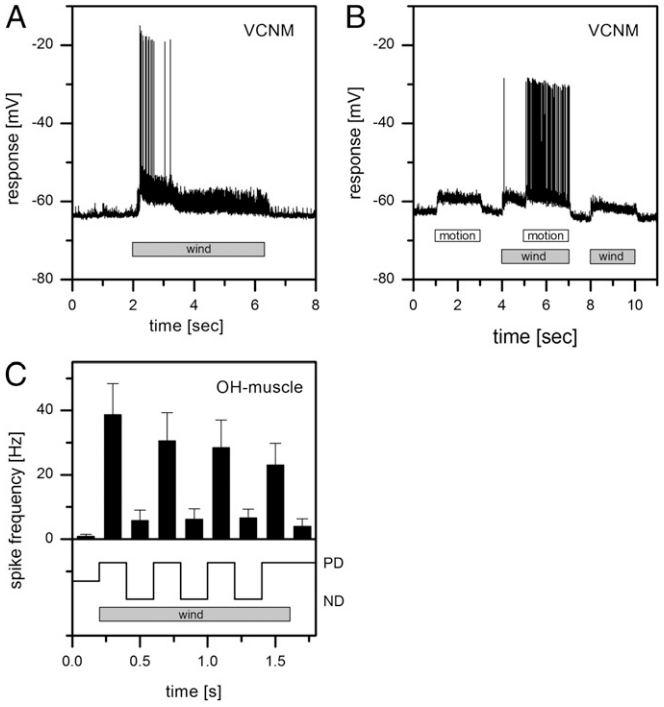

Fig. 3. VCNM and neck muscle responses to wind and motion stimuli. (*A*) VCNM response to a wind puff (gray bar) directed toward the antennae. The VCNM responds with a transient increase in firing frequency. (*B*) Combination of wind and visual motion stimuli led to a supralinear response. Neither the visual stimulus along the PD (white bar) nor the wind stimulus (gray bar) was sufficient to elicit spiking in the VCNM. Only the simultaneous presentation of both stimuli elicited spiking in the VCNM. (*C*) Switching between PD and null direction (ND) motion stimuli during head wind stimulation led to a strong directionally selective response in the OH muscle. Note that visual stimuli alone elicited only a few spikes (rightmost bar). Data represent the mean ± SEM of three OH recordings.

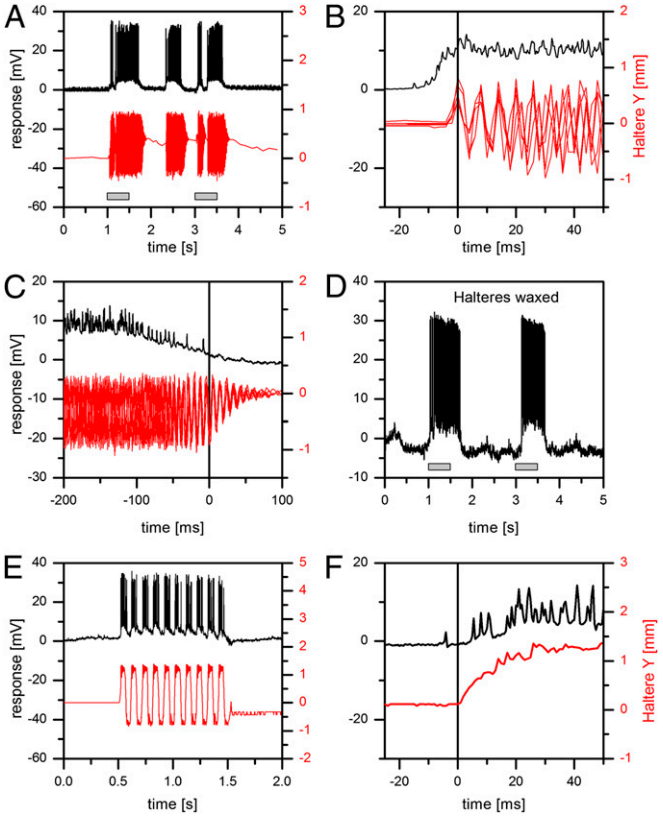

Fig. 4. VCNM responses during haltere beating. (*A*) Simultaneously recorded VCNM membrane potential and vertical position of the halteres. During active beating of the halteres, a strong depolarization in the VCNM is observed. The same data have been obtained from recordings of 11 flies. (*B*) Haltere-triggered average (active haltere beating) of the VCNM potential. The first haltere stroke (red line) was used to trigger and average the membrane potential of the VCNM (black line) over 25 sweeps in four different flies. The response in the VCNM precedes the first haltere stroke. (*C*) Same as in *B*, but the last haltere stroke was used as a trigger signal. The membrane potential of the VCNM declines about 100 ms before the last full-blown haltere stroke over an average of 12 sweeps. The same delay has been found in three additional recordings. (*D*) Strong depolarizations in the VCNM also occur when the halteres are waxed. The same data have been obtained from two more flies. The gray boxes in *A* and *D* indicate wind puffs directed toward the abdomen of the fly. (*E*) Response of the VCNM to passive movement of the halteres with a frequency of 10 Hz. (*F*) Haltere-triggered average (passive haltere beating) of VCNM potential. Here, the response in the VCNM is delayed over an average of 5 sweeps in three different flies.

the third antennal segment (Fig. S1*B*). Both led to a complete loss of the response of the VCNM to the wind puff. In addition, the response was abolished when we waxed the distal tip of the antennae, thereby blocking the torsion of the third segment (Fig. S1*C*). The response of the VCNM to wind stimuli applied to the antennae is in accordance with the findings of a previous study in which cobalt staining of the VCNM, HSN cell, and HSE cell was reported following injection of cobalt into the antennae (40).

A recent study has shown that the responsiveness of visual interneurons in *Drosophila* depends on the behavioral state of the animal (16). The authors of that study found that vertical sensitive tangential cells respond much stronger if the fly is flying. To measure the influence of the behavioral state on the response properties of the VCNM, we elicited active beating of the halteres by directing wind puffs to the abdomen of the fly (41). During the active haltere oscillation, the VCNM responded strongly with a depolarization and high-frequency spiking (Fig. 4*A*). The wind puff directed to the abdomen itself did not move the halteres directly and did not elicit any response in the VCNM if the fly did not actively move its halteres. The temporal coincidence between the response of the VCNM and the oscillation of the halteres indicates a direct input of haltere afferents onto the VCNM. However, the response of the VCNM started a few milliseconds before the onset of haltere movements (Fig. 4*B*) and declined almost 100 ms before the last full haltere stroke (Fig. 4*C*). Finally, the VCNM still responded in a characteristic fashion to the wind puffs directed to the abdomen, even when the halteres were immobilized by wax (Fig. 4*D*). The time course of the responses and the experiments with immobilized halteres speak against an afferent input of the haltere nerve on the VCNM. These results rather indicate that VCNM as well as haltere movements are driven by a central input that is activated by wind puffs directed to the abdomen but sometimes become spontaneously active as well (Fig. 4*A*, second burst).

In a recent article, the response of HS cells to active movement of the halteres was investigated (42). The authors of that study found that the membrane potential change in HS cells preceded the onset of haltere motion. This result is in accordance to the observed time course of the VCNM response.

To investigate whether the central input projects directly to the VCNM or to HS cells, we performed experiments in which we first measured the response of HS (HSN and HSE) cells to active haltere beating and then, in the same flies, the response of the VCNM to active haltere beating. The membrane potential changes found in HS cells (2.3 ± 0.7 mV, $n = 3$) were much smaller than in the VCNM (20.2 ± 2.4 mV, $n = 3$), suggesting that excitation spreads from the VCNM to HS cells through the

electrical synapses. Therefore, these results indicate a direct input from central neurons onto the VCNM.

In addition, the VCNM responds to passive deflection of the ipsilateral haltere (Fig. 4*E*). However, in contrast to active haltere movement, the VCNM response was delayed to the start of the haltere movement (Fig. 4*F*). Furthermore, the timing of spikes in the VCNM is different for active and passive haltere movement. We found a phase locking of the spikes in the VCNM to the beating cycle of the haltere if the halteres were moved passively (Fig. 5 *A*–*C*). At the base of the halteres, campaniform sensillae respond to deflections of the halteres. Recordings from the haltere afferent nerve showed that the activity of the nerve is tightly phase-locked to the beating cycle (43–45). In contrast, during the active haltere movement, the spikes in the VCNM did not occur at a certain phase of the haltere beating cycle (Fig. 5 *D*–*F*), except for rather short periods (trials 80–90; Fig. 5*E*). The difference in spike timing between passive and active haltere beating is not attributable to the different haltere beating frequency. By waxing a small drop of beeswax onto the haltere knob, and thereby increasing the weight, the frequency during active haltere beating was reduced to 70 Hz (Fig. 5 *G*–*I*). Nevertheless, the spikes occurring in the VCNM were not phase-locked to the haltere beating cycle.

Because active haltere beating went along with such strong responses in the VCNM, we asked whether the direction of visual motion is still encoded in VCNM responses during those active episodes. We therefore stimulated the fly with motion stimuli of alternating direction and elicited active haltere beating by a wind puff directed to the abdomen (Fig. 6*A*). The visual motion stimulus alone did not elicit action potentials in the VCNM (Fig. 1*A*). As expected, the active beating of the halteres elicited a strong depolarization and high-frequency spiking of the VCNM. However, the spike rate during this episode was strongly modulated by the direction of the motion stimulus (Fig. 6*B*).

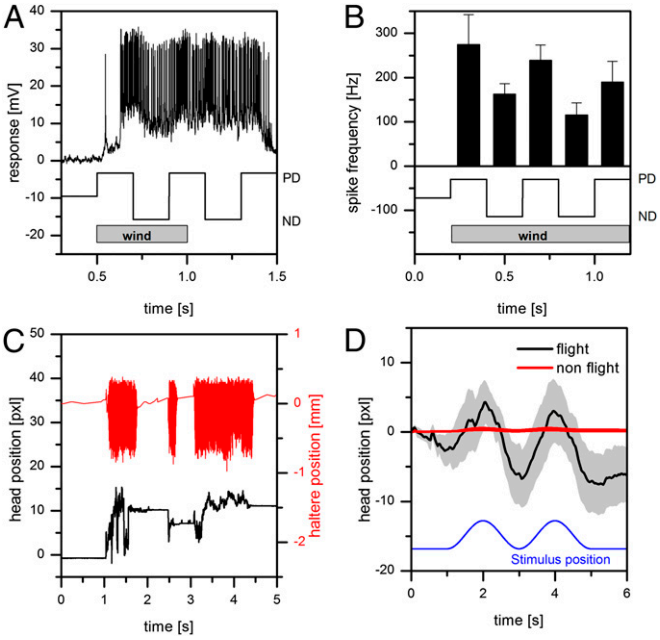

Fig. 6. VCNM responses and behavior. (*A*) Wind puff (gray bar) elicits haltere beating and strong spike activity in the VCNM (black). During this episode, the VCNM is stimulated by visual motion (black line) alternating between the PD and null direction (ND) every 200 ms. (*B*) Spike frequency of the VCNM averaged over five stimulus repetitions during a haltere beating episode and simultaneous motion stimulation as in *A*. The VCNM modulates its spike activity in a directionally selective way. (*C*) Head movements of a fixed fly in pixel (pxl). The head (black trace) moves only during the haltere beating episodes (red trace); otherwise, it is fixed and does not move at all. (*D*) Yaw head response of flies during flight (black) and during rest (red). During flight, the amplitude is, on average, 30- to 40-fold larger than during rest. Data show the mean ± SEM of eight flying flies and seven nonflying flies.

Because the VCNM is a motoneuron connecting to neck muscles, the response properties of the VCNM described above should be reflected in the behavior of flies as well. We used a preparation of the fly comparable to the one used for electrophysiological recording with the main difference that the head was not waxed to the thorax, and thus was free to move. We filmed the head of the fixed fly as well as the halteres from the side. Beating of the halteres was again elicited by wind puffs directed to the abdomen. We found that the head only moved when the halteres were moving: Between the haltere beating episodes, the head was rather fixed without any observable movement (Fig. 6*C*).

This is reminiscent of observations in the flesh fly, where the head of a tethered walking or flying fly was found to compensate immediately for passive head rolling, whereas the compensation took much longer when the fly was at rest (46). To test whether this is also true for the optomotor head yaw response, we waxed a small cardboard to the thorax of intact flies, placed them in a holder in front of a stimulus monitor, and recorded the position of the head while we displayed a visual pattern moving with a sinusoidal velocity profile. This was done both when the fly was flying as well as when it had stopped flying. When the fly was not flying, its head did not follow the pattern motion (Fig. 6*D*, red trace). However, during flight, the head moved along the direction of the pattern motion with a large amplitude (Fig. 6*D*, black trace).

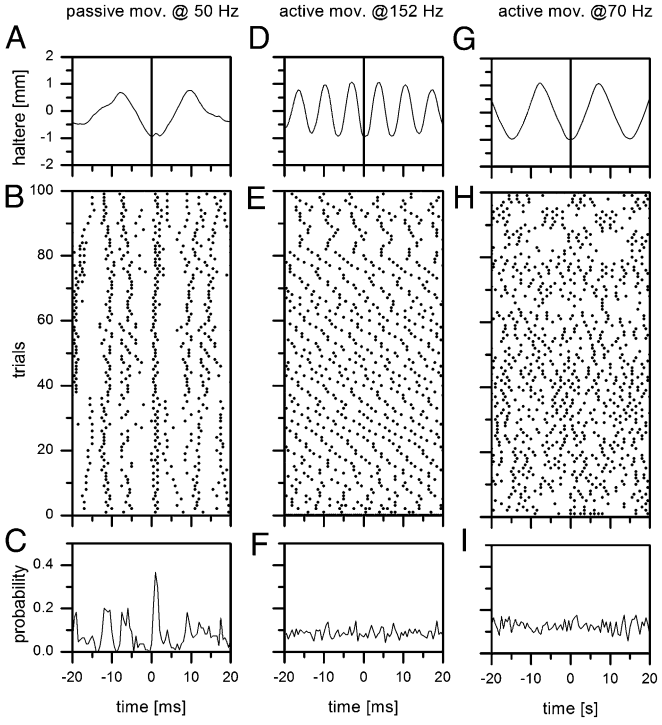

Fig. 5. Haltere beating and spike timing. Spike timing for passive haltere movement (*Left*; *A*–*C*), active haltere movement at an oscillation frequency of 152 Hz (*Center*; *D*–*F*), and active haltere movement at an oscillation frequency of 70 Hz (*Right*; *G*–*I*). (*A*, *D*, and *G*) Averaged vertical haltere movement. (*B*, *E*, and *H*) Position of the haltere was used to align the spike traces of the VCNM. Plotted are 100 trials for passive (*B*) and active (*E* and *H*) movement of the haltere. Whereas the spikes occurring during passive movement are aligned, the spikes elicited during active movement do not show a specific pattern. (*C*, *F*, and *I*) Spike probabilities for data shown in *B*, *E*, and *H*.

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Discussion

The results presented here demonstrate that the VCNM receives synaptic input from four different sources: a subset of large-field motion-sensitive neurons of the lobula plate (the HSN and HSE cells), the Johnston organ of the antennae, campaniform sensillae at the base of the halteres, and a central input affecting the halteres.

This latter input deserves further discussion. From Pringle's work (47), it is known that the halteres are driven by an asynchronous muscle. In general, asynchronous muscles are innervated by motoneurons, the spike frequency of which is much lower and does not correlate with the contraction frequency of the muscles. Calcium is thought to play a permissive role of maintaining the muscle fibers in a "stretch-activatable" state (48, 49). Because of the small size of the sarcoplasmatic reticulum, and therefore slow reuptake of cytosolic calcium, asynchronous muscles exhibit slow deactivation. The slow deactivation of the haltere muscle could explain the observed time course of the VCNM response at the end of an active haltere beating episode (Fig. 4*C*).

These results on the spike timing of the VCNM for active and passive movement of the halteres indicate that the VCNM receives input from the campaniform sensillae at the base of the halteres. The ≈400 campaniform sensillae found in *C. vicina* are arranged in three dorsal and two ventral fields (50). Some fields are oriented parallel to the haltere's longitudinal axis and could monitor the large forces resulting from oscillation in the stroke plane. One field (dF2) (50) and the haltere's large chordotonal organ are oriented such that they could detect lateral distortions resulting from Coriolis forces that arise during body rotations (51). Single fiber recordings from the haltere nerve showed that some of the afferent neurons responded only to deflections of the haltere in the orthogonal plane and not in the stroke plane (45). These highly specific responses have also been observed in recordings of neck motoneurons of the frontal nerve (25), where deflections of the haltere in the stroke plane did not elicit responses. However, deflections orthogonal to the stroke plane elicited compound action potentials. Given the fact that finding the correct stroke plane for passively moving the halteres with the electromagnets is rather difficult, it seems feasible that the passive deflection of the haltere resulted in the activation of a different set of campaniform sensillae than the active oscillation induced by wind puffs directed to the abdomen. This implies that the VCNM receives input only from the haltere field that detects deflection orthogonal to the stroke plane and not from the fields that sense motion of the halteres within the stroke plane. Thus, during active haltere beating (i.e., within the stroke plane), the only input to the VCNM would be from the central neuron. This could explain the different timing of spikes in the VCNM for active and passive haltere motion (Fig. 5).

A recent study (28) showed that some neck motoneurons of the frontal nerve receive direct afferent input from the halteres. In the study of Huston and Krapp (28), a class of neurons responded to visual stimuli with an increase in spike frequency only when the halteres were moved passively at the same time. From this result, the authors concluded that haltere movements are gating the visual stimuli. However, our recordings of the VCNM show that although this neuron receives direct input from the campaniform sensillae of the halteres (Fig. 4*E*), the more important input comes from a central neuron (Fig. 4 *C* and *D* and Fig. 5). Activity of this central neuron affects both the beating of the halteres and the depolarization of the VCNM. In addition, experiments by Rosner et al. (42) showed that HS cells did not respond stronger to visual motion stimuli along their PD during active haltere beating. Therefore, the observed gating of the visual responses in the VCNM cannot be attributable to stronger input from HS cells; rather, they result from an integration of central and visual input on the dendrite of the VCNM.

The experiments with tethered flying flies (Fig. 6*D*) demonstrate the importance of the behavioral state of the animal, as expressed in its haltere beating, for the performance of a simple visual reflex. When stimulating the fly with visual motion mimicking yaw rotation, the gain of the head optomotor response was a 30- to 40-fold stronger if the fly was flying compared with nonflying flies. A similar phenomenon has been observed for the head pitch response in blowflies (52). In those earlier studies, the investigators made use of the fact that the head of the fly was locked in the low-activity state (nonflying) and was jittering with a high frequency in the high-activity state (flying). The gain of the head pitch movement was up to 30-fold higher when the fly was in the high-activity state. The high-activity state correlated with the beating of the halteres but was not dependent on the actual movement of the halteres. In addition, the head started jittering before the halteres started oscillating and stopped jittering about 100 ms before the halteres stopped oscillating. Our electrophysiological recordings provide a mechanistic explanation for this improved performance based on multimodal input onto a single motoneuron innervating a neck muscle. Whereas visual input alone rarely elicits action potentials in the VCNM because of its low resting potential, additional input either from a central neuron or from the wind-sensitive organ in the fly's antennae pushes the membrane potential above threshold, allowing for strong and reliable modulation of spike frequency by visual motion and resulting in contraction of its neck muscles, and thus in compensatory head movements. When the fly is at rest, this mechanism is switched off, the head is locked, and any movement sensed by the lobula plate neurons must be caused by objects moving in the external world.

In summary, our results add to the increasing body of evidence that the behavioral state of an animal affects the response properties of sensory neurons. Further studies need to clarify the neuronal correlate of the central input.

Methods

Experiments were performed on 3- to 5-d-old blowflies, *Calliphora vicina*, taken from our laboratory stock. The procedures for preparation and electrophysiological recording in flies follow those described by Haag and Borst (31). The details of induction and measurement of haltere and head movements, methods for the neurobiotin histology, and two-photon microscopy are described in *SI Methods*.

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