Convergent Mechanosensory Input Structures the Firing Phase of a Steering Motor Neuron in the Blowfly, *Calliphora*

AMIR FAYYAZUDDIN AND MICHAEL H. DICKINSON

Department of Integrative Biology, University of California, Berkeley, California 94720; and Committee on Neurobiology, University of Chicago, Chicago, Illinois 60637


The first basalar muscle (B1) is 1 of 17 small steering muscles in flies that control changes in wing stroke kinematics during flight. The B1 is often tonically active, firing a single phase-locked action potential in each and every wingbeat cycle. Changes in activation phase alter the biomechanical properties of B1, which in turn cause aerodynamically relevant changes in wing motion. The phase-locked firing of the B1 motor neuron (MNB1), is thought to arise from an interaction of wingbeat-synchronous inputs from the wings and from specialized equilibrium organs called halteres that beat antiphase to the wings and function to detect angular rotation of the body during flight. We investigated how the wing and haltere inputs interact to determine the firing phase of MNB1. Our results indicate that both wing and haltere afferents make strong monosynaptic connections with MNB1, consisting of fast electrical and slow Ca2+-sensitive components. Although both the wing and haltere-evoked excitatory postsynaptic potentials (EPSPs) display the two components, their relative contribution is different for the two inputs. Whereas the haltere-evoked EPSP is dominated by the fast electrical component, the wing-evoked EPSP is dominated by a large chemically mediated component and displays an additional prolonged Ca2+-dependent component that is absent in the haltere-evoked EPSP. Both inputs display an activity-dependent fatigue affecting both electrical and Ca2+-sensitive components, from which the haltere synapse recovers more rapidly. The net result of these synaptic differences is that the two pathways differ significantly in their relative ability to evoke action potentials in MNB1. Although the haltere pathway displays greater temporal precision, the wing pathway is stronger, judged by its ability to entrain MNB1 within a background of haltere stimulation. We propose a model by which these physiological differences play a functional role in tuning the firing phase of MNB1 during flight. The wing input may serve primarily to set the background firing phase of MNB1, whereas the haltere input serves to transiently advance the firing phase during equilibrium reflexes.

INTRODUCTION

Since the pioneering work of Wilson (1961) the flight behaviors of insects have served as important model systems for the study of rhythmic pattern generation. The best characterized flight circuit is that of the locust, *Schistocerca gregaria*, which consists of a central oscillatory network that is phasically tuned by peripheral mechanosensory feedback to pattern the activity of wing muscles (for review, see Burrows 1996). Many insects, including flies, employ a quite different strategy in which the basic back and forth motion of the wings is driven myogenically by alternating contractions in antagonist sets of stretch-activated muscles (Pringle 1949; for review, see Dickinson and Tu 1997). These asynchronous power muscles are maintained in a stretch-activated state by tonic input from their motor neurons (Roeder 1951). Although the motor neurons innervating the power muscles fire in a regular pattern (Harcombe and Wyman 1977; Ikeda 1977), their firing rate is well below the wingbeat frequency (~120–150 Hz in *Calliphora*). Spikes in the power muscle motor neurons bear no fixed phase relationship with the wingbeat cycle and do not require sensory feedback on a cycle-by-cycle basis (Heide 1974). This lack of direct neural control coupled with the fact that the power muscles do not directly attach to the wing hinge make them ill-suited for producing rapid changes in the wingstroke necessary for flight control. Instead, steering maneuvers in flies are controlled by a set of small synchronous muscles that insert directly onto the sclerites of the wing base. In contrast to the power muscles, the motor neurons innervating the control muscles receive strong excitatory input from wingbeat synchronous afferents (Heide 1974, 1983). When active during steering maneuvers, the control muscles tend to fire within characteristic phases of the wingstroke cycle (Egelhaaf 1988; Heide 1983; Heide and Götz 1996). The proprioceptive feedback to the steering motor neurons is necessary to ensure proper mechanical activation of their target muscles (Dickinson and Tu 1997; Tu and Dickinson 1996). In this paper, we investigate how two discrete sets of mechanosensory afferents interact to set the firing phase of an important identified steering muscle.

The first basalar muscle, B1, is known to fire during nearly every wingstroke cycle (Egelhaaf 1989; Heide 1971, 1983; Heide and Götz 1996). This phase-locked behavior remains even if wingbeat frequency is artificially elevated by removing distal sections of the wing—a manipulation that increases the resonance frequency of the thoracic muscular-skeletal system. This result suggests that wingbeat-synchronous afferents play a central role in the temporal patterning of B1. The B1 also receives mechanosensory input from the halteres, club-shaped equilibrium organs derived evolutionarily from the hindwing. During flight, the halteres beat up and down in strict anti-phase with the wing (Pringle 1948; Sandeman 1980). Fields of campaniform sensilla at the base of the haltere detect both its basic up and down oscillation as well as deviations caused by Coriolis forces induced by angular rotation of the body during flight (Dickinson 1999; Nalbach 1994). Ablation of sensory feedback from the wings and halteres completely abolishes the
phasic firing of B1 (Heide 1983). Input from a single haltere, however, can entrain the ipsilateral B1 while input from one wing can entrain both ipsi- and contralateral B1s. This result implies that, although both haltere and wing afferents influence the firing of the B1 motor neuron (MNB1), the wing mechanoreceptors may entrain the muscle with greater efficacy.

In a previous study (Fayyazuddin and Dickinson 1996), we have characterized the synapse between haltere afferents and MNB1 in the blowfly, Calliphora vicina. The afferent input to MNB1 is monosynaptic and consists of both electrical and chemical synapses. With repetitive stimulation, the chemical component of the compound excitatory postsynaptic potential (EPSP) rapidly fatigues. These basic physiological results have been independently confirmed for the homologous connection in the fruit fly, Drosophila melanogaster (Trimarchi and Murphy 1997). In Calliphora, the afferents providing input to B1 originate from a single field of campaniform sensilla at the base of the haltere (Chan and Dickinson 1996; Fayyazuddin and Dickinson 1996). Based on the physiology of the haltere-B1 synapse and the firing patterns of B1 during flight (Heide 1983; Tu and Dickinson 1996), we proposed a simple model for the phase tuning of MNB1. In the absence of any external perturbation, mechanoreceptors at the wing base entrain MNB1 to phase tuning of MNB1. In the absence of any external perturbation, mechanoreceptors at the wing base entrain MNB1 to firing pattern of MNB1 under experimental conditions that mimic those that occur during flight. In an attempt to better understand the cellular basis underlying these phenomena, we compare the strengths and dynamics of the EPSPs evoked in MNB1 by stimulation of wing and haltere afferents. The results provide an example of a mechanism for the integration of phasic sensory signals to control motor output.

METHODS

Preparation and recordings

The blowflies (Calliphora vicina) used in this study originated from a culture maintained in our laboratory. Several times a year, we restock the culture with wild-caught individuals to maintain its genetic diversity. We used 1- to 3-day-old male and female blowflies in all experiments. The dissection and general recording procedures have been described previously (Fayyazuddin and Dickinson 1996). Briefly, flies were cold anesthetized and waxed ventrally to a brass rod. The notum was removed along with the indirect flight muscles to reveal the thoracic ganglion, which was then mechanically desheathed. Recordings were made at room temperature using sharp electrodes filled with a solution of 3 M K-acetate and 0.1 M KCl (resistances 50–60 MΩ). Intracellular signals were recorded using an Axoclamp 2A intracellular amplifier (Axon Instruments, Foster City, CA) and filtered at 10 kHz. All extracellular recordings were made through suction electrodes connected to A-M systems model 1800 extracellular amplifiers (band-pass filters set at 0.3 and 5 kHz). Signals were recorded on videotape and analyzed off-line.

Stimulation of mechanosensory afferents

Afferents originating on the wing and haltere were electrically stimulated through suction electrodes placed on the appropriate peripheral nerves (Fig. 1A). Wing afferents enter the thoracic ganglion via the anterior dorsal mesothoracic nerve (ADMN) (Power 1948), which also contains the motor axons of several flight muscles including B1. As the ADMN travels distally, a series of small motor nerves branch off to innervate their respective muscles. We placed a stimulating suction electrode on the ADMN at a site distal to the branch point of the small B1 nerve (Heide 1983). Voltage pulses applied to the electrode activated afferent fibers, but not the B1 axon. The sensory fibers within this distal portion of the ADMN comprise three major submodalities of the wing: bristle sensilla of the marginal vein, large distal campaniform sensilla of the wing blade, and the numerous small campaniform sensilla of the wing base (Dickinson and Palka 1987; Gnatzy et al. 1987; Palka et al. 1979). An additional suction electrode placed on the B1 nerve served three purposes. First, it enabled us to unambiguously identify penetrations of the MNB1 within the thoracico-abdominal ganglion. Second, by monitoring the delay between B1 action potentials recorded centrally with those in the B1 nerve, we could discount cases in which current spread from a wing nerve stimulus directly activated the B1 motor axon. Third, the electrode on the B1 nerve was used to antidromically stimulate MNB1, providing a convenient means of examining subthreshold synaptic potentials.

Stimulation of wing or haltere afferents produces EPSPs in MNB1 of sufficient strength to elicit overshooting action potentials. Because...
certain aspects of this study required visualization of the underlying EPSPs, it was at times necessary to prevent the MNB1 from firing in response to sensory input. Unfortunately, the high impedance of the recording electrodes did not typically allow passage of sufficient hyperpolarizing current to inhibit the large MNB1 axon from firing in response to sensory nerve stimuli. Although it was possible in several experiments to raise spike threshold by perfusing the desheathed preparations in saline containing an elevated concentration of divalent cations, this method proved unsatisfactory for three reasons. First, in some experiments we wished to examine the effects of low external Ca$^{2+}$. Second, because the ionic milieu of insect ganglia is regulated by a complex blood-brain barrier, ion exchange experiments require removal of the ganglionic sheath. Although we removed the sheath by dissection using 30G hypodermic needles, the precise extent of desheathing is difficult to control, and in some preparations spike thresholds remained unchanged after superfusion with saline containing elevated concentrations of divalent cations. Third, the Ca$^{2+}$ and Mg$^{2+}$ concentrations required to raise threshold could potentially influence synaptic properties such as short-term depression and facilitation (Thies 1965). For these reasons, we employed an alternate method to study subthreshold events (Fig. 1B). By stimulating the B1 axon within its peripheral nerve just before activation of the afferent fibers, the evoked EPSPs occurred within the refractory period of antidromic action potentials. The results from experiments where the B1 was antidromically activated were consistent with ones in which elevated concentrations of divalent ions were used to block action potentials. Antidromic stimulation of MNB1 did, however, raise one experimental complication. Each antidromic spike was followed by a calcium-dependent afterhyperpolarization. These afterhyperpolarizations summed with repetitive antidromic stimulation to produce a 1- to 5-mV sag in the membrane potential. To separate any temporal changes in EPSP size from the time dependence of this sag, we activated the MNB1 with 10 antidromic stimuli before activating the wing or haltere nerve. By using this procedure, the slow hyperpolarization in MNB1 reached a steady-state level before the arrival of the first afferent stimulus. The waveform of the antidromically activated action potential was digitally subtracted before any measurements were made on the EPSPs. In addition, stimulation artifacts have been either digitally subtracted or cut in all figures to clarify the data.

**Solutions**

During experiments, the preparations were continually perfused in saline containing (in mM) 150 NaCl, 10 KCl, 4 CaCl$_2$, 2 MgCl$_2$, 4 NaHCO$_3$, 90 sucrose, 5 trehalose, and 5 HEPES. In some experiments, we used a low calcium saline containing 150 NaCl, 10 KCl, 1 CaCl$_2$, 6 MgCl$_2$, 4 NaHCO$_3$, 90 sucrose, 5 trehalose, and 5 HEPES; a calcium-free saline containing 150 NaCl, 10 KCl, 1 EGTA, 6 MgCl$_2$, 4 NaHCO$_3$, 90 sucrose, 5 trehalose, and 5 HEPES; or a saline with an elevated concentration of divalent cations containing 150 NaCl, 10 KCl, 12 CaCl$_2$, 6 MgCl$_2$, 4 NaHCO$_3$, 54 sucrose, 5 trehalose, and 5 HEPES.

**R E S U L T S**

**Temporal precision and strength of wing and haltere input to MNB1**

Single electrical stimuli applied to either the wing or haltere nerve are sufficient to evoke action potentials in MNB1, which are easily monitored by extracellular spikes in the B1 nerve. Although MNB1 follows a haltere nerve stimulus with a precise delay, the spike elicited by a wing nerve stimulus follows with a more variable latency (Fig. 2A), suggesting that the wing afferents might provide a weaker excitation of the motor neuron. To gain a rough measure of the relative synaptic strengths of the wing and haltere inputs, we determined the minimum interstimulus interval at which the second in a pair of homosynaptic stimuli could drive MNB1 past threshold. The motor neuron could follow a pair of wing stimuli at intervals as short as 10.7 ± 0.9 ms (mean ± SE, n = 3) and haltere stimuli with intervals as brief as 10.2 ± 2.1 ms (n = 5). Although the

![Fig. 2](http://jn.physiology.org/)

**FIG. 2.** MNB1 is able to follow heterosynaptic stimulation at a much shorter interstimulus interval than homosynaptic stimulation. A: homosynaptic stimulation of mechanosensory afferents. Left panel: extracellular recordings from the B1 axon in response to stimulation of haltere afferents with a 2-pulse protocol. Four traces with different interstimulus intervals are depicted. Right panel: a similar experiment with wing afferent stimulation. The mean values of the minimum interstimulus intervals that could produce a spike in MNB1 were 10.2 ± 2.1 ms (n = 5) and 10.7 ± 0.9 ms (n = 3) for homosynaptic haltere and wing stimulation, respectively. B: heterosynaptic stimulation of mechanosensory afferents. Left panel: haltere afferents were stimulated 1st, followed by stimulation of the wing afferents at different interstimulus intervals. Right panel: wing nerve stimulation was followed by haltere nerve stimulation. The mean values for minimum heterosynaptic interstimulus intervals that could produce a spike in MNB1 were 4.0 ± 1.2 ms (n = 4) when the wing stimulus followed the haltere stimulus and 4.5 ± 0.9 ms (n = 3) when the haltere stimulus followed the wing stimulus. The timing of each stimulus is indicated below the recordings by open circles for haltere nerve stimulation and filled circles for wing nerve stimulation.
response to wing stimulation was more variable, the results indicate that the refractory period of MNB1 is no longer between two wing stimuli than it is between two haltere stimuli.

Heterosynaptic experiments, in which a stimulus applied to one sensory nerve is followed with variable delay by a stimulus to the other, are shown in Fig. 2B. The motor neuron could follow a wing stimulus preceded by a haltere stimulus at intervals as short as 4.0 ± 1.2 ms (n = 4) and a haltere stimulus preceded by a wing stimulus at intervals as short as 4.5 ± 0.9 ms (n = 3). Both of these heterosynaptic minimum intervals are substantially shorter than the comparable homosynaptic values, suggesting that the homosynaptic intervals are limited by an attenuation in the strength of the synaptic input and not by the absolute refractoriness of the motor neuron.

Because the suction electrode used to stimulate the wing afferents was close to the B1 muscle, large stimuli applied to the wing nerve could result in a direct excitation of MNB1 if the stimulus voltage was too high. Therefore, despite the ease of examining threshold events in MNB1 with extracellular recording, we continued our analysis using intracellular recordings. By comparing the relative timing of the intracellular MNB1 spike with the extracellular spike in the B1 nerve, it was possible to unambiguously determine whether the motor neuron’s response was due to a synaptic input or to direct axonal excitation. This procedure allowed us to keep the stimulus voltage as high as possible for maximum recruitment of wing afferents, while making sure that we did not activate MNB1 directly. Figure 3 shows intracellular recordings from MNB1 in response to stimulation of the haltere and wing nerves at frequencies of 40 and 130 Hz. At a stimulus frequency of 40 Hz, both the wing and haltere nerves can drive MNB1 without failure. As with the extracellular experiments, these results demonstrate that MNB1 follows haltere stimuli with a greater temporal precision than it follows wing stimuli. Whereas there is no significant temporal jitter in haltere-evoked spikes at 40 Hz, the timing of wing-evoked spikes in MNB1 varies by as much as 700 μs (Fig. 3B). At a stimulus frequency of 130 Hz, MNB1 begins to skip occasional stimulus pulses within the train (Fig. 3, A and B). At this high frequency, the response latencies of both haltere- and wing-evoked spikes become more variable, although MNB1 continues to follow haltere input with greater temporal precision than it does wing input. However, the failure rate of MNB1 at high frequency was no greater for wing stimuli than it was for haltere stimuli, again indicating that the strength and temporal precision of the wing and haltere inputs do not appear correlated.

Phase-dependent entrainment of MNB1 by mechanosensory afferents

The results described in the previous section indicate that the summed excitatory inputs from the wing and haltere nerves onto MNB1 differ somewhat in strength and temporal reliability. How might these physiological differences relate to the functional roles played by the two modalities during flight? To examine this question, we attempted to simulate the convergent interactions that might take place between the two inputs during flight (Fig. 4). While driving the motor neuron with a train of stimuli at 100 Hz (close to the normal wingbeat...
frequency of 120–150 Hz) to one afferent nerve, we began stimulating the other nerve at the same frequency but with a fixed phase delay relative to the stimulus train driving the first nerve. The responses of MN1B to different phases of haltere nerve stimulation within a background of wing nerve stimulation are shown in Fig. 4, A–D. At phase delays less than −70%, the superimposed haltere stimulus arrives in each cycle when the MN1B is still refractory from the wing-evoked spike, and the motor neuron remains locked to the wing stimulus. At phase delays of ≥70%, the motor neuron is sufficiently recovered from the wing-evoked spike when the haltere stimulus arrives. The entrainment is weak, however, and the motor neuron occasionally skips the haltere stimulus and follows the wing input. In contrast, superimposed wing input is capable of entraining MN1B within a background of haltere input over a much broader range of phase delays (Fig. 4, A and E–G). At phase delays between 5 and 15%, the first wing stimulus does not drive MN1B to fire, but it produces enough of a depolarization to raise firing threshold so that the subsequent haltere stimulus is unable to elicit a spike (Fig. 4E). At phase delays of ≥25% (Fig. 4, F and G), MN1B completely switches from haltere input to wing input. In sum, these experiments indicate that the wing nerve input to MN1B is stronger than haltere input, as measured by its competitive ability to entrain MN1B over a wider range of phase differences. Further, whereas the superimposed haltere input shows signs of fatigue within the permissible phase range, the motor neuron remains faithfully entrained to the wing stimulus for the duration of the superimposed train. These results indicate that the wing input might be capable of tonically entraining MN1B during flight. In contrast, the haltere input could at best only transiently advance the firing phase of the motor neuron. The results also suggest that the two modalities can only drive MN1B in an “either/or” fashion during flight. In no case did MN1B fire in response to both a haltere and a wing stimulus in the same cycle. This constraint presumably results from the fact that the refractory period of the motor neuron is roughly of the same order as the stimulus interval of 10 ms (cf. wingbeat interval of ~7–8 ms for flight).

Comparison of the wing and haltere inputs

The results described above indicate that, although the sensory cells in the haltere nerve can drive MN1B to fire with greater temporal precision, the afferents within the wing nerve provide a stronger competitive excitatory input. To investigate the mechanistic bases for these differences, we compared the magnitude and time course of the underlying synaptic potentials evoked by stimulation of the two afferent populations. As described in METHODS, we elicited reliable subthreshold responses in MN1B by backfiring the B1 axon just before stimulation of the mechanosensory afferents. Under these conditions, the sensory stimuli arrive when the motor neuron is still refractory.

In a previous study (Fayyazuddin and Dickinson 1996), we have shown that electrical stimulation of the haltere nerve results in a compound EPSP that consists of a fast electrical and a slow chemical component in MN1B. These results for haltere input were confirmed in the present study (Fig. 5A).

FIG. 4. Wing input is capable of entraining MN1B over a much greater range of stimulus phase relative to the haltere nerve in phase resetting experiments. A: raster plots of 2 representative phase-competition experiments. These experiments are designed to measure the ability of the wing and haltere modalities to entrain MN1B. In each sweep, a background train of 20 pulses is applied to either the wing or haltere nerve. At the midpoint of this background train, a short test stimulus train of 10 pulses (phase shifted relative to the background train) is applied to the other sensory nerve. In each subsequent sweep, the relative phase of the background and test trains is increased by 5%. Top panel: the wing nerve is stimulated in the background train while the haltere nerve is stimulated in the test train. Bottom panel: data from the inverse experiment where the haltere nerve is stimulated in the background train while the test train is applied to the wing nerve. Each raster line shows the spike occurrences in MN1B at a particular superimposed stimulus phase. ●, wing-elicited spikes; ○, haltere-elicited spikes. Diagonal dashed lines indicate the expected position of the MN1B spike if it were to follow the test stimulus. Phase delay values have been corrected for differences in conduction delay between the 2 pathways. B–G: sample intracellular recordings of the stimulus train experiment at the phase values indicated in A. Under each trace, the timing of the stimuli applied to the 2 sensory nerves is indicated by vertical lines. All data in this figure is from experiments performed in the same preparation. The frequency of stimulation was chosen to be the maximum frequency MN1B could follow in response to either wing or haltere homosynaptic stimulation. In the experiments shown, the stimulation frequency was set at 100 Hz.

FIG. 5. Comparison of haltere and wing EPSPs. Haltere (A) and wing (B) EPSPs are displayed in the presence and absence of calcium to separate the electrotonic and chemical components. The same data as in the left panels are presented with an expanded time scale in the right panels to better show the differences in the 2 types of inputs. The haltere EPSP consists of 2 components that can be distinguished by latency and time course. In comparison, the wing EPSP consists of 3 components. The relative contributions of the components is significantly different for the 2 EPSPs. The peak of the haltere EPSP is determined by the fast component, whereas the middle component dominates the wing EPSP. The late component of the haltere EPSP and the latter 2 components of the wing EPSP disappear in the presence of calcium-free saline, indicating that they are mediated by chemical synapses. The residual components differ significantly for the 2 modalities with the electrical component of the haltere EPSP being considerably larger in amplitude than that generated by the wing.
Amplitude of Ca$^{2+}$ 1966). For this reason, we compared the integral of the wing and of an EPSP and its peak amplitude (Hill 1936; Noble and Stein threshold of a postsynaptic target is a function of both the duration inputs (Table 1) are quite similar (Fatt and Katz 1951). Another times of both the electrical and chemical components, respectively. * $P < 0.001$, † $P < 0.01$; significance levels of a means comparison using a paired sample $t$-test with unequal variances.

showed a latency of 2.51 ± 0.11 ms ($n = 4$) and may be analogous to, although larger than, the slower, Ca$^{2+}$-sensitive component of the haltere EPSP. Wing stimuli elicit an additional prolonged depolarization that persists for up to 25 ms following the falling phase of the slow component. This prolonged component is either absent or very small in the haltere-evoked EPSP. Although the time course of the fast and slow components of the wing and haltere EPSPs are similar, their relative contributions are significantly different as summarized in Table 1. Whereas the electrical component dominates the haltere EPSP, the slow component makes up the largest portion of the wing-evoked EPSP. As with the slow component of the haltere EPSP, both the slow and prolonged components of the wing EPSP require calcium, consistent with their resulting from chemically mediated synapses. As with the haltere input, the fast component of the wing-evoked EPSP persists in Ca$^{2+}$-free saline, suggesting that it too represents an electrotonic input from sensory afferents. As shown in Fig. 5, the early components of both the wing- and haltere-evoked EPSPs grow larger in the absence of calcium. This increase in the magnitude of the fast component in low Ca$^{2+}$ is discussed in greater detail in a following section.

Although stimulation of the wing nerve can elicit spikes in MNB1 with greater efficacy than stimulation of the haltere nerve (Fig. 4), the peak amplitudes of the two compound EPSPs are nearly equal in size (Table 1). One possible explanation could be that the wing afferents synapse closer to the spike initiating zone in comparison with the haltere afferents. Although we do not specifically know the location of the wing synapses on the MNB1 relative to the haltere synapses, we can surmise that the electrotonic distances between the two is not very large because the rise times of both the electrical and chemical components of the two inputs (Table 1) are quite similar (Fatt and Katz 1951). Another explanation is that the difference in shape of the haltere and wing EPSPs could itself be responsible for the difference in their respective abilities to drive MNB1 past threshold. The spike threshold of a postsynaptic target is a function of both the duration of an EPSP and its peak amplitude (Hill 1936; Noble and Stein 1966). For this reason, we compared the integral of the wing and haltere EPSPs, calculated from the onset to the time of peak voltage (Table 1, Fig. 6E). The time-to-peak areas of the wing EPSPs were nearly twice as large as the haltere EPSPs; a difference due to the greater contribution of the slow, chemically mediated component.

**Characterization of frequency dependence of mechanosensory inputs**

The paired-pulse experiments in Fig. 2 show that the MNB1 can follow the second of two inputs at a shorter latency if the two inputs originate from different sensory pathways. This result is most parsimoniously explained by a rapid decay in the strengths of the haltere and wing inputs with repetitive stimulation. Figure 6A shows 10 consecutive responses of MNB1 at the start of a 130-Hz stimulus train applied to the wing and haltere nerves. The size of the haltere EPSP decreases after the first stimulus and then remains stable for the duration of the train. Chemical synapses often show fatigue at high stimulus frequencies due to the dynamics of calcium entry and vesicular release (Zucker 1989), whereas electrical synapses are generally less sensitive to repeated activation (Jaslovec and Brink 1987). This does not appear to be the case with the wing and haltere synapses onto MNB1, however, because both the early and late components show a frequency-dependent decrement in size.

Although the dynamics of the wing- and haltere-mediated synapses are similar, there are a few notable differences. First, the wing EPSP shows a partial recovery following the second stimulus in the train (Fig. 6B). Second, following the partial recovery, the wing EPSP exhibits a slow gradual decrease throughout the rest of the stimulus train. The decay in the peak amplitude of the wing EPSP is proportionally larger than the change in the amplitude of the haltere EPSP (Fig. 6B). However, this increased decay in relative amplitude does not necessarily indicate that the steady-state strength of the wing input is less than that of the haltere input. Figure 6, D and E, shows the absolute peak amplitude and time-to-peak integrals of the wing and haltere EPSPs. Although the average peak amplitudes of wing- and haltere-evoked EPSPs are similar throughout a train of stimuli, the time-to-peak integrals of the wing-evoked EPSPs are significantly greater than those of the haltere-evoked EPSPs.

Figure 7A shows that the decay in the magnitude of the wing- and haltere-evoked EPSPs is Ca$^{2+}$-dependent. Superfusion with zero or reduced Ca$^{2+}$ saline significantly diminished the attenuation of the fast component of both synapses during a stimulus train ($n = 4$). Not only do the amplitudes remain constant throughout the stimulus train, the rise times of each EPSP within the train are identical. As shown previously (Fig. 5B), although Ca$^{2+}$-free saline abolished the late and prolonged components of the compound EPSPs, it actually increased the magnitude of the remaining electrotonic component.

**Recovery from depression of mechanosensory EPSPs**

We quantified the time course of recovery of the haltere- and wing-evoked EPSPs by stimulating each nerve with a train of 10 stimuli at 200 Hz followed, with variable delay, by a single stimulus. Figure 8A shows superimposed EPSPs

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**Table 1. Comparison of wing- and haltere-evoked EPSPs in MNB1**

<table>
<thead>
<tr>
<th></th>
<th>Haltere</th>
<th>Wing</th>
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<tbody>
<tr>
<td>Amplitude of electrical component, mV</td>
<td>11.46 ± 1.40</td>
<td>5.24 ± 0.56*</td>
</tr>
<tr>
<td>Amplitude of Ca$^{2+}$-sensitive component, mV</td>
<td>5.42 ± 1.60</td>
<td>10.75 ± 1.20*</td>
</tr>
<tr>
<td>Half width of EPSP, ms</td>
<td>1.11 ± 0.36</td>
<td>3.76 ± 1.70†</td>
</tr>
<tr>
<td>Integral to time-of-peak, mV ms</td>
<td>34.1 ± 8.1</td>
<td>71.6 ± 4.0*</td>
</tr>
<tr>
<td>Rise time of electrical component, ms</td>
<td>0.60 ± 0.03</td>
<td>0.58 ± 0.03 n.s.</td>
</tr>
<tr>
<td>Time-to-peak of chemical component, ms</td>
<td>1.65 ± 0.19</td>
<td>1.58 ± 0.07 n.s.</td>
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Values are means ± SE; number of haltere EPSPs is 6 and wing EPSPs is 4. EPSPs, excitatory postsynaptic potentials; MNB1, B1 motor neuron. Rise time of electrical component and time-to-peak of chemical component are defined as the time delay between the beginning of the EPSP and the peak of the electrical and chemical components, respectively. * $P < 0.001$, † $P < 0.01$; significance levels of a means comparison using a paired sample $t$-test with unequal variances.
elicited in response to the test pulses. Figure 8B shows the recovery plotted as a function of the delay between the end of the stimulus train and the test pulse. The mean time constants of recovery (defined as the time required for the EPSP to recover to 63% of its original value) for the wing and haltere inputs were 25 and 15 ms, respectively, indicating that the haltere EPSP recovers more quickly from depression than does the wing EPSP. In addition, whereas the
haltere EPSP returns to control levels, the wing EPSP only partially recovers. The partial recovery may be related to the slow gradual depression illustrated in Fig. 6. The data suggest that the wing EPSP depresses in two stages: a rapid phase similar to that seen in the haltere EPSP and a slower, long-lasting stage reminiscent of classical synaptic fatigue due to transmitter depletion. Further, although the depression of the wing input is relatively long-lasting, the haltere input can recover fully within two to three wing strokes. Nevertheless, as indicated in Figs. 4 and 6 the steady-state strength of the wing input to MNB1 appears greater than that of the haltere input.

**DISCUSSION**

In a previous paper, we proposed the following model to explain how mechanosensory afferents on the wing and haltere interact to determine the firing times of B1 spikes during flight (Fayyazuddin and Dickinson 1996). In straight flight, the phase-locked input from wing afferents entrains MNB1 to fire at the start of the downstroke. During flight perturbations, transient input from haltere afferents in campaniform field dF2 advances the firing of MNB1 so that B1 is activated earlier in the cycle, during the upstroke. The findings presented in this paper, in which we compare the interaction of wing and haltere afferents on MNB1, are consistent with this model. Both inputs are strong enough to entrain spikes in the ipsilateral MNB1 at frequencies approaching the animal’s natural wingbeat frequency (~120–150 Hz). The haltere afferents, however, can drive MNB1 with much greater temporal accuracy than the wing afferents. On the other hand, the wing input is significantly stronger than the haltere input as judged by its competitive ability to entrain MNB1 at high frequencies. If the wing afferents are stimulated at different phases within a train of haltere nerve stimuli, MNB1 follows the wing input, even if the delay between haltere stimuli and wing stimuli is as short as 2 ms. To entrain MNB1 within a background of wing stimuli, the haltere input requires a much larger delay.

In this paper we explore the synaptic basis for the functional differences between the wing and haltere inputs to MNB1 by comparing the structure of the underlying compound EPSPs. The haltere EPSP in MNB1 consists of both electrical and chemical components (Fayyazuddin and Dickinson 1996; Tri-marchi and Murphey 1997). We have found that the wing input, similarly, consists of both electrical and chemical components. However, the relative contribution of the two components to the EPSP generated by the wing afferents is much different from that generated by the haltere afferents. Although the electrical component dominates the haltere input, the chemical contribution to the EPSP is larger for the wing input. In addition, a wing nerve stimulus elicits a slow, prolonged Ca$^{2+}$-dependent response that is entirely missing from the haltere response.

**Frequency-dependent fatigue of mechanosensory EPSPs**

Both the wing- and haltere-evoked EPSPs fatigued when elicited at frequencies approaching wingbeat frequency. This fatigue affected both the fast electrical and the slow chemical components of the EPSP, which suggests that the decay in the size of the EPSP is not entirely due to a depletion of the vesicular pool, receptor desensitization, or some other transmitter-related process. Both the haltere and wing EPSPs recover from this depression rather quickly: the time constant of recovery is 15 ms for the haltere EPSP, and 25 ms for the wing EPSP. Frequency-dependent reduction of electrotonic coupling has been described in the frog primary afferent–motor neuron synapse (Shapovalov and Shiriaev 1980), in which both the presynaptic spike and the postsynaptic EPSP were monitored. In that preparation, the decrease in the postsynaptic electrical EPSP occurred only with a concomitant reduction in the size of the presynaptic action potential due to the refractory period of the afferent axon. This reduction might result from Ca$^{2+}$-dependent hyperpolarizing channels in the presynaptic terminal that mediate a shunting conductance. This interpretation is consistent with both the absence of activity-dependent fatigue and increase in size of the electrical synaptic potential in Ca$^{2+}$-free saline. The presence of Ca$^{2+}$-dependent K$^+$ conductances has been reported at larval neuromuscular junctions of Drosophila, where they can modulate synaptic facilitation (Mallart 1993).

Several other possibilities may account for the fatigue of the electrical EPSP with repetitive stimulation. The depression might result from recruitment failure of sensory afferents within the haltere and wing nerves in response to electrical
stimuli. However, because increasing the magnitude of the stimulus voltage had no effect on the shape or time course of the EPSP, we do not believe afferent failure is responsible for the depression. Furthermore, afferent failure could not easily explain the absence of fatigue in Ca\(^{2+}\)-free saline. Another possibility is that presynaptic inhibition via an interneuron might shunt currents resulting in a narrower afferent spike (Bennett et al. 1985; Wolf and Burrows 1995). Although the depression is present even when the ganglion is bathed in high divalents saline (personal observation), we cannot rule out the possibility that the effect is mediated by nonspiking inhibitory interneurons. Buchanan and co-workers (Buchanan et al. 1992) have suggested that dendritic filtering may account for the activity-dependent depression of the Müller cell to spinal neuron synapses in the lamprey. Because the haltere afferents make a large calyx-like connection with the primary dendrite of MNB1 close to the recording site, dendritic filtering should not play a major role in the reduction in the size of the electrical synaptic potentials observed in our preparations. Finally, the presynaptic terminal may contain feedback autoreceptors that activate shunting conductances when transmitter is released from the afferent terminals or, alternatively, such receptors may be present on the postsynaptic membrane. Because the transmitter of the wing and haltere mechanosensory neurons is likely to be acetylcholine (Gorczyca and Hall 1987; Trimarchi and Murphey 1997), muscarinic receptors might serve this feedback role. In summary, the most likely explanations for the depression of the electrical synapse invokes shunting conductances activated either by Ca\(^{2+}\) entry into the afferent terminals or the release of acetylcholine, which could act either pre- or postsynaptically. Without additional data, we have no way of distinguishing among these different mechanisms.

In addition to the rapid drop in EPSP size at the start of a stimulus train, the wing EPSPs showed an additional slow phase of depression, which might represent classical fatigue of the chemical component (Zucker 1989). Another peculiar feature of the wing input is the partial recovery of the EPSP following the second pulse within each stimulus train. Although we did not investigate this phenomenon further, it might result from facilitation superimposed on the classical depression of the chemical synapse.

Role of haltere inputs in shifting phase of MNB1

During straight flight, B1 fires at a fixed phase relative to the wingbeat cycle (Heide 1971, 1983). In all species of flies so far examined, the phase of firing is advanced on the outside of the turn during turning maneuvers (Musca: Egelhaaf 1989; Calliphora: Heide 1983; Drosophila: Heide and Götz 1996). Based on ablation experiments, the phase tuning of B1 is thought to be determined entirely by mechanosensory inputs from the wing and haltere (Heide 1983). Transecting the sensory component of the wing and haltere nerves removes all phase-locked firing of MNB1. Mechanosensory input from a single ipsilateral haltere or wing is, however, enough to entrain B1 to some degree within the wingstroke. Under conditions where the wingbeat frequency is increased by reducing the wing mass, the B1 muscle continues to be phase-locked to the wingstroke (Heide 1983). This is an informative result, because under these conditions of altered mass the wing beats at a faster frequency than the haltere (Buddenbrock 1919; Sellke 1936). This suggests that during straight flight MNB1 is entrained by the wing afferents and not haltere afferents. Nalbach and Hengstenberg (Nalbach 1994; Nalbach and Hengstenberg 1994) have demonstrated that the haltere system in Calliphora activates neck-motor reflexes and changes in stroke frequency. In Drosophila, haltere-mediated reflexes cause compensatory changes in wingstroke amplitude (Dickinson 1999), which are known from other studies to involve phase changes in the firing of the B1 muscle (Egelhaaf 1989; Heide and Götz 1996; Tu and Dickinson 1996). Thus the haltere afferents might function to transiently advance the firing phase of B1 during compensatory equilibrium reflexes.

Based on the evidence presented above, we developed a model for the phase tuning of MNB1 (Fayyazuddin and Dickinson 1996) in which the mean phase of firing of the motorneuron is set by proprioceptive feedback from the wing. If the fly is perturbed during flight, Coriolis forces generated at the end knob of the haltere produce strains in the cuticle that are transmitted to the base where they are encoded by campaniform sensilla. Our previous studies indicate that the afferents in the haltere nerve responsible for the input to MNB1 originate in a single campaniform field, d2 (Fayyazuddin and Dickinson 1996). As first proposed by Pringle (1948), d2 appears morphologically arranged so as to be most sensitive to the lateral deflection of the haltere, and thus may be the most important field for detecting Coriolis forces. During angular perturbation, the campaniform sensilla in d2 could transiently entrain MNB1 and shift the phase of firing of the B1 muscle. The physiology of the haltere afferent to MNB1 synapse presented in this paper is consistent with this model. The wing input is indeed stronger and entrains MNB1 under most conditions. When the haltere input is activated, MNB1 can transiently phase-lock to the haltere afferents but returns within a few cycles to following the wing input.

Although in this study we have found that the haltere input shows much greater temporal precision than the wing input, this may in fact be a reflection of our stimulation protocol. In our experiments the wing nerve was stimulated electrically so that all the mechanosensory afferents were activated simultaneously. This may not reflect the condition in a flying animal in which afferents from different individual sensilla may be activated during different phases of the wing stroke. Under natural flying conditions, the electrical component of the wing EPSP may, for example, occur at the apex of the chemical component and thus restrict the spike to a narrower phase range.

Our findings may also offer an explanation for why MNB1 spikes appear restricted to a relatively narrow phase band (Heide 1983; Heide and Götz 1996; Tu and Dickinson 1996), despite the fact that under different stimulus conditions the afferent spikes from the haltere might arrive within a broad window of the stroke cycle. The spike in MNB1 produced by the wing EPSP has a refractory period during which the relatively weak input from the haltere is unable to elicit a spike. Thus the haltere input must arrive at MNB1 within a permissible window. This mechanism can be compared with the visually driven course corrections in the locust flight system (Reichert et al. 1985). In this system, the phase-independent visual information carried by descending deviation detector interneurons is converted into phase-specific information by thoracic interneurons. These premotor interneurons integrate...
the synaptic drive from the central pattern generator for flight with the descending input from the deviation detectors to produce spikes only during the depolarizing phase of the central rhythm. In the case of the fly, the integration of two competing timing inputs takes place directly on the membrane of the steering motor neuron.

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